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POSSIBLE FUNCTIONS OF CHANNEL SUBUNIT FAMILIES
B. Sakmann

Functional characterization of membrane channels by patch clamp techniques has revealed great diversity of transmitter or voltage gated channels in native membranes. Concomitantly recombinant DNA techniques revealed a plethora of genes encoding channel subunits. Thus functional diversity within a particular class of channels may be generated by families of genes encoding homologous channel subunits that assemble in various combinations into functionally distinct channel subtypes. For most channels the subunit composition and stoichiometry of a particular functional subtype is not yet established except for the nicotinic acetylcholine receptor of Torpedo. One way to identify the subunit composition of channels in native membranes is to compare their functional properties with those of channels expressed in a host membrane, following introduction of subunit coding nucleic acids (cRNA or cDNA) into a host cell. In the case of ligand-gated channels, such as channels gated by acetylcholine (AChR channels) or γ-aminobutyric acid (GABAR channels) and voltage gated K⁺ channels mediating delayed or transient outward currents (RCK channels) it has been shown that particular functions of the channel can be attributed to particular channel subunits. Examples are the γ- and ε-subunits of skeletal muscle AChR channels or the β- and γ-subunits of GABAR channels, which specify channel subtypes with different pharmacological, kinetic and conductance properties. In the case of voltage gated K⁺ channels single RCK subunits specify functionally diverse homomultimeric K⁺ channels, which mediate transient and delayed K⁺ currents. However, heteromultimeric channels with novel properties can also assemble from different RCK subunits. The constituent RCK subunits specify sensitivity to K⁺ channel blockers, gating and conductance properties.

A clear correlation between particular channel phenotypes in the native membrane, their subunit composition and gene regulation of the respective mRNAs has been established for the nicotinic AChR channel in skeletal muscle. Here a developmental switch in the expression of the γ- and ε-subunit genes causes a change in end-plate channel properties from the fetal type, composed of αβε- subunits to the adult type subtype composed of αδε-subunits. Northern blot and in situ hybridisation analysis of AChR subunit specific mRNAs in fetal, adult and denervated skeletal muscle indicate that the expression of subunit specific mRNAs is regulated by multiple transcriptional mechanisms. First, in a mechanism which is restricted to the end-plate, subsynaptic nuclei become "imprinted" early during synaptogenesis to express subunit specific mRNAs. The expression of α- and β-subunit specific mRNAs is downregulated in the absence of nervous or muscular signals. Second, a more generalized mechanism operates on extrasynaptic nuclei and is dependent on the electrical activity of muscle fibres. Each AChR subunit gene is under multiple transcriptional controls each having different importance for each subunit.

The functional diversity of channels may allow control of gene expression by multiple transcription mechanisms. A switch in the expression of genes encoding particular subunits can occur in response to external stimuli causing a change in the channel phenotypes. This may be required for longterm adaptive changes in synaptic efficacy and in electrical excitability of neurons during development or differentiation.

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ROLE OF NITRIC OXIDE (NO) AND ADENOSINE IN THE REGULATION OF CORONARY BLOOD FLOW.
J. Schrader

The oxygen supply via the coronary circulation normally exactly matches the oxygen requirements of the heart so that cardiac energy usage relative to the delivery of oxygen is in equilibrium. Thus, metabolic regulation of coronary blood flow in essence is maintaining cardiac energy metabolism balanced (14). This concept was recently broadened by the demonstration that vasodilatory and vasoconstrictory factors are continuously formed by vascular resistance vessels (1). To what extent the endothelium-derived factors are under metabolic control of the cardiomyocytes is presently not known.

Criteria for the involvement of any metabolite in the control of coronary flow are: 1. Quantitative studies should indicate that the amounts and kinetics of transmitter release is appropriate to give the indicated effect. 2. Exogenously applied transmitter should mimic the physiological response. 3. Rapid mechanism of transmitter inactivation should be present. 3. Inhibitors of transmitter action should have effects consistent with the hypothesis.

Palmer et al. (1987) provided the first evidence for a parallelism between the release of endothelium-derived factor (EDRF) and nitric oxide (15). Using a specific difference-spectrophotometric assay based on the rapid oxidation of oxyhemoglobin to metmyoglobin we have confirmed the formation and release of NO into the coronary effluent perfusate of isolated guinea pig hearts (6-8). Authentic NO applied into the coronary circulation dose-dependently decreased vascular resistance and enhanced coronary release of cGMP. During single passage through the intact coronary system 86% of the infused NO was converted to nitrite ions. Increasing oxygen tension in aqueous solution from 150 to 700 mmHg decreased half life of NO (5.6s) by 32%. During passage of NO through the coronary circulation half life of NO is shortened to less than 130 ms. Isolated hearts constantly release NO and cGMP at a rate of 16 fmoles/min and 342 fmoles/min, respectively. The NO-scavenger oxyhemoglobin and methylene blue increased coronary resistance and decreased cGMP release. L-arginine, a putative precursor of NO (16), slightly increased coronary resistance while release of cGMP was enhanced. L-Nmonomethylarginine (10⁻⁴M) reduced basal release of NO and cGMP and this was associated with coronary vasoconstriction. Omission of NO release in bradykinin stimulated hearts preceded onset of coronary vasodilation in all cases. Amounts of bradykinin-induced NO release were within the dose-response curve for exogenously infused NO. Similar findings were obtained with acetylcholine, ATP and serotonin. Collectively these findings suggest that basal formation of NO is likely to play an important role in setting the resting tone of coronary resistance vessels. Nitric oxide appears to fulfill all of the above mentioned criteria for a chemical transmitter for the modulation of coronary vascular tone.
Adenosine is another potent coronary vasodilator the physiology and biochemistry of which has been intensively studied over the past twenty years (5) without finally defining its physiological role. The reasons for the remaining uncertainties reside in the fact that 1. the transmethylation pathway has been identified as an additional important intracellular route for the adenosine formation in the well oxygenated heart (4, 11). 2. The coronary endothelium constitutes a metabolic barrier for intracoronarily applied adenosine so that the true sensitivity of the coronary vascular smooth muscle might be higher than dose–response curves suggest (10, 13). 3. The coronary endothelium itself is capable of producing adenosine (9). 4. A substantial fraction of the released adenosine is derived from extracellular degradation of adenine nucleotides primarily liberated from isolated hearts and cultured endothelial cells (18). 5. The turnover of plasma adenosine is extremely short (less than 1 s in humans) which precludes any precise quantification of adenosine formation from arteriovenous differences (12).

Assessment of the physiological role of adenosine is further hindered by imprecise knowledge of the interstitial concentration of adenosine, to which vascular smooth muscle cells are exposed. Most of the cardiac adenosine is protein bound (S-adenosylhomocysteinehydrolase (SAH)) and small changes in the fraction of free adenosine may go undetected by conventional tissue extraction procedures. A new approach was recently developed in our laboratory (2, 3) to estimate the free intracellular adenosine concentration that makes use of the kinetic properties of SAH-hydrolase. Since the equilibrium constant of SAH-hydrolase favors synthesis, SAH formation prevails in the presence of increased levels of homocysteine. Enzyme kinetics predict that with saturating concentrations of l-homocysteine the rate of SAH formation is directly proportional to the free intracellular adenosine concentration. Using the principles of the SAH-method and C-11-homocysteine, a positron emitting isotope, cardiac adenosine formation was also assessed with PET. In the dog model, cardiac ischemia and coronary stenosis resulted in the local accumulation of C-11-SA H. Thus, an enhanced regional formation of adenosine can now be measured noninvasively. During pressure autoregulation no additional adenosine is formed by the heart. Only when the autoregulatory reserve is exhausted does the adenosine formation steeply increase. These and additional findings argue for the hypothesis that tissue oxygenation and not energy turnover is the most important stimulus which finally determines cardiac adenosine formation (17). Coronary autometry must be regulated accordingly, if adenosine is the mediator.

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THE MOLECULAR MECHANISM OF MUSCLE CONTRACTION. CONTROVERSIAL ISSUES AND RECENT IDEAS ABOUT CROSS- BRIDGE ACTION IN MUSCLE
D. Brenner

It is now generally accepted that muscle contraction occurs when the actin and myosin filaments slide past each other. According to the cross-bridge theory, each cycle of filament sliding, or force generation under isometric conditions, are driven by domains of the myosin molecule, the cross-bridges. In the hypotheses developed up to the mid-seventies (e.g. A.F. Huxley, Proc. Nat. Acad. Sci. 71, 1974), cross-bridges were considered to represent some oar-like mechanism involving several key features:
(i) A coordinated cycle of attachment, structural change of the attached cross-bridge (leading to force generation or filament sliding), detachment, and finally a structural change (reverse of the structural change of the detached cross-bridge) which returns the detached cross-bridge to the starting point of the cycle. (ii) With each attachment/detachment cycle one molecule of ATP was assumed to be hydrolyzed (i.e., coupling of attachment/detachment to ATP-hydrolysis). (iii) The structural change of the attached cross-bridge was thought to include filament sliding of some 10nm (i.e., coupling of 10nm of filament sliding to ATP-hydrolysis). (iv) Release of hydrolysis products was assumed to induce the structural change of the attached cross-bridge, while the actual hydrolysis step was thought to induce the large scale structural change returning the detached cross-bridge to its starting point in the cycle. The actual hydrolysis step should only occur while cross-bridges are detached from actin, release of products only while cross-bridges are attached to actin. (c) Regulation of muscle contraction was postulated to occur via control of the number of cycling cross-bridges (recruitment: Podoléky and Talcholj, J. Physiol. 201, 1970), provided by steric blocking and unblocking of attachment prior to force generation (Hasselgrove, Cold Spring Harb. Symp. 37, 1973; Huxley, ibid.; Parry & Squire, JBO 72, 1973).

Most of these postulated basic properties, however, are in conflict with recent experimental results or could not yet be confirmed:
(1) Biochemical studies revealed that the actual hydrolysis step does not induce a structural change and occurs both while cross-bridges are attached to or detached from actin (Stein et al., Biochem. 18, 1979).
(2) Many experimental approaches failed to reveal a large-scale structural change in attached cross-bridges. Only in EM studies and some X-ray diffraction work evidence for structural differences between attached cross-bridges in various states was found (e.g. Craig et al., PNAS 82, 1985; Yu & Brenner, Biophys. J. 40, 1986). However, it is not at all clear whether these differences arise from an "active" change in the structure of the attached cross-bridge or from a "passive" deformation imposed onto the attached cross-bridge. (3) Under high-speed shortening, ATP-hydrolysis is not several-fold larger than under isometric conditions, as expected from the theories of the seventies (e.g. Kushmerick & Davies, Proc. R. Soc. Lond. B 172, 1965). This finding, and recent in vitro studies (Tannagida et al., Nature 316, 1985) suggest that the sliding distance while a crossbridge passes through the force-generating state(s) is several fold larger than 10nm. (4) For all states it was found that cross-bridges can be attached to or detached from actin. In fast attachment and detachment are very fast reactions on the time scale of a cross-bridge cycle, i.e., a cross-bridge can detach and reattach many times as it completes one cycle, hydrolyzing one molecule of ATP (Brenner et al., PNAS 79, 1982; Biophys. J. 52, 1986; Brenner, PNAS 85, 1988). (5) Regulation of muscle contraction was considered to be due to release of hydrolysis products of ATP (Brenner, Nature New Biol. 238, 1972) which in turn determines the magnitude of the rate constants for active turnover (Brenner, PNAS 85, 1988). Thus, at least at partial activation, force-generating cross-bridges do not act completely independent of each other.

This summary illustrates that almost all of the key features of the generally accepted scheme for cross-bridge action need reconsideration. A working hypothesis will be discussed which was developed from the recent findings of mechanical, biochemical, and structural studies (Brenner, in: Molec. Mech. of Muscle Contr., J.M. Squire ed., Macmillan, 1989) in short, cross-bridges are assumed to (a) cycle between two main groups of states as ATP is hydrolyzed: the weak-binding states in which cross-bridges cannot generate active force, and the strong-binding states which represent the main force-generating states. In both groups of states cross-bridges dynamically interact with actin (detached or attached) more or less rapidly but always fast compared to active turnover. In the strong-binding states upon reattachment cross-bridges rapidly reassume a force-generating configuration. Thus, this conformational change after reattachment does not occur, leaving cross-bridges in a weakly-attached non-force-generating configuration. As ATP is hydrolyzed, cross-bridges cycle between these two main groups of states. (b) Rapid detachment which is necessary during high speed shortening to avoid impedance of filament sliding is provided by (transient) detachment with reattachment to a subsequent site on the actin filament while cross-bridges remain in the strong-binding states. Thus, different from the previous hypotheses, no large increase in ATPase activity with shortening velocity is expected and filament sliding while occupying the strong-binding states is not limited to 10nm, the expected range of a permanently attached cross-bridge. (c) Concerning regulation, we propose that P, release, the step which leads into the strong-binding state, only occurs when weak-binding cross-bridges are attached to the "turned on" (activated) form of actin, whereby the equilibrium between the turned on and turned off form of actin is determined by the Ca" binding to TnC along the actin filament.

We shall discuss the essential differences to previous hypotheses and their implications not only for our understanding of muscle contraction and cell motility but also for resulting new pharmacological approaches for modulation of contractile function, especially in sarcodinum and smooth muscle.

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PTH-dependent inhibition of proximal tubular transport: cellular mechanisms

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The kidney is one of the target organs of parathyroid hormone (PTH); PTH stimulates gluconeogenesis, ammoniagenesis as well as the production of 1,25(OH)₂Vit D₃ in cortical (proximal tubular) tissue, has effects on glomerular filtration rate and affects tubular reabsorption. Three 'direct' transport effects of PTH are of main importance: 1) it inhibits proximal tubular phosphate-reabsorption; 2) it inhibits proximal tubular bicarbonate-reabsorption and 3) it stimulates distal tubular calcium-reabsorption.

In vivo and in vitro micropuncture/microperfusion studies have well documented that proximal tubular phosphate-reabsorption is Na-dependent and secondary active (2, 4, 9). Similarly, it was found, that proximal tubular bicarbonate-reabsorption is to its large extent Na-dependent and transcellular transport is mediated by a secondary (tertiary?) active transport (3). The elements of transcellular Na-dependent transport of bicarbonate (Zelle A) and phosphate (Zelle B) are summarized in Figure 1.

Figure 1: Cellular scheme for bicarbonate-reabsorption (Zelle A) and phosphate-reabsorption (Zelle B).

These models are based on studies on intact cellular preparations as well as on studies with isolated membrane vesicles (2-10). A significant part (30-40%) of bicarbonate reabsorption does not involve Na/H-exchange but is driven by a primary active H-ATPase located in the brush border membrane (3). From studies on physiologically altered bicarbonate- and/or phosphate-reabsorption (PTH; P₁⁻ deprivation/overload; acidosis/alkalosis) it was concluded that altered transport is mainly related to altered transport rates of membrane transport mechanisms located in the apical membrane (2, 3, 4, 9).

As already indicated, increased PTH levels lead to inhibition of bicarbonate- and phosphate-reabsorption; studies with isolated membrane vesicles have suggested that these transport alterations are related to a reduced Vₘₐₓ of either Na/Pᵢ cotransport (2, 4, 9) or Na/H exchange (3, 10). It has been generally accepted, that PTH-dependent inhibition is mediated by basolateral hormone/receptor-interactions and stimulation of adenylate cyclase and that protein kinase A mediated phosphorylation reactions at the brush border membrane level lead to reduced transport rates of Na/H exchange and Na/Pᵢ cotransport rates (2, 3, 4, 9, 10). However, there is a gap between PTH concentrations occurring at 'normal' (physiological) conditions (= 10⁻¹² M) and those required for stimulation of the basolaterally located receptor/adenylate cyclase system (half maximal activation: = 10⁻⁸ M). Thus, the search for a second, cAMP-independent pathway in PTH dependent control of proximal tubular transport was initiated. Indeed, already several years ago evidence for PTH-dependent alterations in phosphatidylinositol-metabolism was presented (e.g. 11); more recently PTH dependent activation of phospholipase C activity was documented in an enriched proximal tubular basolateral membrane preparation (12). Furthermore, in suspended mouse renal tubules a phorbol ester dependent activation of protein kinase C led to a reduced Na-dependent phosphate uptake (13).

We and others have used over the last several years an established renal cell line derived from opossum kidney as a model system to study PTH regulation of proximal tubular transport functions. This cell line (OK-cells) shows a polar distribution of transport systems: Na/Pᵢ cotransport, Na/H exchange and Na/hexose cotransport in the apical and Na/K-ATPase, Na/Pᵢ cotransport and Na-independent phosphate transport in the basolateral membrane; the apical membrane Na/Pᵢ cotransport is different from that in the basolateral membrane and shows kinetic properties similar to that in the renal proximal tubule; also Na/H exchange is similar to that in the renal proximal tubule and shows allosteric control by intracellular pH (5-8). Furthermore, apical Na/H exchange and Na/Pᵢ cotransport (but not the basolateral transport systems) are inhibited by PTH, half maximal inhibition is observed at extremely low PTH concentrations, i.e. lower than 10⁻¹¹ M (8, 14-17). PTH inhibition of Na/Pᵢ cotransport and Na/H exchange is mimicked by pharmacological activation of either kinase A or kinase C (5, 6, 15). PTH also leads to activation of phospholipase C and production of diacylglycerol (DAG) and inositoltrisphosphate (IP₃) (12, 17). Dose response relationships (see figure 2) suggest that phospholipase C activation and subsequent protein kinase C activation is of particular importance in control of transport activities at low (physiological) PTH concentrations.

Figure 2: "Correlation" between PTH-dependent production of intracellular messengers and inhibition of Na/H exchange and Na/Pᵢ cotransport.
Although there is a close similarity in the regulatory cascades for PTH-dependent inhibition of Na/H exchange activity and Na/Pi cotransport, we have obtained evidence that the 'final' inhibitory mechanisms may be different: Na/Pi cotransport inhibition, but not Na/H exchange inhibition, seems to involve an endocytotic mechanism (18). The inhibition of Na/H exchange by activation of kinase C is surprising; experiments using a different cell line (LLC-PK1/PK20), which has apical as well as basolateral Na/H exchange, documented that apical activity ('epithelial' Na/H exchange) is inhibited by kinase C whereas basolateral exchange ('housekeeping') is stimulated (19).

**SUMMARY:** Proximal tubular brush border membrane Na/H exchange and Na/Pi cotransport are inhibited by PTH initiated regulatory cascades. It is/was generally accepted that cAMP-mediation plays a role in cellular control of these transport activities. However, recent studies suggest that also a cAMP independent pathway, i.e. phospholipase C/protein kinase C, is able to mediate PTH action. Studies on cultured epithelial cells suggest that the cAMP-independent pathway is of particular importance at low PTH concentrations; thus, a dual receptor concept for PTH action on proximal tubular transport has to be envisaged (see figure 3).

![Figure 3: Schematic representation of cellular mechanisms in PTH-dependent control of proximal tubular transport.](image)

Further work should focus 1) on the verification in intact tubular preparations of the model presented in figure 3 (based on observations made in cultured epithelia), 2) on the identification and location of (2) PTH receptor(s) including their cellular location (apical vs basolateral) as well as 3) on the structural identification of the transport systems known to be targets of PTH action.

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NEUROPHYSIOLOGY OF OLFACTION

J. Boeckh

The introduction of modern methods and approaches into the area of olfactory physiology and the impact of an increasing number of researchers especially in the US have brought about considerable progress into this field which has long been notorious for its poor development in comparison to other major sensory pathways. Even if there is no convincing report on the isolation or identification of receptor proteins, odor induced changes in second messenger systems have been detected, and membrane studies have revealed a number of ionic channels as well as receptor currents and other details of electrogenesis. Odor binding proteins have been identified, and the inventory of known cell specific proteins increases permanently. Tracer studies and immunohistochemistry of the developing, regenerating and adult mucosa and central stages resulted in identification of cell types, projection patterns and formation of local circuits and pathways. Electrophysiology and micro-pharmacology have provided more and extended inside into modes of interactions between neuronal elements and have partly confirmed, extended or corrected prior and current hypotheses about modes of coding of quality, quantity, time course and spatial distribution of odor stimuli. A host of recent evidence exists about the neurochemistry and function of centrifugal innervation which casts new light upon modulation of responsiveness, specificity and coordination especially of bulbar activity. Ablation experiments, application of metabolic markers as well as fastly working voltage-sensitive dyes have demonstrated specific cooperation of many local circuits and parts of the bulb during processing of odor input. A special contribution on this topic came from comparative studies on vertebrates and invertebrates. Experimental neuroanatomical studies have enlarged our view about secondary and tertiary central connections of the olfactory pathway. Recently, first data have been published on the neurobiology of olfactory learning and imprinting. Such data will further promote our understanding of neurophysiological events underlying odor induced, and odor guided behaviour.

CURRENT CONCEPTS OF NOCICEPTOR FUNCTIONS

H.O. Handwerker

Two decades ago nociceptors were simply regarded to be slowly conducting afferent units specialized to signal tissue damage to the central nervous system. The method of micro-neurography opened the possibility to study the stimulus induced activity of single slowly conducting cutaneous afferents in human subjects together with the concomitant pain experiences. The close correlations found in numerous studies supported the notion that pain is mediated by a distinct group of afferents. However, recently the concepts of the function of small primary afferents have been modified under several aspects.

With the advent of neuropeptide histochemistry, a subpopulation of small dorsal root ganglion cells with slowly conducting axons has been specified which synthesize tachykinins such as substance P, CGRP, and somatostatin. Initiation and transmission of impulses mediating pain may be but one of several functions of these peptidergic primary afferents. In particular in visceral organs many of them seem to be involved in the local neuroeffector control of the smooth muscles.

Another important function of slowly conducting primary afferents is the stimulus induced release of vaso-active substances, probably tachykinins, from the peripheral nerve endings causing vasodilatation and plasma extravasation (1). Since unmyelinated afferents cause these effects at frequencies less than 1 Hz, which may not be consciously perceived, it has been proposed that they may often have a pro-inflammatory action without producing any reflex or sensory effects.

In contrast to fast conducting primary afferents most unmyelinated and some thin myelinated units are sensitive to chemical stimuli, in particular to endogenous agents which are released in inflammation. The sensor and transducer functions underlying the chemosensitivity of slowly conducting afferents are now being studied with newly developed in-vitro techniques. For the study of membrane currents requiring intracellular and patch clamp recording techniques, the cell bodies of dorsal root ganglion cells have been used as models. No unique membrane receptor or second messenger system characteristic of nociceptors has been found hitherto, but rather a multiplicity of agents and potential second messengers (2). Though most of the small primary afferents are more or less sensitive to the whole spectrum of inflammatory agents, qualitative differences have been found between different populations.
Thus, the population mediating itch sensations from the superficial skin layers probably differs from another population mediating sensations of burning pain (3).

Characteristically slowly conducting afferents are sensitized by inflammatory processes for chemical, thermal and mechanical stimuli. Probably this sensitization is a basis of inflammatory pain and hyperalgesia. The membrane mechanisms of the sensitization are still unknown. However, it has recently been shown that in inflammation opioid receptors are expressed in the membrane of nociceptors (4). This may be but one example of membrane plasticity.

Until recently nociceptor sensitization was understood as a merely quantitative phenomenon leading to enhanced input to central neurons and hence to temporal summation. However, there is now increasing evidence from studies in different tissues that a large proportion of these units are virtually unexcitable in normal tissue. These "silent" units may turn to an active state when the tissue is inflamed. Thus, different populations of primary afferents mediate pain in inflammation.

The nature of the neurotransmitter(s) released by slowly conducting primary afferents at their central synapses is not yet clearly established. However, existing evidence does not support the notion that tachykinins like substance P are classical neurotransmitters. Instead, the release of tachykinins from the central endings of primary afferents seems to have conditioning functions on the spinal neurons. This may be the basis of plasticity changes in spinal circuitry occurring in the course of prolonged pain states. One sign of plasticity induced by ongoing activity in nociceptive primary afferents is the induction of the proto-oncogene C-fos in those units which are involved in the processing of nociceptive impulses.

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ACTIVATOR COMODIFICATION: A NEW METHOD TO MEASURE MICROSCOPI
C REACTION DYNAMICS OF SODIUM CHANNEL SITE 2 ACTIVATORS
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Sodium channel comodification with batrachotoxin or other activa-
tors has frequently been used to measure microscopic reaction
dynamics of channel blockers like tetrodotoxin, saxitoxin, local anaesthetics or antiarrhythmic drugs.

We now show that activator comodification can also be used to
determine the reaction dynamics of sodium channel activa-
tors, if the two activators act allosterically and induce
different conductance states of the sodium channel.

Outside-out patches were obtained from dissociated and
cultured ventricular myocytes of late-fetal rats. 1 mM
BDF 9145 was added to the extracellular solution containing
140 mM Na, test drugs were added to the intracellular solu-
tion containing 148 mM Cs (20°C). BDF 9145 induced "full"
activation of the sodium channel by imposing a normal open-
channel current of -1.2 pA (at -30 mV) for hundreds of
milliseconds after depolarization from -110 mV holding
potential. Repetitive pulsing was halted at -30 mV as soon
as a single channel became modified by BDF 9145. The ad-
ditional binding of veratridine, a partial activator, was
manifested by a sudden reduction of open-channel current
to -0.3 pA, veratridine unbinding by a sudden return to
-1.2 pA. Dwell-time histograms of bound and unbound channel
states showed exponential distributions, and the rate con-
stant for leaving the unbound state increased linearly
with veratridine concentration (0.3-30 µM) while that for
leaving the bound state was unaffected by veratridine con-
centration. The method was also successfully applied to
four other ceveratum alkaloids (veracevine, cevadine,
germine-3-acetate, and germitrione) and to grayanotoxin-I.

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EXPANSION OF EPITHELIAL Na + CHANNELS IN XENOPUS OOCYTES FROM
SIZE FRACTIONATED mRNA OF BOVINE TRACHEAL EPITHELIUM
B. Kroll, S. Bremer, B. Tümmeler, B. Frommer

Poly(A)⁺-RNA was prepared from native bovine tracheal epithelium and
size-fractionated by saccharose density gradient centrifugation. A total of 9
fractions spanning the entire gradient was injected into full grown
immature oocytes of Xenopus laevii to test for their ability to induce
epithelial Na⁺ channels. Two days after injection of 25 ng of the
fractionated mRNA the current response to bath application of 0.01
mmol/l amiloride was monitored while the oocytes were clamped to -70
mV. Whereas water-injected control oocytes did not respond, mRNA
injected oocytes showed amiloride-inhibitable currents of up to 190 nA.

Expression maxima were found in oocytes injected with either fraction 0
(mRNA length up to 10 kb) or fraction 5 (up to 4.4 kb). To better identify
the ion channels responsible for the amiloride-inhibitable current flow a
number of tests was performed. At a holding potential of -100 mV the
dose/response curve for amiloride was measured. It followed a single-site
inhibition model with half maximal inhibition at 44 nM/ll. This value
agrees well with amiloride inhibition of Na⁺ channels in native respiratory
epithelia. To test for Na⁺ over K⁺ selectivity, the current-voltage relation
of the amiloride-inhibitable current was tested after reducing extrae-
cellular Na⁺ concentration from 85 to 27, 8.5 and 0.85 mmol/l by substitution
with N-methyl-D-glucamine. In these experiments the reversal potential
agreed well with amiloride inhibition of Na⁺ channels in native respiratory
epithelia. To test for Na⁺ over K⁺ selectivity, the current-voltage relation
of the amiloride-inhibitable current was tested after reducing extracellular
Na⁺ concentration from 85 to 27, 8.5 and 0.85 mmol/l by substitution
with N-methyl-D-glucamine. In these experiments the reversal potential
shifted by an average of 55.2 mV (n = 9) for a tenfold change in extracellular Na⁺
concentration indicating a Na⁺ over K⁺ selectivity of at least 10. This
result was confirmed by replacing all bath Na⁺ by K⁺ which virtually
abolished the current response to amiloride. We conclude that oocytes are
able to express highly selective epithelial Na⁺ channels in their cell
membrane from large size fractions of mRNA of tracheal epithelium.

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DIFFERENTIAL EFFECTS OF EXTERNAL CALCIUM ON GATING AND OPEN-CHANNEL CURRENT OF CLONED NA CHANNELS EXPRESSED IN OOCYTES

Michael Pusch

Macroscopic and single-channel currents through rat brain type II, type III and 'K226Q' (a mutant of type II) Na⁺ channels expressed in oocytes have been measured using the patch-clamp technique. Mutant K226Q differs from the wild-type at homologous position 226 where the positive amino-acid lysine (K) is replaced by an uncharged glutamine (Q). Position 226 is located at the C-terminal end of segment S₆ of homologous repeat I of the Na⁺ channel sequence.

The blocking effect of extracellular Ca²⁺ on single-channel inward currents was examined for the three channel types. Affinities turned out to be similar for wild-type II and K226Q channels. However, the form of the concentration- as well as the voltage-dependence of the Ca²⁺ block was different for all channel types. In particular the form of the concentration-dependence for wild-type II channels could not be accounted for by a simple block model, suggesting the existence of several Ca²⁺ binding sites.

In addition to the blocking effect, it is known that external Ca²⁺ shifts steady-state activation curves to more positive voltages. This effect was confirmed for wild-type II and K226Q channels. Interestingly, K226Q channels are significantly more sensitive to external Ca²⁺ than wild-type II channels with respect to shifts of steady-state activation.

From these observations it is concluded that mutant K226Q differs from wild-type in a region of the protein which is located close to the extracellular side of the membrane, accessible to external Ca²⁺.

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15 INTERACTION BETWEEN TTX AND MONOVALENT CATIONS AT NODAL Na CHANNELS
U. Lonnendonker and B. Neumcke

Na currents and Na-current fluctuations were measured in myelinated frog nerve fibres to study interactions between the Na channel blocker tetrodotoxin (TTX) and monovalent cations in the external solution. In hyperosmolar solutions containing 12 mM TTX and various permeant cations the number N of Na channels per node not blocked by TTX was higher than in Ringer + 12 mM TTX. The relief of TTX block depended on the cations in the external solution. N increased by the following factors upon addition of 110 mM chloride salts to TTX Ringer: 1.75 (Na), 1.64 (Li), 1.88 (hydrazine), 1.15 (guanidine), 1.20 (K). No significant changes of N were observed in TTX-Ringer + 110 mM TMA-C1 or 250 mM sucrose. Hence, the efficacy of TTX displacement by Na:Lii hydrazine:guanidine:K:Cl is 1:0.85:1.17:0.20:0.27. This sequence reflects a weaker cation selectivity than the ion selectivity 1:0.93:0.59:0.13:0.086:0 of nodal Na channels (Hille J Gen Physiol (1971) 58: 599, (1972) 59: 637). The results are interpreted by a TTX receptor in a superficial prefilter to the Na channel contributing to cation discrimination at the outer channel region.

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16 ULTRASLOW SODIUM CHANNEL INACTIVATION PRESENT IN RAT SKELETAL MUSCLE IS ABSENT IN CARDIAC MUSCLE. LOOSE-PATCH MEASUREMENTS.
R. Eickhorn, D. Hornung, H. Antoni

An inactivation process of the fast sodium system developing with a time constant in the range of minutes was described in loose-patch experiments on rat skeletal muscle by Simoncini & Stühmer and by Ruff et al. (J Physiol 383 (1987) 327 and 339). We searched for a similar process in cardiac muscle using right and left ventricular papillary muscles of Wistar rats (n=10). Skeletal muscle fibres from the same animals were used for comparison. The muscles were voltage clamped by means of the loose-patch at 25°C in Krebs-Henseleit solution containing 1.8 mM Ca 2+ and 5.9 mM K + . Steady state fast inactivation from a holding potential of -100 mV with conditioning prepulses of 250 ms showed a midpoint of the Boltzmann distribution at -79.8 ± 1.9 mV with a steepness of 11.1 ± 0.75 per e-fold. If instead of conditioning pulses of only 250 ms those of 10 s were given the peak current at -140 mV increased by 14 ± 10%. The position and steepness of the inactivation curve were, however, not changed significantly.

In a series of 4 experiments the holding potential was stepped from -100 mV to -140, -120 or -80 mV for 5 minutes during which every ten seconds a test pulse (5 ms, 0 mV) was given. The change of current amplitude was complete within the first 10 s. The peak current elicited 4 minutes after a step from -100 mV to -140 mV differed only by 0.06 ± 0.54 nA from that between 10 and 40 s (not significant). These results in cardiac muscle differ markedly from skeletal muscle, in which the peak sodium current changes with a time constant of 19 ± 50 s at -80 mV. Hence, ultraslow inactivation is absent in cardiac muscle.

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17 FUNCTIONAL EXPRESSION OF THE CALCIUM RELEASE CHANNEL FROM SKELETAL MUSCLE RYANODINE RECEPTOR cDNA
R. Penner, E. Neher, H.Takeshima*, S. Nishimura* & S. Numa*

Combined patch-clamp and fura-2 measurements were performed to study the calcium release properties of Chinese hamster ovary (CHO) cells transfected with the rabbit skeletal muscle ryanodine receptor cDNA carried by an expression vector.

Both caffeine (1–50 mM) and ryanodine (100 μM) induced release of calcium from intracellular stores of transformed CHO cells but not from control (non-transfected) CHO cells. The calcium responses to caffeine and ryanodine closely resembled those commonly observed in skeletal muscle. Repetitive applications of caffeine produced characteristic all-or-none rises in intracellular calcium:

\[ \text{Inositol 1,4,5-trisphosphate (IP3)} \rightarrow \text{Ca}^{2+} \text{release} \]

The activation of the Ca current in skeletal muscle, which is known for its slow kinetics, can be markedly accelerated by a conditioning current activation (Feldmeyer et al. J Physiol. 415:115P. 1989). We measured Ca inward currents in cut muscle fibres of the frog and determined their voltage dependence in the normal slow and the fast gating mode. Fast gating was established by a large depolarizing pulse causing considerable current activation but no inactivation: it was followed by repolarization to a subtreshold potential of -50 mV for 100 ms and a test pulse to different superthreshold potentials. Although the conditioning pre-pulse caused a strong increase in the activation speed of the test current at each potential the current peaks nearly coincided. This indicates that no new channel population with different kinetic characteristics became available but that these channels which normally give rise to the slow Ca current exhibited an altered gating behaviour.

Intramembrane charge movements which have previously been correlated with Ca release are sensitive to Ca antagonists (Eickhorn et al. J Physiol. 399:585. 1987). We applied the pulse protocol described above after eliminating the Ca-current by removing Ca from the bathing solution and adding 2 mM Cd. The charge movements measured during the test pulses remained virtually unaffected by the conditioning pulse.

A model in which a fast reaction (possibly accompanied by a Ca antagonist-sensitive charge movements) in conjunction with an additional slow reaction leads to channel gating can account for most experimental observations.

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18 CA CURRENT AND INTRAMEMBRANE CHARGE MOVEMENTS AFTER CONDITIONING DEPOLARIZATION IN FROG SKELETAL MUSCLE
By W. Helger, D. Feldmeyer, B. Pohl and P. Zölner

The activation of the Ca current in skeletal muscle, which is known for its slow kinetics, can be markedly accelerated by a conditioning current activation (Feldmeyer et al. J Physiol. 415:115P. 1989). We measured Ca inward currents in cut muscle fibres of the frog and determined their voltage dependence in the normal slow and the fast gating mode. Fast gating was established by a large depolarizing pulse causing considerable current activation but no inactivation: it was followed by repolarization to a subtreshold potential of -50 mV for 100 ms and a test pulse to different superthreshold potentials. Although the conditioning pre-pulse caused a strong increase in the activation speed of the test current at each potential the current peaks nearly coincided. This indicates that no new channel population with different kinetic characteristics became available but that these channels which normally give rise to the slow Ca current exhibited an altered gating behaviour.

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A model in which a fast reaction (possibly accompanied by a Ca antagonist-sensitive charge movements) in conjunction with an additional slow reaction leads to channel gating can account for most experimental observations.
Many neurosecretory cells of the hypothalamus display synchronous phasic activity which appears to be important for the regulation of hormone secretion (D.A. Foulaín and J.B. Wakerley, Neurosci. 7:773 1982). Embryonic hypothalamic neurons have been shown to grow synchronously bursting networks when cultured on a glial background monolayer (U. Miegald and D. Swendulla, Neurosci. Lett. 28: 281 1985). While synchronization and desynchronization of phasic activity has been shown to rely on quisquulate-type receptors coupled to channels selective for monovalent cations and GABA-controlled chloride channels, little is known about voltage-activated currents that may be involved in burst generation and control of transmitter release.

In the present study we recorded voltage-activated currents from embryonic (E14-E15) hypothalamic neurons after up to 7 days in culture (DIC) on a glial monolayer. Both low- and high-threshold calcium currents were found to develop gradually between 7 and 21 DIC. This correlates with the proliferation of dendrites and the formation of complex networks which occurs during this time. By contrast, TTX sensitive sodium currents developed within 5-10 DIC and potassium currents could be observed after as little as 6 hours. Low-threshold calcium currents were drastically reduced by external application of Ni²⁺ in low concentration (70% reduction at 50μM) where high-threshold currents were not affected. Neurons were also dissociated after DIC or later and calcium currents recorded 4 to 8 hours after replating. After this treatment the neurons usually had a spherical shape and lacked dendritic processes. High-threshold calcium currents were still present in almost all of the neurons, whereas transient low-threshold currents could not be observed.

The ability of the in vitro networks to generate synchronous phasic activity parallels the development of calcium currents and is most pronounced after 21 DIC, suggesting that calcium currents are required for bursting or synchronization of neuronal activity. In particular, low-threshold calcium channels appear to be located primarily in the dendrites where they could be involved in the control of synaptic potentials. The ability of the in vitro networks to generate synchronous phasic activity parallels the development of calcium currents and is most pronounced after 21 DIC, suggesting that calcium currents are required for bursting or synchronization of neuronal activity. In particular, low-threshold calcium channels appear to be located primarily in the dendrites where they could be involved in the control of synaptic potentials. 

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REGULATION OF CARDIAC CA⁺ CHANNELS BY THE GTP-BINDING PROTEIN Gₛ AND PHOSPHORYLATION

A. Cavalié, T.J.A. Allen and W. Trautwein

In heart cells, coupling of β-adrenergic agonists to their receptor enhances the Ca current via cAMP-dependent phosphorylation of L-type Ca channels and/or via a direct action of the GTP-binding protein Gₛ. Simultaneous activation of both pathways by 1 μM isoprenaline produces a 3–4 fold increase of the Ca current, but the isoprenaline effect is reduced to 1.7 fold if the cell was previously dialysed with 0.5 mM EGTA. The latter result supports the suggestion that Gₛ can modulate Ca channels in intact myocytes when the cAMP-dependent phosphorylation was suppressed. Furthermore, we studied the relationship between these two signalling pathways by stimulating them sequentially. After cell dialysis with 100-200 μM cAMP, bath application of 20-60 μM IBMX produced a 3–4 fold increase of the Ca current, suggesting saturation of the cAMP cascade. Under this conditions 1 μM isoprenaline did not enhance the current further, indicating that Gₛ cannot modulate maximally activated Ca Channels. However, simultaneous application of 20 μM IBMX and 1 μM isoprenaline increased the Ca current up to 7–8 fold, an effect much stronger than exerted by isoprenaline alone or in combination with cAMP. These results suggest that a direct action of Gₛ may act to prime Ca channels for up-regulation by cAMP-dependent phosphorylation.

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FREQUENCY-DEPENDENCE OF T-TYPE CALCIUM CURRENTS IN SINGLE SMOOTH MUSCLE CELLS ISOLATED FROM GUINEA PIG CORONARY ARTERIES

V.Ya. Ganilkevich and G. Isenberg

Myocytes from coronary arteries have two types of Ca-currents which are carried through Ca-channels similar to T-type and L-type. The types of Ca-current can be distinguished by their voltage-dependence. With 10 mM extracellular BaCl₂ as charge carrier, the current at -50 and -30 mV is mostly T-current whereas the current at -80 mV is mostly L-current. This difference in holding potentials, the current fell along a negative staircase as expected from the long recovery time of the L-current. At negative potentials, where the Ca-current was mostly T-current, 3 Hz pulsing increased the amplitude of Ca-current up to three times. Repriming of T-current was studied with a two-pulse protocol at a holding potential of -100 mV. At pulse-intervals of 100-200 ms, the current was facilitated beyond the control current by a factor of 1.8 - 2.8 (n=17). This overshoot in repriming was attenuated at -90 mV, and it was abolished by holding potentials less negative than -80 mV. Frequency-dependent facilitation of T-current does not require Ca influx, i.e. the overshoot was similar in media with 10 mM BaCl₂ or 10 mM CaCl₂. The facilitation is unlikely to be mediated by changes in Lc. Ca-concentration since, the overshoot was not modified by caffeine-induced SR-Ca-release of cell-dialysis with 40 μM EGTA plus 10 mM BAPTA. Preliminary analysis of single T-channel events suggest that frequency facilitation is a channelflops mechanism. That is, the T-Channel spends more time in the open state (1.7-1.9 times) when the frequency is increased from 0.2 to 1.6 Hz.

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Previous studies have shown that a variety of agonists binding on functional phosphoinositide receptors of control and peripheral neuron induce inhibition of voltage-dependent Ca²⁺ currents (ICa) that is related to a decrease in development of neurosecretion. In neuroblastoma glioma hybrid cells (10C15), the synthetic opioid D-Ala²-D-Leu⁵-enkephalin (in the presence of propranolol) induced a fully reversible inhibition of ICa in a dose-dependent manner, whereas bradykinin was not effective. The following inhibition values were obtained: DADLE-74.72 ± 6.43 % (n=3), somatostatin-87.59 ± 3.67 % (n=3), bradykinin-8.88 ± 2.40 % (n=14). Effects of intracellularly applied guanine nucleotides and the sensitivity towards pertussis toxin indicate that a pertussis toxin-sensitive G-protein (Gα, sα, or Gβγ) are involved in the inhibition modulation of ICa. In order to identify the endogenous G-protein activated via inhibitory receptors, the effects of receptor agonists on photolabeling of G-protein α-subunits with the photoreactive OTP analogue, (α³²P)GTP αs-adeno-nucleotide, were examined in membranes of Nb cells. The data obtained in a series of experiments showed that the ability of receptor agonists to stimulate photolabeling of a protein comigrating with the α-subunit of Gα (39 kDa, correlated wL) with their ability to induce ICa-inhibition (DADLE, somatostatin, adrenalin, bradykinin). The data suggest that Qlo is involved in the inhibitory control of ICa. This hypothesis is supported by findings that antibodies against the Gα-α-subunit attenuate the adrenaline-induced inhibition of ICa in Nb cells (McPadden et al., Nature 3: 277-282, 1991).

Bodevist et al. (Gen. J. Physiol. and Biophysics 4: 113-127, 129-141, 1985) have shown that chA is not involved in the fast regulation of Ca⁺⁺ channels, but in the long-term modulation of Ca⁺⁺ current magnitude. Our studies show an exact time course of the development of a fast and slowly inactivating current in 10C15 cells during incubation with cells in dHOMO (1 mM) or forskolin (10 μM). As a result, when calcium blockers were included in the culture medium and could be restored by further treatment with DADLE, ICa was suppressed. Under these conditions, the ICa associated with the dHOMO-induced suppression disappeared. The faster effect of forskolin on ICa blockade was prevented by cycloheximide, a potent protein-synthesis blocker. The results suggest that the results indicate that a hypothesis of Na⁺ blockade during low-term incubation with cells in Na⁺⁺-inhibited ICa in Nb cells containing Ca⁺⁺-channel blockers. This hypothesis was supported by binding studies that showed that the number of dihydropryline (DRP) binding sites was significantly reduced in WVT-treated cells (WVT 10 μM protein, n=4) compared with the control cells (204.4 ± 10.3 pmol/mg protein, n=4).

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Calcium currents from neonatal rat cardiac myocytes were recorded from cardiac myocytes grown in primary culture were examined using the whole-cell voltage clamp technique. The calcium current (ICa) was investigated in conditions when other components of the cell membrane permeability were excluded. Only the high-threshold (L) calcium current was found in cultured neonatal rat ventricular myocytes. An inward current characterized by large amplitude and slow inactivation decay was induced when the extracellular Ca²⁺ concentration was reduced by EGTA. This current was suppressed by extracellular Na⁺ removal, or by calcium antagonists, and increased by epinephrine and BAY K 8644. These findings suggest that this current is carried by sodium ions through Ca²⁺ channels. Both Ca and Na currents through calcium channels were reversibly blocked by omega-conotoxin (10μM). Complete blockade developed 10 - 15 minutes after toxin introduction in the extracellular solution. At the same time the genuine rundown of calcium current in cultured neonatal rat ventricular myocytes takes much more time (40 - 45 minutes for complete current abolishing). Blockade of Na currents through calcium channels was characterized by a transient increase of current amplitude without any changes in its kinetics and voltage-dependent properties. Structural differences between calcium channels in rat and guinea-pig and frog cardiac myocytes were suggested. A. A. Bogomolets Institute of Physiology, Bogomolets St. 4, Kiev-24, GSP 292601, Ukraine, U.S.S.R.

**OMEGA-CONOTOXIN BLOCKADE OF CALCIUM CURRENTS IN CULTURED NEONATAL RAT CARDIOMYOCYTES.**

**BY ALEXIJ N. SAVTCHENKO & ALEXIJ N. VERKHARSKY**

1. Ion permeation through L-type Ca-channels has been described with a 2-site 3-barrier model [1]. The model predicts a high Qlo for open channel conductance of Ca- and Ba-ions that bind with high energy, but a low Qlo for Na- and Li ions that bind only weakly. 

2. However, no details on the ion-channel interactions that might play an important role in signal transduction during target recognition of the developing glial cell.

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**L-TYPE Ca-CHANNELS IN URINARY BLADDER MYOCYTES: SIMILAR Qlo OF Ca-, Ba- AND Na-CONDUCTANCE POINTS TO THE IMPORTANCE OF ION-CHANNEL INTERACTION**

**A. Scheier, U. Klöckner and A. Schranz**

Ion permeation through L-type Ca-channels has been described with a 2-site 3-barrier model [1]. The model predicts a high Qlo for open channel conductance of Ca- and Ba-ions that bind with high energy, but a low Qlo for Na- and Li ions that bind only weakly.

Myocytes were isolated from the urinary bladder of the guinea-pig. Elementary Ca, Ba, Li or Na currents through L-type Ca-channels were recorded from cell attached patches at 22°C or at 35°C. The higher temperature increased the open channel conductance for Ca-ions from 8.5 to 16 pS (Qlo = 1.63 ± 0.07, mean ± S.D.), for Ba-ions from 24 to 43 pS (Qlo = 1.55 ± 0.6), for Li from 27 pS to 50 pS (Qlo = 1.61 ± 0.06), and for Na from 74 to 131 pS (Qlo = 1.55 ± 0.09). The differences in the Qlo's are not significant, that is, the predictions of the 3 barrier model with 2 intra-channel binding sites are not fulfilled.

**To model our results, we gave up the concept of discrete binding sites.** We postulated the Qlo to result from multiple ion-channel interactions over a 1.1 nm long channel part. Part of the ionic hydration shell was assumed to be substituted by polar groups of the channel-protein until rehydration in the exit. On this level of negative free energy a permeation barrier was superimposed. The energy of this barrier was calculated from multiple ion-channel interactions that were assumed to occur with rates similar to those describing replacement of water molecules in the inner water shell of the cation [2].

**With these theoretical values we calculated Qlo's that approximated the experimental Qlo's for all ions.**

1. Almers, W. and McCleskey, E.W. (1984) J Physiol. 353, 585-608

2. Pieber, H., Eigen, M., Ilgenfritz, G., Maas, G., Winkler, R. (1986) Atemz. Chir. 20: 93-115
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Currents through calcium-activated nonspecific cation (CAN) channels were studied in the fast bursting neuron of Helix aspersa and Helix pomatia. CAN currents were activated by intracellular injection of Ca using a fast quantitative pressure injection technique (see for details Swandulla, Bürgers & Lüttjohann, Pflügers Arch. 406, 1986). External application of Forskolin (10-25 μM), an activator of adenylate cyclase, caused a transition from endogenous bursting activity of the cells to beating activity. These same concentrations of forskolin reduced CAN currents reversibly to about 50%. External application of the membrane permeable cAMP analogues 8-bromo-cAMP and dibutyryl-cAMP (100-500 μM) almost completely blocked the CAN current. A marked reduction in the CAN current was also observed following quantitative injections of cAMP (internal concentrations up to 50 μM) directly into the cells from a second pressure injection pipette. Similar results were obtained with quantitative injections of the catalytic subunit (C-subunit) of the cAMP-dependent protein kinase (internal concentrations 10^{-5} M) units of enzyme) directly into the cells. Injection of the nonhydrolysable GTP analogue, GTP-γ-S (internal concentration 100 μM), which stimulates G-proteins, produced a prolonged increase in CAN current amplitude by as much as 300%. External application of serotonin at concentrations of 100-200 μM caused a transition from bursting to beating activity of the neurons and mimicked cAMP’s effects on CAN currents. Two other neurotransmitters tested, dopamine and acetylcholine, were not significantly effective in reducing CAN currents.

Our results indicate that CAN currents in Helix burster neurons are modulated by calcium, cAMP, and G-proteins. The physiological transmitter that induces this second messenger action may be serotonin. The dual control of CAN channels by two second messengers, Ca and cAMP, has functional implications. While Ca activates CAN channels in these neurons (Swandulla & Lux, J. Neurophysiol. 54, 1985; Partridge & Swandulla, Pflügers Archiv. 410, 1987) cAMP-dependent phosphorylation downregulates them thereby resulting in modulation of neuronal bursting activity.

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M. Current Inhibition in N1018-15 Neuroblastoma X Glioma Hybrid Cells

S. Schäfer and H. Neves

In N1018-15 cells, bradykinin (BK) inhibits the M-current, a voltage dependent K+ current. Since phorbol dibutyrate (PdBu) and oleoyl acetylglyceryl (OAG), activators of protein kinase C (PKC), mimic this effect, it has been suggested that M-current inhibition by BK is mediated by the activation of PKC (Brown & Hijghashida, J. Physiol. 397, 1987). Using the whole-cell mode of the patch clamp technique we found that even 1 μM PdBu only partially depressed the M-current, and that it did not prevent further inhibition by BK. Bath application of H7 (50-70 μM), a relatively unspecific inhibitor of PKC, partially reversed the otherwise irreversible effect of PdBu but did not affect M-current inhibition by BK. Further studies using more potent and more specific PK inhibitors are required to reveal which PKC really mediates BK induced M-current inhibition in N1018-15 cells.

The M-current was also depressed by millimolar concentrations of methylnarcaines: 10^{-6} M was the most effective followed by 1,7-dimethylxanthine, caffeine, and theophylline. The threshold for the caffeine response was at 2 mM and inhibition was nearly complete at 20 mM. It was not prevented by prior application of 1 μM PdBu, nor did it affect M-current inhibition by BK. Adding 10 μM ryanodine to the pipette solution abolished neither the caffeine nor the BK effect. Taken together with the observation that Ca^{2+} injections do not affect the M-current (Brown & Hijghashida, 1988) it seems unlikely that Ca^{2+} release from intracellular stores plays a role in mediating M-current inhibition in N1018-15 cells.

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TRH MODULATES AN INWARD-RECTIFYING K+ CURRENT IN RAT PITUITARY CELLS

C.K. Bauer and J.R. Schwarz

Thyrotropin-releasing hormone (TRH) is known to induce a biphasic secretion of prolactin in anterior pituitary cells of the rat (GH3 cells). It has been postulated that phase II of secretion is mediated by the closing of voltage-dependent K+ channels (e.g. Ozawa & Sand, Phys Rev 1986).

Using the whole-cell configuration of the patch clamp technique we could demonstrate an inward-rectifying K+ current, activated by hyperpolarizing pulses from a holding-potential of -40 mV in isotonic KCl solution. Peak currents increased and depolarization-induced pre-pulses indicating the existence of open K+ channels at the holding potential. The K+ currents showed time- and voltage-dependent inactivation at potentials more negative than -60 mV. Recovery from inactivation was slow and voltage-dependent (>10 s at -40 mV). The inward current was reduced by Ca^{2+} and Ba^{2+}, but not by Ni^{2+} and Co^{2+} (5 mM). Drugs like quinidine, 4-aminopyridine and TEA blocked the current, in contrast to dendrotoxin. Single channel recordings from inside-out patches revealed an inward-rectifying K+ current, activated by Ca^{2+} ions, the other is blocked by ATP.

The first channel has a single-channel conductance of 122 pS with 105 mM-K on both sides of the membrane at 15 °C. The reversal potential in Ringer solution is below -50 mV, indicating that this channel is selective for K+ over Na+ ions. The maxi-K channel is activated both by micromolar concentrations of intracellular Ca^{2+} ions and by depolarization. The second channel has a single-channel conductance of 42 pS, is blocked by internal ATP (10 μM) and shows little voltage dependence. The reversal potential depends on extracellular K concentration, indicating that it is also a K channel. Openings of this ATP-sensitive K channel typically occur in bursts. Both channels are blocked by millimolar concentrations of external tetraethylammonium ions. They may link axonal metabolism and excitability and may be part of the complex feedback system regulating membrane potential under physiological and pathophysiological conditions.

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TRH INHIBITS THE M-CURRENT IN N1018-15 CELLS

S. Schäfer and H. Neves

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The M-current was also depressed by millimolar concentrations of methylnarcaines: 10^{-6} M was the most effective followed by 1,7-dimethylxanthine, caffeine, and theophylline. The threshold for the caffeine response was at 2 mM and inhibition was nearly complete at 20 mM. It was not prevented by prior application of 1 μM PdBu, nor did it affect M-current inhibition by BK. Adding 10 μM ryanodine to the pipette solution abolished neither the caffeine nor the BK effect. Taken together with the observation that Ca^{2+} injections do not affect the M-current (Brown & Hijghashida, 1988) it seems unlikely that Ca^{2+} release from intracellular stores plays a role in mediating M-current inhibition in N1018-15 cells.

INTRACELLULAR ATP AND CALCIUM MODULATE TWO K-CHANNELS IN MYELINATED NERVE FIBRES

P. Jonas, O.S. Koh, K. Kampe, H. Hermsteiner and W. Vogel

Different types of potential-dependent K channels in myelinated nerve fibres have been demonstrated on the macroscopic level and, more recently, on the single-channel level applying a specific enzymatic treatment and patch-clamp technique (Jonas et al. 1989, PNAS 86: 7238). This new method allows access to the inner side of the membrane. For the first time we present evidence for axonal K channels controlled by cytoplasmic factors in amphibian nerve fibres: one is activated by Ca^{2+} ions, the other is blocked by ATP.

The first channel has a single-channel conductance of 122 pS with 105 mM-K on both sides of the membrane at 15 °C. The reversal potential in Ringer solution is below -50 mV, indicating that this channel is selective for K+ over Na+ ions. The maxi-K channel is activated both by micromolar concentrations of intracellular Ca^{2+} ions and by depolarization. The second channel has a single-channel conductance of 42 pS, is blocked by internal ATP (10 μM) and shows little voltage dependence. The reversal potential depends on extracellular K concentration, indicating that it is also a K channel. Openings of this ATP-sensitive K channel typically occur in bursts. Both channels are blocked by millimolar concentrations of external tetraethylammonium ions. They may link axonal metabolism and excitability and may be part of the complex feedback system regulating membrane potential under physiological and pathophysiological conditions.

Supported by the Deutsche Forschungsgemeinschaft.
POLYMYXIN B BUT NOT POLYMYXIN B MODIFIES Ca2+-ACTIVATED K+ CHANNELS IN MOUSE SKELETAL MUSCLE
R. Weik

The effect of the polycationic peptide-antibiotic polymyxin B was studied using excised inside-out patches from dissociated mouse toe muscle fibres. Polymyxin B, dissolved in an artificial solution containing (mM) 155 KCl, 2 CaCl2, 1 MgCl2, and 10 HEPES, produced a block of high conductance (~250 pS) Ca2+-activated K+ channels found in native sarclemma vesicles of differentiated mammalian skeletal muscle (R.H.A. Fink et al., Proc. SPIE 17187, 1989). Supported by NIH/NCRR and NHF (Australia).

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ION CHANNELS IN THE LUMINAL MEMBRANE OF ISOLATED PERFUSED RAT CORRECTING DUCTS (CCD)
E. Göhler, R. Rödel, and E. Gruber

As a result of the permeability changes, we observed Ca+-dependent K+-channel with a conductance of 30-40 pS. Its P0 is larger at depolarized membrane voltages. The Cl--channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) inhibits the channel. The absence of a colocalization with Na+ or K+-channels suggests that this Cl--channel may be derived from intercalated cells. Supported by DFG 480/9.

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Apical and basolateral membrane conductances in A6 cells
M. Granitzer, T. Leal, W. Nagel, J. Crabbé

Confluent monolayers of a renal distal tubule cell line (A6), grown on a permeable support, were mounted in a horizontal Ussing type chamber, short-circuited and impaled with microelectrodes. Specific membrane conductances were calculated from equivalent circuit equations using amiloride (10-5M) during intracellular recording. Transport properties were analyzed under control conditions and during short-term increases in basolateral [K+] from 2.5 to 20 mmol/l without or in the presence of 2 mmol/l serosal Ba2+.

As in most other epithelia, the apical membrane represents the major resistive barrier. Transcellular, apical and basolateral membrane conductances (gA and gB), obtained from 12 acceptable microelectrode studies, averaged 43, 57 and 297 μS/cm², respectively. There is a highly significant positive correlation between short-circuit current (Isc) and gB, whereas gA was uncorrelated to ISc. The Isc averaging 43 μA/cm², was almost completely blocked by amiloride. This was associated with fast hyperpolarization of the intracellular potential (Vh) and increase in fractional apical membrane resistance to almost 100%.

Appreciable K+-conductance of the basolateral membrane can be derived from the depolarization of VA after application of high [K+] or Ba2+ to the basolateral side. Using the values of VA during amiloride at normal and high K+, an apparent transfer number of 0.72 can be calculated for K+ at the basolateral membrane. This value conrasts with the decrease in gB to about 30% after blockade of the K+-channels by Ba2+. The nature of the remaining conductance is presently unclear. The amiloride sensitive current decreased during high K+ and Ba2+. In part, this is explained by the reduction of the electro-chemical gradient for apical Na+-uptake due to the depolarization. In addition, gA decreased to less than 40%, which is considerably smaller than predicted by the GHK constant-field equation. Whether it results from voltage sensitivity of the apical Na+-permeability, requires further investigation.

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IN CELLS EXPRESSING RAS ONCOGEN BRADYKININ AND BOMBESIN LEAD TO SUSTAINED OSCILLATIONS OF CELL MEMBRANE POTENTIAL.

F. Lang, E. Kuhn, F. Friedrich, M. Paulmichl, M. Hammerer, K. Maly, H. Gunnicke

The products of ras oncoproteins are GTP-binding proteins which are resistant to inactivation by GTPase activating protein. The functional significance of this property and its significance for malignant transformation remains largely elusive. The present experiments have been performed to test, whether expression of ras oncoproteins alters electrical properties of 3T3 fibroblasts and their modification by mediators such as bradykinin (BK) or bombesin. Experiments were performed on 3T3 fibroblasts transfected with a transforming Ha-ras MMTV-LTR construct expressing the oncogene upon treatment with demecaxon (ra) as controls. The carried transfected cells without demecaxon (ra) and nontransfected cells with demecaxon (or) were used. All cells were kept in media almost devoid of serum (0.5 %) for 48 - 72 h. In all cells, the cell membrane is hyperpolarized by calcium ionophore A 23187. Patch clamp experiments indeed reveal K+ channels (30 pS at 0 potential), which are activated by calcium from the intracellular side. According to Fluor3 fluorescence BK leads to transient increase of intracellular calcium. In ra- and or, BK induces a single, transient hyperpolarization (r: 10 pS from 22 ± 2 to 48 ± 3 mV; or: from 16 ± 2 to -59 ± 5 mV). In ra, BK elicits oscillations of cell membrane potential from 24 ± 2 to -59 ± 3 mV throughout the presence of the hormone. The peak of the oscillations is decreased to -31 ± 2 mV by increasing extracellular K+ concentration from 5.4 to 20 mmol/l. The oscillations are partially blocked by 1 mmol/l verapamil and abolished by removal of extracellular calcium. The oscillations are not abolished in the presence of furosemide, amiloride or ouabain in or cells pretreated with pertussis toxin. Isoproterenol (I) and dexamethason (II) are know to exert anti-inflammatory and analgesic effects, we also investigated possible effects of indomethacin, ibuprofen and salicylic acid. However, no pronounced effects were detected with 100 µM of these drugs. It is concluded that the blocking effects of mefenamic and flufenamic acid are related to their specific structure, containing two phenyl rings linked by an amino bridge.

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ION CHANNELS IN HUMAN MELANOMA CELLS

E. Bohm and B. Willms

Ion channels in the membrane of cells from a human melanin producing melanoma cell line (I) (1981) has been investigated with the patch clamp technique. In cell attached patches the most frequently observed channel is a potassium channel with similar properties as a delayed rectifier known e.g. in pinal cells (2) or osteoclasts (3). The channel has a single channel conductance of approximately 10 pS. The permeability ratio between Na and K is about PNa/PK = 0.03. The open probability is increased at positive potentials. Whole cell currents and averaged currents show slow activation but no inactivation. The open probability of the channel is down regulated by isoproterenol. Isoproterenol also delays activation. A second type of potassium channels shows rapid inactivation. This channel has a conductance of approximately 12 pS (A-type potassium channel). It is less frequent to observe than the delayed rectifier. Melanocytes also possess a channel with properties similar to the inward rectifier that is activated at negative potentials. A fourth channel is a non-selective, supposedly Ca activated cation channel. The channel has a conductance of about 19 pS and cannot discriminate between Na and Ca channels.

(1) Albert C, Chriseacu Rev Inst Pest (Lyon) 6, 265, 1973
(2) Aguyo LG and Weight FF J Physiol (Lond) 405, 397, 1989
(3) Reicosaitis JH et al Proc Natl Acad Sci USA 86, 6021, 1989

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METHANIC ACID AND FLUFENAMIC ACID BLOCK THE NONSELECTIVE CATION CHANNEL IN RAT EXOCRINE PANCREAS

H. Gögelein and D. Dahlem

After stimulation with secretagogues Ca2+ dependent nonselective cation channels are activated in the basolateral membrane of rat exocrine pancreatic cells. This channel type was also observed in a number of other secreting epithelia, such as the salivary gland and the distal colon. Recently it was reported that the channel is blocked by 3,5-dichlorodiphenylamine-2-carboxylic acid (DCDPC, Gögelein and Flammüller (1989), Pflügers Arch. 413:287). Now we investigated some drugs which are structurally related to DCDPC. Isolated pancreatic acini or single cells were prepared in the laboratory of Dr. J. Schütz in our institute. Inside-out oriented cell-attached membrane patches were obtained from the basolateral cell membrane. The nonselective cation channel was evoked by exposing the cytosolic side to NaCl-solution containing 1.3 mmol/l Ca2+. The channel appeared mostly in clusters where each channel had a mean open-state probability P∞ of about 0.7. Addition of either 10 µmol/l of flufenamic acid or mefenamic acid to the bath decreased P∞ to about 50% (n=7 and 5, respectively), whereas 100 µmol/l of these drugs caused complete and reversible inhibition of the channel. The effect of niflumic acid was less pronounced. 10 µmol/l of this drug decreased P∞ only slightly, whereas 100 µmol/l decreased P∞ from 68±0.07 to 21±0.8 (n=5). As these drugs are known to exert anti-inflammatory and analgesic effects, we also investigated possible effects of indomethacin, ibuprofen and salicylic acid. However, no pronounced effects were detected with 100 µM of these drugs. It is concluded that the blocking effects of mefenamic and flufenamic acid are related to their specific structure, containing two phenyl rings linked by an amino bridge.

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ION CHANNELS IN RAN EXOCRINE PANCREAS

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After stimulation with secretagogues Ca2+ dependent nonselective cation channels are activated in the basolateral membrane of rat exocrine pancreatic cells. This channel type was also observed in a number of other secreting epithelia, such as the salivary gland and the distal colon. Recently it was reported that the channel is blocked by 3,5-dichlorodiphenylamine-2-carboxylic acid (DCDPC, Gögelein and Flammüller (1989), Pflügers Arch. 413:287). Now we investigated some drugs which are structurally related to DCDPC. Isolated pancreatic acini or single cells were prepared in the laboratory of Dr. J. Schütz in our institute. Inside-out oriented cell-attached membrane patches were obtained from the basolateral cell membrane. The nonselective cation channel was evoked by exposing the cytosolic side to NaCl-solution containing 1.3 mmol/l Ca2+. The channel appeared mostly in clusters where each channel had a mean open-state probability P∞ of about 0.7. Addition of either 10 µmol/l of flufenamic acid or mefenamic acid to the bath decreased P∞ to about 50% (n=7 and 5, respectively), whereas 100 µmol/l of these drugs caused complete and reversible inhibition of the channel. The effect of niflumic acid was less pronounced. 10 µmol/l of this drug decreased P∞ only slightly, whereas 100 µmol/l decreased P∞ from 68±0.07 to 21±0.8 (n=5). As these drugs are known to exert anti-inflammatory and analgesic effects, we also investigated possible effects of indomethacin, ibuprofen and salicylic acid. However, no pronounced effects were detected with 100 µM of these drugs. It is concluded that the blocking effects of mefenamic and flufenamic acid are related to their specific structure, containing two phenyl rings linked by an amino bridge.

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REGULATION AND INTERACTION OF RAT BRAIN POTASSIUM CHANNEL PROTEINS
J.P. Ruppersberg, K.H. Schröer, B. Sakmann, *M. Stocker and **O. Pongs

Rat brain potassium channel (RCK) proteins were expressed in the membrane of Xenopus oocytes after injection of the corresponding cRNA into the oocyte or in HeLa cells after transient with a vector derived from SV40 containing cDNA encoding for RCK proteins. Currents mediated by the potassium channels formed by the RCK proteins were measured with two-microelectrode voltage clamp or cell-attached patch clamp in oocytes and in the whole-cell recording mode of the patch clamp method in the transfected HeLa cells.

With these methods we tested for the effect of c-AMP and phorbol ester on potassium currents under various conditions. We found that RCK1, RCK2, RCK3 and RCK4 potassium channels decreased in their activity within minutes in consequence to the dialyzation of the intracellular space by the micropipettes used for the measurements. In contrast to this decrease in RCK1, RCK2 and RCK3 but not in RCK4 the currents could be markedly increased by intracellular application of c-AMP or phorbol esters indicating a stimulation of the channels by phosphorylation. When RCK1 and RCK4 proteins were simultaneously expressed in the same cell heterooligomeric channels called RCK1,4 formed which had distinct functional and pharmacological characteristics. The heterooligomeric RCK1,4 channels seemed to be as insensitive to c-AMP dependent phosphorylation as the RCK4 channel.

We assume phosphorylation and changing the subunit structure to be major mechanisms for RCK potassium channel regulation in the mammalian brain.

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DIFFERENT INTERACTION EFFECTS IN K+ CHANNELS FOLLOWING CO-EXPRESS OF WILD TYPE AND SHAKER MUTANT SUBUNITS OF DROSOPHILA MELANOGASTER
D. Wittka & G. Bohrman (1); R. Lichtenthaler, M. Stocker & O. Pongs (2)

The shaker locus of Drosophila melanogaster codes for a variety of related K+ channel subunits, which constitute a subunit family, and of which probably each member by itself forms functional K+ channels. All Shaker proteins share a common core region with 6 putative membrane spanning helices (S1-S6) but have variable NH2-(A,B,G,D) and COOH-(A,B,G,D) termini. There is evidence that all termi face the cytoplasmic membrane side.

Shaker mutants of Drosophila melanogaster lack or have abnormal IA-currents in voltage clamped muscle cells as well as abnormal action potentials with delayed repolarization in nervous tissue. One of the Shaker alleles, which eliminates the current completely, is ShKslz. Another mutant, ShKsls, shows only 28% of wild type current. Gene dosis experiments indicated that ShKslz belongs to the antimorphic type and, furthermore they showed that the product of ShKslz significantly interferes with intact K+ channel subunits, whereas the product of ShKsls does not.

In the genomic sequence of ShKslz a single base exchange was found, which leads to an amino acid exchange of V to D within the putative extracellular loop between S5 and S6. On the other hand, a defect splicing mechanism in ShKslz causes a translation step in the class 1 terminus, while class 2 transcripts remain unaffected. By molecular-biological techniques both mutations were introduced into the corresponding cDNA templates. Transcribed cRNA and cRNA-mixtures were then injected into Xenopus oocytes. The potassium channel protein expression, whole cell IA-currents were measured. In both cases IA was completely abolished when only one of the mutated cRNAs. Injection of 1:1 cRNA-mixtures of either ShKslz or ShKsls revealed a reduction of the peak current amplitude to 32% of the expected wild type current amplitude (ShKslz) or no change of that wild type current (ShKsls), respectively. Consequently, ShKslz produces a defective K+ channel subunit, which seems to interfere with and to deactivate the normal product, whereas ShKsls does not interact with the intact wild type subunit. This result is confirmed by injection of further cRNA-mixtures (3:1, 1:2).

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SINGLE CHANNEL RECORDINGS OF ONE TYPE OF NA CHANNEL AND MULTIPLE K CHANNELS IN MYELINATED AXONS
P. Jonas, M. Herrnsteiner and W. Vogel

The tibial nerve of the toad Xenopus was desheathed and the myelinated fibres were treated for 135 min with 3 mg/ml collagenase in Ringer's and for 35 min with 1 mg/ml protease at 22 °C. Axons with retraction of the myelin sheath were patch-clamped in the nodal and paranodal region with 30-80 MΩ pipettes (Jonas et al. 1989,PNAS 86: 7238-7242). In Ringer's current events are found which reverse sign at the calculated reversal potential for Na+. They are blocked by tetrodotoxin. The single channel conductance is 11 pS (15 °C). Averaged events show the typical activation and inactivation kinetics of macroscopic Na currents. Three potential-dependent K channels were also identified (I-, F- and S-channel). The I-channel, which is the most frequent type has a single-channel conductance of 23 pS (inward current, 105 mM K on both sides of the membrane) and activates at potentials between -60 and -30 mV. It deactivates with intermediate kinetics and is blocked by 50-500 nM tetrodotoxin. The F-channel has a conductance of 30 pS, activates between -40 and 90 mV, and deactivates with fast kinetics. The former inactivates within tens of seconds, the latter within seconds. The third type, the S-channel has a conductance of 7 pS, and deactivates slowly. All three channels can be blocked by external tetraethylammonium ions. Three components of macroscopic K current have been described recently (Brä et al. 1980, J. Physiol. 420: 365-385). We suggest that the I-, F- and S-channels form the basis for these current components.

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Cultured Schwann cells are characterized by a strong outward rectification of the membrane with a threshold close to the resting membrane potential of about -50 mV. With the patch-clamp technique we identified single channels with a conductance of 10 - 12 pS; the kinetic behavior of these channels can account for the two different types of membrane components recorded from different cells in the whole cell recording configuration: while some single channels displayed inactivation, other were non-inactivating. Moreover, the time constant of averaged single channel and whole-cell current inactivation were similar and both showed a voltage dependency. These channels are K⁺ selective since changes in extracellular [K⁺] resulted in changes of the reversal potential as predicted for an exclusively K⁺ selective pore. The reversal potentials also predicted an intracellular [K⁺] of 60 mM indicating that the K⁺ equilibrium potential is slightly negative to the membrane potential. We conclude that cultured Schwann cells express either two types of K⁺ channels with similar conductance or a channel which can acquire two functional states and that these channels can account for the membrane currents observed in this cell.

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A RISE IN [K⁺], SHIFTS THE ACTIVATION CURVE OF THE DELAYED RECTIFIER K⁺ CHANNELS IN CULTURED MOUSE SCHWANN CELLS: A POSSIBLE MECHANISM FOR K⁺ HOMEOSTASIS.
BY ALEXEJ N. VERKHRATSKY¹, D. HOPPE AND HELMUT KETTERNANN.

Cultured mouse Schwann cells are characterized by the expression of an outward rectifying K⁺ channel as previously described. In this study we analyzed the effect of the K⁺ gradient on channel properties applying the patch-clamp technique in the whole cell and cell attached configuration. In normal [K⁺]₀ (5.6 mM), channels are activated at potentials more positive than -50 mV thus close to the resting membrane potential. When [K⁺]₀ was elevated, the threshold of activation shifted to more negative values; in 145 mM [K⁺] the threshold of activation was -75 to -60 mV. Moreover the steepness of the activation curve increased with increasing of extracellular [K⁺]. The sensitivity of the K⁺ channel for [K⁺]₀ was only apparent at normal intracellular K⁺ levels (60 mM); with high intracellular [K⁺] (140 mM) channel gating was not affected. This property of the Schwann cell K⁺ channel may serve to facilitate the uptake of K⁺ in times of activity induced elevations.

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PEPTIDE MEDIATED INACTIVATION OF A POTASSIUM CONDUCTANCE IN LOCUST SKELETAL MUSCLE: INVOLVEMENT OF A SECOND MESSENGER?
K.E. Zittel and C. Walther

In locust jumping muscle both proctolin (Arg-Tyr-Leu-Pro-Thr) and YGGFMRFamide (Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH₂) lower the resting membrane conductance (by up to -50%) by inactivation of a not voltage dependent, ohmic K⁺-conductance (Murck et al., in: Dynamics and plasticity of neuronal systems, eds. N. Elmen and W. Singer, p. 433, Stuttgart:Thieme Verlag, 1989). Half-maximal effects are achieved with ca. 10⁻⁴M and 5·10⁻⁶M, respectively. Intracellular injection under voltage clamp of the known G-protein activator GTP·S renders the response to either peptide irreversible. In order to find out whether the peptides act via a secondary messenger and not directly via G-protein(s) the effects of various bath-applied drugs were investigated. Neither 8-bromo-cAMP nor 8-bromo-cGMP (10⁻⁴M) nor the respective phosphodiesterase blockers 3-isobutyl-1-methylxanthine (IBMX, 10⁻⁴M) and Na-nitroprusside (10⁻³M) had an effect on the peptide response or the resting membrane K⁺-conductance. 10⁻⁶M TPA (12-O-Tetradecanoyl-phorbol-13-acetate), an activator of protein kinase C (PKC), induced a slowly progressing reduction of the resting membrane K⁺-conductance, while 10⁻⁴M 4e-phorbol (12,13-didecanoyl) did not affect the peptide effect or the resting membrane K⁺-conductance. 10⁻⁷M 4c-phorbol (12,13-didecanoyl), a threshold close to the resting membrane potential. When [K⁺]₀ was replaced by Na⁺ the ATP-block could be relieved by addition of 10⁻⁷M 4c-phorbol (12,13-didecanoyl) while 10⁻⁷M 4c-phorbol (12,13-didecanoyl) was without effect. The opening probability of a K⁺-channel (ca. 100 pS maximum conductance; gating and conductance not markedly voltage sensitive) which is a good candidate for the peptide sensitive conductance was not obviously affected by bath application of either peptide during cell-attached recording. These results point to an involvement of PKC as a mediator for the action of these peptides on the resting K⁺-conductance.

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ACTIVATION OF ATP-SENSITIVE K⁺ CHANNELS FROM HUMAN SKELETAL MUSCLE: A NEW METHOD ALLOWS SINGLE CHANNEL RECORDING WITHOUT ENZYME TREATMENT
H. Hafe, C. Franke and S. Guasthoff

Up to now recordings with the patch clamp technique from vertebrate skeletal muscle were possible only after enzyme treatment. We report here measurements from membrane blebs formed on skeletal muscle fibres obtained from specimens of human skeletal muscle. We routinely performed open muscle biopsies from patients. Only a small amount of material was necessary. The blebs were induced by only mechanical irritation of the fibres without any enzyme treatment. After excision of inside-out patch-channels, single channel activity could be regularly observed in symmetrical solution (in mmol/l: K⁺ 140, Ca⁺⁺ 10⁻⁴, Cl⁻ free). The conductance was 20 pS. The channels were reversibly blocked by ATP (> 0.2 mmol/l) or dilibemidine (> 1 μmol/l) applied to the sarcolemmal side. The block was concentration dependent and the ATP-block could be relieved by addition of EMD 52692 (> 1μmol/l), one of the recently characterized K⁺ channel openers (Quast and Cook, TIPS 10:431-425, 1989). If K⁺ was replaced by Na⁺, single channel amplitude decreased, indicating selectivity for K⁺ ions of the channel. These data indicate that the recently reported enhancement of membrane K⁺ conductance (Guasthoff et al., Pflügers Archiv. 414:179, 1989) by EMD 52692 in human skeletal muscle is based on the direct activation of ATP sensitive K⁺ channels. Furthermore, membrane blebs are seen to contain channel proteins and the method will allow further studies of normal and diseased membranes of human skeletal muscle. Supported by the Herrmann-und-Lilly-Schilling Stiftung, Physiologisches Institut der TU München, Biedersteinerstr.29, D-8000 München 40

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HEREDITARY MYOTONIA IN THE MOUSE INVOLVES QUALITATIVE CHANGES OF SARCOLEMMLAL POTASSIUM CHANNELS. A PATCH CLAMP STUDY.
E. Wichmsmeyer and H. Jockusch

Like in myotonic of man, the hyperexcitability of the muscle of the myotonic ADR mouse is caused primarily by a reduction of chloride conductance to <10% of the wildtype (Mehrae et al., Muscle and Nerve 11, 440-446, 1988). In the allicic MTO mutant an additional reduction of the potassium conductance by 50% has been demonstrated (Byant et al., Soc. Neurosci. Abstr. 13, 1681, 1987).

Using the patch clamp technique, we have recorded single channel currents of enzymatically dissociated toe muscle fibres from normal and myotonic mice. In the cell attached mode we found two types of the inwardly rectifying potassium channels, with current amplitudes of 0.3 and 0.6 pA at resting potential. Whereas both types of channels were present in wildtype fibres, only the 0.3 pA channel was found in ADR muscle. This finding was confirmed on inside out patches and explains the lowered K+-conductance in myotonic mouse muscle.

A qualitative change of K+-channels in addition to the lowered chloride conductance points to a generalised membrane defect in myotonic muscles, as has been suspected by several authors (cf. Kuhn & Seller, Klin. Wochenschr. 48, 1134-1136, 1970; H. Brinkmeier, Doctoral Thesis, Bielefeld 1988). Supported by DFG, SFB 223.

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Single channel properties of Opossum Kidney cells (OK).
F. Hollunder-Reese, M. Bleich, M. Mohrmann, R. Greger

OK cells were grown on glass cover slips in DMEM/10%FCS in an atmosphere of 5% CO₂ and 95% air. After two days the cells were sub confluent. A coverslip was transferred into a bath chamber mounted on an invertoscope. Standard patch clamp techniques were used. Most experiments were done in excised inside/out patches. Two types of K+-channels (big and intermediate conductance) and nonselective as well as (Cl-)channels were found. The big K+ channel was Ca²⁺ dependent and weakly pH dependent. The channel had a mean conductance of 166 ± 12 (n=16) pS at clamp voltage Vc=0mV (pipette KC1/bath NaCl). This channel was blocked reversibly by Ba²⁺, 10⁻⁵ mol/l, n=3. The voltage dependence of this block suggests that Ba²⁺ acts from the cytosolic side. Verapamil 10⁻⁵ mol/l and quindine 10⁻⁴ mol/l block reversibly. TEA 10⁻⁵mol/l blocked only from the outside. The intermediate conductance K⁺ channel had a mean conductance of 63 ± 13 pS, n=4, (Vc=0mV), and was Ca²⁺ independent. Unlike the big K⁺ channel, this channel was also observed in cell attached configuration (n=1). In some excised membrane patches both types of K⁺-channel coexisted (n=4). One other type of channel (most likely Cl-channel) had a mean conductance of 77 ± 14 pS (g(+)), and 49 ± 9 pS (g(-)), n=4 and was blocked reversibly by 5-nitro-2-(3-phenyl-propylamino)-benzoate (NPPB) 10⁻³ mol/l. Supported by DFG Gr 480/9 and Mo/398/3-1.

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LOCALIZATION, CHARACTERIZATION, AND RECONSTITUTION OF CHLORIDE CHANNELS FROM MAMMALIAN SKELETAL MUSCLE
S. Weber-Schürholz, H. Jockusch, T. Schürholz, M. Akabas, D. Landry, Q. Al-Awadi

Membrane vesicles were prepared from rabbit skeletal muscle and separated by sucrose gradient centrifugation. Fractions obtained (in the order of increasing density) were outer sarcosome (SL), T-tubules (TT), sarcoplasmatic reticulum (SR), triads and mitochondria. SL and TT were characterized by high specific binding capacity for 3H-hexaxin (Na-channel) and 3H-PN 200 (DHP-receptor), respectively. Highest activity in potential sensitive 3CΙ-transport and binding of the chloride channel ligand, 3H-indanyloxyacidic acid (IAA 94; Landry, D. et al., Science 224, 1469, 1989),were found in the SL. We conclude that chloride channels are predominantly localized in the SL and not in the TT as previously proposed (Dulhunty, A., J. Membrane Biol. 45, 293, 1979).

SL vesicles were solubilized with n-octyl-β-D-glucopyranoside and subjected to IAA sepharose affinity chromatography. Bound protein was eluted with 100μM IAA 94 and either analyzed by SDS gel electrophoreses or reconstituted into planar lipid bilayers. The eluate contained a selected set of polyepptides and yielded highly specific chloride channels with four different conductance levels.

These findings may lead to an understanding of myotonia, a hereditary disease affecting chloride conductance.

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DOES IP₄ PLAY A ROLE AS SECOND MESSENGER IN OOCYTES OF XENOPUS LAEVIS?

In oocytes previously injected with poly(A)+RNA from cloned receptors activates a signal transduction pathway in which the hormone (TRH) could be expressed. Binding of TRH to these receptors results in the formation of inositolphosphates. Inositol(1,3,4,5)tetrakisphosphate (IP₄), which is also formed, is still uncertain.

Injection of oocytes with IP₃ elicited Ca²⁺-dependent chloride currents. These currents could be blocked by co-injection of heparin, which also blocked the TRH-induced current response. Injection of the (2,4,5)IP₃-analogue resulted in pronounced membrane currents, indicating the activation of the IP₃ receptors. Recently we have shown that injection of IP₃ also elicits membrane currents, distinct from those produced by IP₃. This suggests that IP₃ plays a role as a second messenger in these oocytes.

The aim of the present study was to examine the effects of various compounds on the IP₃-induced Cl⁻ transport pathways. The blockers were tested in patch clamp experiments on Cl⁻ channels in excised inside/out membrane patches of cultured HT₉₋₄ colon carcinoma cells and respiratory epithelial cells. The properties of these Cl⁻ channels have been described in previous reports. They have (i) a conductance of 50-70 pS with (ii) outward rectification and are independent of (iii) cytosolic calcium and (iv) pH. All compounds tested, although of entirely different chemical structure, reduced the open probability (Pₒ) of the examined Cl⁻ channels reversibly by inducing flickering. The half-maximal concentration for inhibition (Pₒ₅₀) of the different blockers were derived from dose-response curves. The obtained sequence of the Pₒ₅₀ values was: 5-nitro-2-(3-phenylpropylamino)-benezoate (NPBP) 74±10⁻⁶ < adenosine 3'10⁻⁶ < indanyloxacetic acid (IAA) 6±10⁻⁶ < 4',6'-dinitrostilbene-2',2'-disulfonic acid (DNDS) 2±10⁻⁵ < 4',6'-dilithio cyanostilbene-2,2'-disulfonic acid (DIDS) 3±10⁻⁵ = 4-acetamido-4-isothiocyanato-stilbene-2,2'-disulfonic acid (SITS) 3±10⁻⁵. The present data do not permit any conclusions as to the mechanism of interaction with the Cl⁻ channel. Supported by DFG Gr 480/9.

EVIDENCE FOR A CL⁻ STIMULATED HCO₃⁻ CHANNEL IN FUSED MDCK CELLS

W. Seliger, H.-J. Westphal, U. Kersting and H. Oberleithner

Madin-Darby-canine-kidney (MDCK) cells in culture resemble properties of intercalated (IC) cells of the cortical collecting duct. The cells transport bicarbonate and base equivalents depending on the specific metabolic conditions. Fused MDCK cells can regulate their volume during hypotonic stress by concomitant net efflux of K⁺ and HCO₃⁻ ions. This control mechanism depends on the presence of extracellular HCO₃⁻ and Cl⁻ and cannot be inhibited by the stilbene derivative DIDS. Furthermore, measurements of cytoplasmic pH and cell membrane conductance reveal the participation of an electrogenic HCO₃⁻ transport. Using the patch-clamp technique, we performed single channel measurements in the excised mode (inside/out configuration) with symmetrical HCO₃⁻ concentrations in the bath medium (24 mM NaHCO₃, 110 mM Na-glucosate, 5% CO₂, pH 7.4) and in the pipette solution (24 mM NaHCO₃, 220 mM mannitol, 1 mM NaCl, 5% CO₂, pH 7.4). We can disclose a channel with the following characteristics: i) a conductivity of about 17 pS; ii) mean open and closed times of 16 and 5 ms, respectively; iii) an open probability (Pₒ) of about 0.1 in the physiological range of the membrane potential, Pₒ increases up to 0.4 at depolarized voltages; iv) a reversal potential near 0 mV; v) a permeability ratio of HCO₃⁻ over Cl⁻ of >2. In presence of a high Cl⁻ concentration (110 mM) in the bath medium (cytoplasmic face) the channel is activated (Pₒ doubles compared to controls).

Based on the existence of a voltage and Cl⁻ sensitive HCO₃⁻ channel we postulate that an increase of intracellular Cl⁻ induced by cell depolarization enhances Pₒ of the HCO₃⁻ channel. This couples HCO₃⁻ efflux to Cl⁻ influx. This mechanism can contribute to cell volume and cell pH regulation.

Supported by SFB 176.

ANION CURRENT RECORDED FROM THE INNER MITOCHONDRIAL MEMBRANE OF BROWN ADIPOCYTES

T. Kittsche & D. Siemen

Due to the chemoosmotic theory the inner mitochondrial membrane should be impermeable to small inorganic ions. Nevertheless ion fluxes have been described by several groups. Recently Sorgato et al. found an ion channel in rat liver cell mitochondria (Nature 330: 498 - 500, 1987). The authors discussed whether this channel could have the same function as the uncoupling protein of brown adipocytes. This protein uncouples the respiratory chain from ATP-synthesis thus effecting nonshivering thermogenesis. It can be blocked by purine di- and trinucleotides. We used mitoblasts (swollen mitochondria with removed outer membrane) from brown adipose tissue of 6 - 8 week Sprague-Dawley rats. Mitochondria were isolated according to the method of Cannon and Lindberg (Methods Ezymol. 55: 65 - 75, 1979) by multiple slow (800 x g) and fast (10,000 x g) centrifugation steps. Current recordings were done by means of the patch clamp method (Hamill et al., Nature 391: 85 - 100, 1981) in the "mitoblast-attached" and in the "whole-mitoblast" mode.

Our results show a 110 pS channel with similar kinetics as observed in liver cells. With K-glucosate inside the pipette no anion inward current was observed. We do not see this channel very often in mitoblast-attached patches but we see it regularly in the whole-mitoblast mode. It can be blocked by 20 µM GDP and by 20 µM GMP as well but not by 20 µM cyclic GMP. From this we conclude that the 110 pS channel is an anion channel. It is present in brown fat cells as well as in liver cells. As the uncoupling protein is known not to be blocked by GMP it is most likely that the anion current flowing through the here described 110 pS channel is not identical with the shunt current causing nonshivering thermogenesis.
Cultured human fibroblasts were transiently transfected with cDNA clones encoding rat α1, α2 and β2 subunits of GABAAR (γ-aminobutyric acid-receptor). GABA-activated currents in cells cotransfected with cDNAs encoding different combinations of GABAAR subunits were measured with patch clamp whole cell technique (asymmetrical 140mM Cl⁻).

In cells cotransfected with cDNAs encoding α1 and β2 subunits the currents were strongly blocked by micromolar concentrations of Zn²⁺ in a non-competitive manner. At 10 μM GABA the current amplitude was reduced by 10 ± 5% at 31 ± 15% of control (n=8).

Cotransfection of cells with cDNAs encoding the α1 and β2-subunits or α1-, β2- or α2-subunits resulted in a much lower sensitivity to 10 μM Zn²⁺ at 10 μM GABA: for the α1-, β2-combination the amplitude was 97 ± 12% of control (n=11); for the α1-, β2-, β2-combination the current under 10 μM Zn²⁺ was 92 ± 14% of control (n=10).

We conclude that the block of GABAAR by divalent cations depends on the subunit composition of the GABAAR channel. Receptors composed of α1- and β2-subunits are susceptible to block by Zn²⁺, GABAAR containing a β2-subunit are much less susceptible.
RESPONSES TO EXCITATORY AMINO ACIDS IN NEURONS ACUTELY ISOLATED FROM SPINAL CORD SLICES OF ADULT RATS

F. Rufall, J. Rosenheimer*, C. Franks, D. O. Smith* and H. Hatt

Up to now, desensitization kinetics and pharmacological properties of glutamate receptors of motoneurons were studied on embryonic neurons. We report here patch-clamp recordings from aged neurons differentiated in vivo. Spinal cord of adult rats was immersed in ice-cold Hapes buffered saline and cut into 400 μm thick cross sections. Sections were then introduced to a chamber containing bicarbonate-buffered saline (pH 7.4, 37°C) with constant stirring. Following a 30 to 60 min incubation, the medium was replaced with 0.1% trypsin and incubated for an additional 35 min. Single cells were isolated from these slices by mechanical treatment. Cells were allowed to settle in the petri-dish for about 1 h before patch-clamp recordings were made. In whole-cell recordings, large inward currents were elicited in response to pressure application of glutamate or quisqualate in the soma and in some cases in the neurites. NMDA or kainate channels were blocked by hyperpolarization. Quisqualate-gated currents desensitized to zero current level with a time constant of a few ms. In control, no desensitization was observed with the agonist kainate. In outside-out patches, fast application of quisqualate rapidly activated and desensitized channels with characteristics similar to those of embryonic neurons. Supported by the DFG (SFB 220).

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GLUTAMINERGIC RESPONSES IN NEUROLEI GLIAL CELLS AND RETZIUS NEURONES OF THE MEDICINAL LEECH

H. Halle

Glial cells have glutamate receptors similar to those found in neurons (e.g. Sontheimer et al., Glia 1:328, 1988). We have used double-barrelled, ion-sensitive microelectrodes to investigate the effects of glutaminergic agonists on intracellular potential (Em) and intracellular ion-activities in neuropile glial cells and in Retzius neurones of isolated leech segmental ganglia. In both types of cell, application of glutamate (Glu) or kainate (Ka) and quisqualate (Qui) elicited concentration-dependent depolarizations accompanied by increases of the intracellular Na+ activity (aNa) and by concomitant decreases of the intracellular K+ activity (aK). In neuropile glial cells, these alterations of aNa and aK were preceded by a transient decrease of aK and an increase of aK upon application of Glu and Qui. These initial aNa and aK transients might be due to glial uptake of neuronal K+ released during the action of Ka and Qui. As found for Ka, the neuronal and the glial responses to glutaminergic agonists persisted during inhibition of synaptic transmission in solutions containing high Mg2+ and low Ca2+. In both types of cells, Retzius neurones and neuropile glial cells, H- and ATP-methylnicotinate (NMNA) did not affect Em, aNa, or aK. These results indicate that leech neuropile glial cells were equipped with non-NMDA glutamate receptors similar to that in vertebrate neurons.

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Andreas König

PROPERTIES OF A NON-SELECTIVE CATION CHANNEL IN HUMAN VASCULAR ENDOTHELIUM

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We report here patch-clamp recordings from aged endothelial cells obtained from human umbilical cord. Ions channels have been studied by the patch clamp method. Besides an inward rectifying potassium channel and a high conductance Ca2+-activated channel, a non-selective cation channel has been recorded. This channel is activated by μM concentrations of histamine and nM concentrations of thrombin. Open probability is voltage independent and shows a slow rundown in cell-attached patches. After excision, channel activity runs down to zero open probability in less than 4 min. However, under these conditions the single channel conductance was unchanged and allowed a detailed permeation analysis. The survival time is prolonged in the presence of GTP S. With symmetrical potassium concentrations, the single channel conductance is 28 pS and the reversal potential is 0 mV. With 140 mM Na and 5 mM K in the pipette, the conductance is 26 pS. A reversal potential of -1.5 mV was measured. With 60 mM Ca and 70 mM Na in the pipette, a reversal potential was -10.5 mV. The single channel conductance is 5.4 pS measured from inward currents with 110 mM Ca (pipette) and 140 mM K (bath). From the analysis of the reversal potentials, a permeation ratio of fCa/fNa = 1:0.7:0.2 was calculated. These data refer to the existence of a Ca permeable non-selective cation channel in human endothelial cells that can be activated by agonists as thrombin and histamine. The non-selective channel might be a tool to induce a sustained agonist mediated Ca influx.

Stimulation of isolated guinea-pig hepatocytes with the α1-adrenoergic agonist phenylephrine (Phe, 1 μM) causes an increase of the membrane conductance for K+ and Cl-. (Gilmour et al. FIBS Lett. 217: 247, 1987) most likely mediated by a rise in [Ca2+]i via IP3-mediated Ca2+-release from internal stores of Ca2+ ions. We have measured the isolated Cl−-current using whole-cell patch-clamp in order to obtain information on mechanisms of [Ca2+]i-regulation. Ca2+-dependent Cl−-current was blocked by Cs+ on both sides of the membrane. Upon superfusion with Phe an inward current was recorded at -30 mV holding potential. This current relaxed within several seconds. In some cells the transient increase in Cl−-conductance was followed by oscillatory changes of gCl, which in line with previous [Ca2+]i-measurements using aequorin (Woods et al. Cell Calcium B:89, 1987) or fura-2 (Kawanishi et al. J. Biol. Chem. 264: 12859, 1989). The Cl−-current could be switched off by voltage steps to positive membrane potentials (Fig. 1). As this effect of depolarization occurred with a delay of several seconds, a direct action of voltage on gCl seems unlikely. Hyperpolarization of the cell had the opposite effect: it delayed relaxation of the current and, in the presence of Phe, could initiate a second Cl−-current. The effect of hyperpolarization was not observed prior to stimulation with Phe. Similar results as with Phe were obtained with extracellular application of ATP (10-4 M). Our results support the hypothesis that Caentry in hepatocytes is facilitated by hyperpolarization and is reduced or abolished by depolarization.

Effect of depolarization on slow oscillation of Cl−-current in the presence of Phe (10-4 M). Calibration: 400 pA; 50 s.

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**BIOPHYSICAL MODELS TO DESCRIBE STRUCTURAL DETERMINANTS OF CONDUCTANCE AND SELECTIVITY IN THE NICOTINIC ACETYLCHOLINE RECEPTOR**

C. Bünch, T. Konno

**ANON CHANNEL FORMING ACTIVITY FROM CLAVIBACTER MICHIGANENSE SUBSP. NEBRASKENSE**

T. Schürholz, M. Willinzig, R. Eichenlaub, E. Neumann

**A23187 AND MELITTIN ACTIVATE A CHLORIDE CONDUCTANCE IN PRIMITIVE RED CELLS OF THE CHICK EMBRYO**

Ch. Reinhardt, W. Kaiser and E. Baumann

It has been previously demonstrated that the membrane potential of primitive embryonic red cells is dominated by a proton conductance and the chloride/bicarbonate exchange by band 3 protein in these cells is impaired (Engelke et al. 1988, J. Cell. Physiol. 133, 67). In consequence there is a disaerialism between chloride and proton distribution; the calculated chloride equilibrium potential is around -15 mV whereas $E_{cl}$ is -44 mV at days 4 of development. We have extended our measurements to earlier stages of development, where red cells are in the log growth phase. At resting conditions the $E_{cl}$ values were between -40 to -50 mV and not changed by variation of external sodium, chloride or potassium; stimulation of cells with ATP, db-cAMP, db-guanosine monophosphate and PMA also did not alter the $E_{cl}$. However incubation with A23187 or melittin caused a fast, long lasting depolarisation of about 20 to 30 mV, bringing the $E_{cl}$ close to the chloride equilibrium potential.

This depolarisation was not inhibited when cells were treated with DIDS, or when external sodium or calcium were removed, but it was never observed in the absence of external chloride. As the size of the depolarisation corresponds to what one observes when cells are treated with the chloride ionophor tributyl-tin we conclude that A23187 and melittin stimulation activates a chloride channel. It may be involved in the volume regulation of proliferating embryonic red cells and can only be activated in the presence of external Cl-.

Ch. Reinhardt, Physiologisches Institut, Universität Regensburg, 8400 Regensburg, supported by DFG Ba 691/4

**H. Hatt, F. Zufall, M. Stengl**

Insect olfactory neurons were identified with two monoclonal antibodies, one of which recognizes specifically pheromone sensitive neurons (Hishinuma et al., 1988, Nature, 335, p. 645). Simulations with barrier models help to understand which equilibrium or kinetic parameters of ion transport could be altered by the mutations. The simulations show that the data are consistent with the assumption of a small negative fixed charge on the extracellular channel mouth and two cation binding sites in the narrow part of the channel separated by a high energy barrier. Within this model, a mutation can - depending on its location - alter the fixed charge, the binding strength at the sites or the energy barriers of the cations. Most of the mutant data can be described with a model in which only a few parameters, chosen according to the mutant position, are altered.

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**SINGLE CHANNEL RECORDINGS ON INSECT OLFACTORY RECEPTOR CELLS**

H. Natt, P. Zufall, M. Stengl+

The initial steps of the transduction mechanism in olfaction by which odorant binding produces the activation of channels in the olfactory cilia is still unknown. Recently, a primary long-term culture system of Manduca sexta antennal cells was developed and olfactory neurons were identified with two monoclonal antibodies. The olfactory neurons differentiate in culture and at least one type of Na+ channels could be identified. Na+ channels recorded in the whole cell mode could be reversibly blocked with 0.1 mM TTX. The predominantly observed K+ channel type was voltage dependent with a conductance of 30 pS. It could be blocked by application of nucleotides (ATP > GTP > cAMP) to the cytoplasmic side of the membrane. Secondly, a Ca++ dependent K+ channel (≈66 pS) with low voltage sensitivity was characterized. Third, a transient type of K+ channel (≈15 pS) could be activated by depolarizing voltage steps. These results indicate that the olfactory neurons differentiate in culture and provide the basis for further studies of pheromone effects.

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* Supported by the DFG HA 1201/2-1

**AN ANION CHANNEL FORMING ACTIVITY FROM CLAVIBACTER MICHIGANENSE SUBSP. NEBRASKENSE**

T. Schürholz, M. Willinzig, R. Eichenlaub, E. Neumann

Clavibacter michiganense subsp. nebraskense (Vidaver et al. Int. J. Syst. Bacteriol. 24:482-485, 1974) is a Gram-positive, coryneform bacterium, which is a pathogen of maize. Infection with Cl. m. subsp. nebraskense causes leaf freckles and wilting (Goss wilt) in the host plant.

Addition of culture medium to the aqueous phase of a planar bilayer chamber, caused formation of ion channels in the lipid membrane. The insertion of the channel component was not voltage dependent. However, the channel conductance showed a two fold voltage dependence. (i) Single channel conductance increased with voltage; at negative voltage (neg. on the side of insertion) no conductivity could be detected. (ii) The probability of channel opening increased with voltage. The channels were strongly selective for anions, Cl- > KSCN > SO4- and impermeable for gluconate. The incorporated channels were sensitive to protamine K+.

Supported by the Deutsche Forschungsgemeinschaft (SFB 223, C2 and D3)

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Our aim is to study how neurons differentiate to different subtypes, characterized by diverse receptors and ion channels. Such studies are complicated in many systems by the finding that development of electrical excitability starts very early in embryogenesis, at a time when neuronal subtypes can hardly be identified by morphological criteria.

Here we report a method that allows one to record from identified embryonic Retzius cells in Hirudo Medicinalis. These cells start to take up monocaines as soon as neurites begin to sprout. Following incubation (4 hours) in 300 µM autofluorescent 5,7-dihydroxytryptamine, 50 µM pargyline, and 30 µM ascorbate added to normal medium (+ 12% FCS) embryonic Retzius cells were visualized in the middle of the forming ganglia. After dissociation in solution containing 45 mM MgCl₂, stained cells could be identified among the largest cells in the culture dish. Cells identified by this method retained their ability to generate action potentials. Using a single-electrode voltage clamp connected to an electrode allowing internal dialysis, serotonin-(200µM) induced currents were recorded, which reversed at the equilibrium potential for Cl⁻. Cl⁻ currents of more than 10 pA were observed starting with the 10th day of development at 24°C. At this time, voltage-gated ion currents and action potential activity already developed. Apart from an increase in amplitude serotonin-induced Cl⁻ currents in embryonic cells were similar to those from freshly cultured adult Retzius cells.

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B.U. Keller, Y. Yaari and A. Konnerth

Excitatory synaptic transmission mediated by glutamate receptors was investigated by applying the patch-clamp technique to visually identified neurons in thin slice hippocampal slices (Edwards et al., Pflügers Arch 414, 600-612). Excitatory postsynaptic currents (EPSCs) were evoked in granule cells by field stimulation of the perforant path in the presence of 100 µM GABA. NMDA-mediated EPSCs were isolated and quantified by bath application of 0.5 &micro;M CNQX, which is a specific antagonist for glutamate receptors. The decay times (EPSCs) were evoked in granule cells by field stimulation of the perforant path in the presence of 100 µM GABA. NMDA-mediated EPSCs were isolated and quantified by bath application of 0.5 &micro;M CNQX, which is a specific antagonist for glutamate receptors.

RECOMBINANT GABA A RECEPTORS

G. von Blankenfeld, S. Tner, D.B. Fritchett, H. Sontheimer, M. Ewert, P.H. Seeberger and H. Rettemann

Recently the primary sequence of several different GABA receptor subunits was determined by molecular cloning. In this study, we compared GABA activated currents and their modulation by benzodiazepines in cultured human cells transfected with cDNA coding for different GABA receptor subunits. Flunitrazepam, a benzodiazepine agonist which potentiates GABA responses in both neurons and astrocytes was only effective in receptor subtypes containing the /2 subunit (q½/2 and q½/2). The 5-carboline DMCM decreased GABA activated currents mediated by GABA receptors composed of q½/1 and q½/2 subunits as described for the neuronal GABA receptor but increased GABA activated currents via receptors containing the q½ subunit (q½/1 and q½/2). A similar result was also observed with astrocytes. These results suggest that flunitrazepam and DMCM do not act on isosteric sites and that differences in the responsiveness of GABA receptors to these compounds are based on different subunit compositions of GABA receptors. It further suggests that the previously observed difference in the responsiveness of astrocytes and neurons to the benzodiazepine agonist flunitrazepam and DMCM could be accounted for by differences in their GABA receptor subunit composition.

Neurobiology of the University: Heidelberg, Im Neuenheimer Feld 345, 6900 Heidelberg.
SYNAPTIC CURRENTS IN IDENTIFIED HORMONE SECRETING CELLS OF THE RAT PITUITARY.

R. Schneggemberger and A. Konnerth

Hormone secreting cells of the intermediate lobe of the pituitary (IL) are innervated by terminals of neurones originating in the hypothalamus. Whole cell and single channel currents were recorded in visually identified IL cells in thin pituitary slices, using a modified version of the method described by Edwards et al. (Pflügers Archiv 414, 1989). Afferent fiber stimulation induced inhibitory post-synaptic currents (i.p.s.cs) with a latency of 1-2 ms. These i.p.s.cs, recorded in symmetrical Cl- solution, had a linear I/V relation, reversed around 0 mV and were blocked by bicuculline (K_0 ~ 50 nM), indicating that the activation of GABA_A-receptor is mediating the i.p.s.cs. At room temperature and a holding potential of ~60 mV the i.p.s.cs had amplitudes of ~100 to ~500 pA and lasted 100 to 150 ms. They had a fast onset (rise time to potential of ~60 mV the i.p.s.cs had amplitudes of ~100 to ~500 pA and lasted 100 to 150 ms. They had a fast onset (rise time to potential of ~60 mV) and lasted 100 to 150 ms. They had a fast onset (rise time to potential of ~60 mV) and lasted 100 to 150 ms. They had a fast onset (rise time to potential of ~60 mV). After a hypotonic shock a transient depolarisation could be measured, which was paralleled by a transient increase of membrane conductance. The maximal cell size was reached within 5-10 minutes. The depolarisation as well as the membrane conductance adopted their maximal values within the same time range. After 20-25 minutes the cells recovered their original cell size. This regulatory volume decrease (RVD) could also be observed in Ca^2+-free hypotonic bath solutions. Under these conditions the time course of RVD as well as the change of membrane conductance was slowed down. Addition of 1mM quinine to the isotonic bath solution caused a sustained depolarisation and a decrease of membrane conductance. A hypotonic shock in the presence of 1mM quinine induced a further depolarisation and an increase of membrane conductance. A delayed RVD could be observed. After 40-50 minutes the cells adopted their original size. Addition of 0.1 mM DIDS to the hypotonic bath slowed down the time course of depolarisation and the change of membrane conductance. The relative change of membrane potential and of conductance was significantly reduced compared to control conditions. RVD could be suppressed by DIDS. The data indicate, that a quinine-sensitive as well as a quinine-insensitive potassium current and a DIDS blockable anion current are involved in RVD of OK-cells.

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SLOW WHOLE-CELL RECORDING OF MEMBRANE CURRENT AND POTENTIAL DURING REGULATORY VOLUME DECREASE

J.M. H. Reuter & H.-A. Kolb

Using the slow whole-cell recording technique, we measured the membrane current and potential, while simultaneously monitoring the size of single Opossum kidney (OK) cells. After a hypotonic shock a transient depolarisation could be measured, which was paralleled by a transient increase of membrane conductance. The maximal cell size was reached within 5-10 minutes. The depolarisation as well as the membrane conductance adopted their maximal values within the same time range. After 20-25 minutes the cells recovered their original cell size. This regulatory volume decrease (RVD) could also be observed in Ca^2+-free hypotonic bath solutions. Under these conditions the time course of RVD as well as the change of membrane conductance was slowed down. Addition of 1mM quinine to the hypotonic bath slowed down the time course of depolarisation and the change of membrane conductance. A delayed RVD could be observed. After 40-50 minutes the cells adopted their original size. Addition of 0.1 mM DIDS to the hypotonic bath slowed down the time course of depolarisation and the change of membrane conductance. The relative change of membrane potential and of conductance was significantly reduced compared to control conditions. RVD could be suppressed by DIDS. The data indicate, that a quinine-sensitive as well as a quinine-insensitive potassium current and a DIDS blockable anion current are involved in RVD of OK-cells.

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KINETIC CHARACTERIZATION OF ENDOGENOUS CELL-TO-CELL CHANNELS BETWEEN PAIRED XENopus LAEVIS OOCYTES

J. Strötchen and G. Boheim

Oocytes from Xenopus laevis were frogs were paired after removal of follicle cell and vitelline layers. The dual voltage-clamp technique was used to measure the junctional cell-to-cell conductances. For reference both cells were clamped to the same holding potential. Stepwise changes of the holding potential of the outer cell led to the appearance of an additional current \( i_j \), which corresponds to ion flow between the cells via gap junction channels in the area of membrane contact. The junctional conductance \( \lambda_j \) is defined as the ratio of \( i_j \) to the transjunctional voltage (difference of holding potentials) \( V_{ij} \).

After pairing a steady increase of \( \lambda_j \) was observed with a time delay of 4-5 h. 7h after pairing 11 cell pairs out of 53 had maximum conductances of \( \lambda_j = 0.4-2.0 \mu S \), whereas in the other cell pairs the conductance did not exceed a value of \( \lambda_j = 0.05 \mu S \). Steady state conductances were symmetrical with respect to both polarities of transjunctional voltage \( V_{ij} \). Maximum \( \lambda_j \) was observed at \( V_{ij} = 0 \). Increasing \( V_{ij} \) lead to a decrease in \( \lambda_j \), whereas a constant residual conductance of 15-20 \% of \( \lambda_j \) remained at high \( V_{ij} \). The time course of cell-to-cell channel inactivation could be described by the sum of two exponential functions. The amplitude of the slow current inactivation exhibited a nonlinear voltage dependence. These properties were independent of the value of the reference holding potential and depended only on \( V_{ij} \).

The complex relaxation amplitude pattern is interpreted in terms of two steps: At low \( V_{ij} \) (20-30 mV) gap junction channels between paired frog oocytes are partially inactivated by a fast and a slow process, which are directly coupled. At higher \( V_{ij} \) (40-50 mV) a further slow inactivation of \( \lambda_j \) occurs.

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STABILISATION AND INDUCED REDUCTION OF INTERCELLULAR COMMUNICATION IN ISOLATED PAIRS OF CULTURED MAMMALIAN CELLS

D. Paschle and D.F. Hilser

The alteration of electrical coupling via gap junctions by cAMP, ATP, Ca^2+, retinoic acid and by antibodies against liver 26 kD and 21 kD gap junction proteins (connexins - Cx32 and Cx30) was measured with the double whole cell patch clamp method in isolated pairs of BRL (Buffalo Rat Liver) and FL (human amnioa) cells. Junctional conductance was monitored throughout the experiment by applying a square pulse of 10 mV at 0.02 Hz to one cell and measuring the resulting current in the other cell. From these records the time course of gap junctional uncoupling was determined during a time span of up to 90 min. Under normal experimental conditions, the cells showed spontaneous uncoupling, where the junctional conductance decreased to about 10% of its initial value after 22 ± 4 min in BRL and after 39 ± 12 min in FL cell pairs.

Filling the patch pipette with buffered saline containing 1 mM db-cAMP and 5 mM ATP results in a stable conductance of about 30% of the initial value after 60 minutes. Addition of 10 mM Ca^2+ to the pipette solution and 0.5 mM retinoic acid in the bath medium blocks electrical coupling completely within less than 5 minutes. With anti-Cx32-antibody in a stabilising pipette solution we achieved 10% coupling in 23 ± 9 min in BRL cell pairs (p<0.1) while with anti-Cx26-antibody a 10% value was reached after 30 min in two of seven experiments. The results with FL cells point in the same direction.

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In rat hepatocytes grown on gas-permeable membranes (C. Petzinger, et al., In Vitro Cell. Dev. Biol. 24: 491-499, 1988) we measured cellular and canalicular potentials and input resistances with double-barelelled electrodes. In bicarbonate-containing solutions we found -30.7±1.2 mV (mean ± SEM, n = 37) and -13.9±1.4 mV (n = 22) for cell and canalicular membrane potentials, respectively. Canalicular input resistance was 31.1±4.7 MΩ (n = 22). There was no dependence of these parameters on culture age. Cellar input resistances, however, continuously decreased from 32.3±3.4 MΩ at 2 hours (n = 11) to 8.2±2.1 MΩ after 3 days of preparation (n = 6). In ion substitution experiments there were no changes in membrane conductances to K⁺, Na⁺, and Cl⁻ that could account for this effect. Cable analysis, however, revealed that the apparent increase in membrane conductance reflects a time-dependent increase in electrical coupling between cells. This coupling was in part sensitive to heptanol (2 mM) but insensitive to octanol (0.5 mM) and 8-Br-cAMP (1 mM). We conclude that for a quantitative analysis of transport mechanisms in cultured cells changes in the degree of cell-cell interaction have to be considered.

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Properties of single gap junction channels were investigated in isolated pairs of BRL (Buffalo Rat Liver), FL (human amnion) and PLC (human hepatoma) cells with the double whole cell patch clamp technique. In BRL and FL cell pairs conductance steps of single channels were observed after spontaneously occurring reductions of gap junctional conductance from more than 20 Ω to about 500 Ω. PLC cells usually show less initial conductance changes, single channel events, therefore may be recorded immediately after establishing the double whole cell configuration.

From these records we conclude that the conductance steps during opening or closing of single gap junctional channels are between 25 - 35 pS and 45 - 60 pS in PLC and 45 - 55 pS and 75 - 90 pS for BRL and FL cells, respectively. Larger conductance steps have also been recorded, yet with a very low frequency.

Furthermore, we investigated the influence of retinoic acid and of dB-cAMP, both modulators of cell-cell communication, on the properties of single gap junction channels.

Our Experiments indicate that at the single channel level neither retinoic acid nor cAMP significantly influence the conductivity of gap junctions. Preliminary results lead us assume that the decrease of cell-cell coupling by retinoic acid may be due to an alteration in the channel kinetics. We cannot exclude, however, the possibility of different unit conductances and varying sensitivities to agents such as retinoic acid, cAMP, Ca²⁺ or transjunctional voltage.

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Voltage clamp instruments are closed loop control systems using electronic feedback. Modern control theory provides a large variety of solutions for the design and optimal tuning of feedback systems. Control systems which are composed only of delay elements can be optimized easily by adequate shaping of the "frequency characteristic magnitude". Using controllers with a proportional-integral characteristic (PI-controllers) it is possible to force the modulus of the frequency characteristic |F(ω)|, associated with the transfer function F(s) (output to input ratio in the frequency domain) to be as close as possible to one over a wide frequency range ("Modulus Hugging"). This means that the controlled variable very rapidly reaches the value required by the command variable. The method provides:

1. Control with steady state errors below 0.5%.
2. System stability (no ringing, predictable overshoot).
3. Easy calculation of system parameters and performance (settling time,
   overshoot, etc.) from the time constants of the control chain according to
   standard optimization rules ("absolute value optimum" and "symmetrical
   optimum")
4. System design using standard graphs and tables.
5. Easy tuning of the controller settings with step commands.

The "absolute value optimum" (AVO) provides the fastest response to a command step with very little overshoot while the "symmetrical optimum" (SO) has the best performance characteristics. We have adopted this method for the design and optimal tuning of voltage clamp systems, since their various components (microelectrodes, buffer amplifiers, differential amplifiers etc.) can be described as delay elements (first or second order delays). These systems have time constants in the range of microseconds. In the case of a time-sharing single electrode clamp system, an additional dead-time element caused by the sample and hold amplifiers was considered, which also was approximated by a first order delay with a time constant related to the reciprocal of the switching frequency. These "small" time constants were added to an equivalent time constant Tₚ (100 μs). The cell capacity was considered as an integrating element with a time constant Tᵢ which is always one order of magnitude greater than Tₚ.

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ELECTRICAL PROPERTIES OF RAT HEPATOCYTES IN PRIMARY CULTURE
F. Wehner, D. Guth and R.K.H. Kinne

EFFECTS OF NYSTATIN AND QUINIDINE IN STUDIES ON AMMONIUM ION TRANSPORT ACROSS RUMEN EPITHELIUM.
D. Baakker, Susanne Hoppe and H. Höller.

Previous studies in nystatin action on biological and artificial membranes indicated that this polyene antibiotic produces small aqueous channels which permit penetration of small monovalent ions. It has been shown that in different epithelia after treatment of the mucosal barrier with such pore-forming antibiotics the remaining electrophysiological characteristics reside mostly in the basolateral membrane.

To study electrophysiological characteristics and the effects of K⁺ and NH₄ on the basolateral membrane of sheep rumen epithelium we applied 400 UI nystatin on the mucosal side in Ussing-type chambers. At this concentration nystatin caused a distinct increase of short circuit current (Iₛₛ) after application which reached after 60 min a stable plateau of 6 μeq·cm⁻²·h⁻¹ above that of untreated controls. The elevated Iₛₛ returned to control levels when ouabain was added to the serosal solution, giving evidence that an electrogenic activity of the Na⁺/K⁺-ATPase at the basolateral membrane was responsible for this increase.

In nystatin treated epithelia addition of K⁺ or NH₄ resulted in an increase of Iₛₛ which was more than threefold higher compared to untreated epithelia. The Iₛₛ increase caused by K⁺ was more than 3 μeq·cm⁻²·h⁻¹, and Iₛₛ stayed constant at this level over 20 min, while an NH₄ induced increase of Iₛₛ was transient with an initial step of 1 μeq·cm⁻²·h⁻¹. All currents could be blocked rapidly and completely by addition of quinidine (1 mmol/l).

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GTP-INDUCED FUSION OF MEMBRANE VESICLES FROM RAT PANCREATIC ENDOPLASMIC RETICULUM, MEASURED WITH FLUORESCENCE DYES AND LIGHT SCATTERING METHODS

W. Hamppe, H. Ruf and J. Schulz

Evidence suggests that GTP controls the Ca\(^{2+}\)-conveyance between intracellular Ca\(^{2+}\) pools. The non-hydrolysable GTP-analogue GTP\(\gamma\)S does not show these effects (Ghosh et al., Nature 340:236, 1989). Insolitin-1,4,5-triphosphate releases Ca\(^{2+}\) from a pool, which is filled with Ca\(^{2+}\) via a Ca\(^{2+}\)/H\(^+\)-countertransporter at the expense of an H\(^+\) gradient, that is established by an H\(^+\)-pump using ATP but not GTP as substrate (Thévenod et al., J Membrane Biol 107:2263-2275, 1989).

We have investigated if GTP induces fusion of vesicles from rat pancreatic endoplasmic reticulum (ER) using light scattering (90\(^\circ\), 640 nm), fluorescence dequenching, and energy transfer methods. Addition of GTP (10\(^{-5}\) mol/l) to ER-vesicles in the presence of the membrane fusogen polyethylene glycol (PEG, 3% w/v) decreased fluorescence light scattering by 10% and increased fluorescence as measured with the fluorescent membrane probes octadecylrhodamin (IR8) and S-(N-octadecanoyl)-aminofluorescin (IF8) by 20% \pm 2.5% S.E. Previous addition of ATP (5 mM) increased the GTP-effect to 38.5% \pm 3.9% S.E.

GTP\(\gamma\)S had no effect on its own and inhibited the GTP induced signals. The protonophore carbonyl-cyanide-m-chlorophenylhydrazone (CCCP 10\(^{-5}\) mol/l) decreased the GTP induced fluorescence increase from 38.5% to 19%, whereas the H\(^+\)-ATPase inhibitors 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-CI 10\(^{-5}\) mol/l) and N-ethylmaleimid (NEM 10\(^{-4}\) mol/l) did not effect the size of the GTP induced signal.

The data indicate that GTP induces fusion of ER-vesicles in which a proton gradient might be involved.

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GTP-INDUCED \(\text{Ca}^{2+}\) MOVEMENTS IN INSOSITOL 1,4,5-TRISPHOSPHATE-SENSITIVE AND -INSENSITIVE INTRACELLULAR \(\text{Ca}^{2+}\) POOLS FROM RAT PANCREATIC ACINAR CELLS

M. Dehlinger-Kremer, T. Ozawa and J. Schulz

In previous studies we have shown the existence of at least two nonmitochondrial intracellular Ca\(^{2+}\) pools: an insolitin 1,4,5-triphosphate (IP\(_3\))-sensitive Ca\(^{2+}\) pool (IsCaP) which takes up Ca\(^{2+}\) via a Ca\(^{2+}\)/H\(^+\) countertransporter at the expense of an H\(^+\) gradient established by an H\(^+\) pump; and an IP\(_3\)-insensitive Ca\(^{2+}\) pool (IisCaP) which takes up Ca\(^{2+}\) via a vanadate-inhibitable Ca\(^{2+}\) ATPase (F. Thévenod et al., J Membrane Biol 107:173, 1989). GTP is proposed to control Ca\(^{2+}\) conveyance between intracellular Ca\(^{2+}\) pools by forming Ca\(^{2+}\)-carrying junctions between membranes (Ghosh et al., Nature 340:236, 1989). We have investigated the mechanism for GTP-induced Ca\(^{2+}\) uptake and Ca\(^{2+}\) release in a fraction from isolated endoplasmic reticulum of rat pancreatic acinar cells, using \(\text{Ca}^{2+}\) and \(\text{Ca}^{2+}\) macroelectrode.

In the presence of oxalate (10\(^{-5}\) mol/l) and the membrane fusogene polyethylene glycol (PEG 3% GTP 3%) Ca\(^{2+}\) (10\(^{-5}\) mol/l), but not GTP\(\gamma\)S, induces overshooting Ca\(^{2+}\) uptake followed by Ca\(^{2+}\) release. This Ca\(^{2+}\) release is higher in the presence of vanadate (10\(^{-4}\) mol/l) indicating that Ca\(^{2+}\) uptake is dependent on a vanadate-inhibitable Ca\(^{2+}\) pump that compensates in part for Ca\(^{2+}\) release. In the presence of the IP\(_3\) analogue insolitin 1,4,5-triphosphorothioate (IPS\(_3\), 3x10\(^{-5}\) mol/l) or of the Ca\(^{2+}\) release agent caffeine (2x10\(^{-5}\) mol/l) GTP-induced overshooting Ca\(^{2+}\) is abolished or decreased, respectively. With GTP\(\gamma\)S the caffeine effect was very small.

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DIFFERENT MECHANISMS OF INHIBITION OF SODIUM PUMP IN XENOPUS LAEVIS OCYOTES BY DIACYLGlycerol ANALOGUES AND PHORBOL ESTER I. Veselska*, G. Schmalzing, H. Häde/fessel, H. Die1 & W. Schwartz

The effects of activation of protein kinase C by phorbol 12-myristate 13-acetate (PMA), 1,2-dioctanoyl-sn-glycerol (dioG) and 1-oleoyl-2-acetyl-sn-glycerol (OAG) on sodium pump current, membrane capacitance, ouabain binding and \(\text{Na}^{+}\)/\(\text{K}^{+}\) uptake in Na\(^+\)-loaded Xenopus oocytes were studied. \(\text{Na}^{+}/\text{K}^{+}\) pump current was monitored as differences in steady-state membrane current, produced by changes from 3 \(\mu\)M K-containing to K-free solution in the presence of K-channel blockers. Protein kinase C activators caused a gradual decline in sodium pump current. PMA (50 \(\mu\)M) was the most potent inhibitor, producing a 80% reduction of pump current. A comparable degree of pump inhibition by diacylglycerols required a 1000-fold higher molar concentration. All three compounds decreased the number of ouabain binding sites on the cell surface in proportion to current inhibition. Staurosporine, an inhibitor of protein kinase C, abolished this effect, whereas H\(_7\) was ineffective.

Reduction of pump current and ouabain binding sites by PMA and OAG were accompanied by a reduction of total membrane surface, estimated from measurement of membrane capacitance. The PMA-induced increase of \(\text{Na}^{+}/\text{K}^{+}\) uptake suggests that the inhibition of pump current was brought about by energy uptake. In contrast to PMA and OAG, triOS did not stimulate endocytosis, as judged from the lack of effect on membrane capacitance and ouabain uptake. Independent of the type of kinase C activator, ouabain binding sites lost from the cell surface after PMA treatment were not re-recovered after 30 min. The number of cellular membranes with digitonin plus 0.02% SDS was increased.

The findings suggest that the inhibitory effect of protein kinase C activators on sodium pump current may involve a selective internalization or direct inhibition of surface sodium pumps in addition to a non-specific removal of pump molecules by endocytosis.

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DEPENDELTY OF Na/K/CIT-COTRANSPORT ON VOLUME AND INTRA-
CELLULAR MAGNESIUM OF HUMAN RED CELLS
Il. Mairhainer and J. F. Hoffmann

The purpose of this study was to analyze the relation of Na/K/Cit-cotransport (cotransport) with steady-state cell volume (MCV). We measured cotransport in unfractionated and density separated red cells (DSC) of different MCV’s from different donors to see if cotransport differences contribute to differences in the distribution of MCV’s in the red cell populations. Also the effects of different cellular Mg concentrations and of acutely altered cell volume (using osmotic and isosmotic techniques) were examined. Cotransport was determined as the bumetanide (10 μM) sensitive 22Na-efflux in the presence of ouabain (50 μM) after adjusting cellular Na (Na0) and K0 by the nystatin technique to achieve Vm0 for cotransport.

We found a significant inverse correlation between MCV and cotransport in both unfractionated red cells and DSC at Vm0 of cotransport. MCV was correlated directly with red cell 2,3-DPG, whereas total red cell Mg was very similar in various cell fractions indicating that intracellular free Mg (Mgi) might be lower in red cells with high 2,3-DPG (i.e. high MCV) and vice versa. Altering Mg0 with the ionophore A23187 showed a high Mg-sensitivity of cotransport. Depletion of Mg0 inhibited and an elevation of Mg0 activated Na/K/Cit-cotransport. The K0 for Mg0 corresponds to a medium Mg concentration of about 0.06 μmol/l, set in the presence of A23187. Thus it appears likely that differences in Mg0 among populations of red cells contribute to differences in cotransport activity and MCV.

Measurements of cotransport following acute changes of cell volume showed that cell shrinkage activates, whereas swelling inhibits cotransport. In DSC this response to immediate volume changes was most pronounced in the fraction of cells with the lowest density. When cell volume was changed osmotically, cotransport was higher in the shrunken cells independent of Na0 (5 to 80 mmol/l, reciprocally altered with K0). The effect of volume persisted, however, when cell volume was altered isosmotically after appropriate adjustment of Na0 and K0 by the nystatin technique. Further, the response of cotransport to acute volume changes appears to occur independently of Mg0 since neither decreasing nor increasing Mg0 alters the volume response. Thus the volume sensitivity of cotransport is dependent on changes in the electrochemical driving force, in Mg0, and on changes in cell volume per se.

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EVIDENCE FOR ELECTRONEUTRAL L-ARGININE TRANSPORT IN CULTURED KIDNEY TUBULE CELLS (OK)
J. S. Schwegler1, E. Schömig2, A. Heuner1, S. Silbernagl1

Recently, we described an electrogenic transport of L-arginine into the established opossum kidney OK cell line [Plüger's Arch 415:543-560]. In the present study, however, radiotracer uptake experiments show that the major component of saturable 3H-L-arginine uptake in OK cells is independent of the membrane potential.

3H-L-arginine [10 μmol/l] uptake was determined on confluent OK cell monolayers which were grown on plastic dishes (0.6 cm2) for 7 days. Uptake was saturable and linear with time for at least 60 s. Initial rates of uptake were measured after an incubation period of 20 s. Intracellular breakdown of arginine is not detectable after that incubation period. Cell membrane depolarization by the addition of 3 mmol/l BaCl2 fails to reduce 3H-L-arginine uptake. The complete substitution of extracellular sodium by choline as well as of extracellular chloride by gluconate reduce 3H-L-arginine uptake to 85 ± 3 % and 90 ± 1 % (n = 3) of control, respectively. Extracellular acidification [pH 6.9] and alkalinization [pH 7.9] does not alter 3H-L-arginine uptake significantly [n = 3]. The replacement of extracellular sodium by potassium inhibits 3H-L-arginine uptake in a concentration dependent manner. However, only 48 ± 1 % (n = 3) of total 3H-L-arginine uptake are blocked by the complete substitution of sodium by potassium.

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INCREASED Na+/H+-ANTIPORT ACTIVITY IN LYMPHOCYTES OF PATIENTS WITH ESSENTIAL HYPERTENSION.
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Increased activity of the Na+/H+-antiport may be a major abnormality in essential hypertension. We investigated the activity of this transport system in lymphocytes loaded with the fluorescent dye BCECF from 9 patients with essential hypertension [EH; 162 ± 25 (SD)/104 ± 11 mm Hg] and 8 normotensive control subjects (C; 116 ± 13/74 ± 10 mm Hg). Cells were acidified by addition of different amounts of Na+-propionate (10–20 μM). The undissociated acid permeates the cell membrane, thereby decreasing intracellular pH to different values below baseline. pH slowly recovered to its initial value, a response that was not seen in the presence of ethylisopropylamiloride indicating involvement of Na+/H+-exchange. pH-recovery (ΔpH/min) was plotted against initial pH-values (after acidification) and a linear regression analysis yielded different slopes in C (0.21 ± 0.07) and EH (0.70 ± 0.49; p<0.001). In contrast, baseline pH-values were identical in both groups (C: 7.02 ± 0.08; EH: 7.02 ± 0.06). Faster pH-recovery rates in EH versus C suggest either an increased pH-sensitivity of the Na+/H+-exchanger or an augmented amount of Na+/H+-exchangers in lymphocytes of EH. The observation that baseline pH was identical in C and EH rules out the possibility that the enhanced activity in EH is caused by "pre-stimulation", e.g. by circulating agents.

INCREASED Na+/H+-ANTIPORT ACTIVITY IN LYMPHOCYTES OF PATIENTS WITH ESSENTIAL HYPERTENSION. B. O. Siffert, D. Rosskopf and U. Osswald

It was recently reported that platelets of essential hypertensives (EHT) show an increased activity of Na+/H+ exchange. The present study aimed at investigating i) whether this increased activity would result in an increased cytosolic pH (pHi) and ii) whether the increased activity is an epiphenomenon rather than a causative factor in EHT. Platelets were loaded with the fluorescent pH dye BCECF and acidified by addition of propionic acid. The recovery of pHi was recorded and the initial slopes of the fluorescence tracings were used to estimate the activity of Na+/H+ exchange. The pHi of platelets in normotension (NT; 7.14 ± 0.04, n=10) did not differ from that in EHT (7.16 ± 0.04; n=8). In contrast, the initial rate of pHi recovery from an artificial acid load was three times faster in EHT as compared with NT. Platelets from patients with renal artery stenosis had both a normal pHi and Na+/H+ exchange activity. We conclude that: i) overexpression of Na+/H+ exchange activity in EHT does not occur as a result of elevated blood pressure, since this phenomenon is absent in renal artery stenosis. ii) overexpression of Na+/H+ exchange activity does not result in an increased pHi. iii) The observation that baseline-pHi was identical in NT and EHT makes it unlikely that the enhanced activity in EHT is caused by "pre-stimulation", e.g. by circulating agonists.

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EXPRESSON OF PROBENECID INHIBITABLE P-AMINO-hippurate transport in Xenopus uocyes
J.Steffgen, W.Schwarz and H.Koepsell

Until now information about the sequence and the molecular structure of the probenecid sensitive p-aminohippurate (PAH) exchanger is missing. To test whether expression cloning employing oocytes of Xenopus laevis can be used to clone the PAH transporter, we injected mRNA from kidney (rat and pig) and intestine (rat) into the oocytes. Total RNA was obtained by homogenization of tissue and later phenol extraction and poly(A) mRNA was separated by oligo (dT) cellulose chromatography. About 50 ng of this mRNA were injected per oocyte (stage V or VI). After 2 or 3 days of incubation at 18°C, uptake of [3H]-PAH into single oocytes was measured in oocyte Ringer's solution in the presence or absence of 5 mM probenecid. Already non-injected oocytes showed a PAH uptake from which about 37 % could be blocked by probenecid. The absolute amount of this uptake varied between different animals. No significant increase of PAH uptake could be detected after injection of mRNA from rat intestine. However injection of mRNA from rat (pig) kidney led to a 2.2 (3) fold increase of PAH uptake in comparison to non or water-injected oocytes which were obtained from the same animal. About 90 % (66 %) of the PAH transport which was increased by mRNA from rat (pig) kidney was inhibited by 5 mM probenecid. Preincubation of the oocytes at about 30°C for 3 hours before influx measurements led to a further increase of PAH uptake in the oocytes injected with mRNA from rat kidney by a factor of about 2.5. Also the endogenous PAH transport was stimulated but only about 1.3 fold. The results suggest that probenecid inhibitable PAH transport can be expressed after injection of mRNA from rat and pig kidney.

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EVIDENCE FOR A DIVALENT CATION SENSITIVE SHORT CIRCUIT CURRENT ACROSS THE ISOLATED RUMEN OF SHEEP
N. Martens, M. Rübbelke and G. Gabel

The isolated rumen epithelium exhibits in Ussing-chamber studies a short circuit current, Isc, of 0.5-1.5 μeq·cm⁻²·h⁻¹. Omitting Mg and Ca on the mucosal side caused a significant increase of the Isc from 1.2±0.2 to 3.8±0.5 μeq·cm⁻²·h⁻¹. This enhanced Isc was significantly reduced by subsequent mucosal addition of MgCl₂ or CaCl₂ (2mM), but not by Mg-EDTA or Ca-EDTA. Further studies showed that other divalent ions (Ba, Sr) reduced the Isc to the same extent. The Ca channel blocker verapamil (0.1 mM) decreased the Isc in the absence of Ca and Mg. The reduction of Isc by divalent ions or verapamil was reversible.

Amiloride (0.1 mM), which inhibits electrogenic Na transport in different tissues, did not change the Isc under these experimental conditions. Ouabain (0.1 mM) in the serosal solution abolished the Isc within 30 - 40 min. Replacement of Na by choline in the mucosal solution reduced or even caused a negative Isc, which was not changed by mucosal addition of Ca.

This divalent cation sensitive Isc of the rumen epithelium shows similarities with a divalent cation sensitive pathway in amphibian epithelia (W. van Driessche et al., Comp. Biochem. Biophys. 90A, 693, 1988). The role of this transport mechanism remains to be elucidated because under physiological conditions the concentrations of Ca and Mg in the ruminal fluid are high enough to block this pathway.

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EXCITATORY AMINO ACID-INDUCED CHANGES IN EXTRACELLULAR CALCIUM CONCENTRATION IN THE RAT HIPPOCAMPUS INDICATE DIFFERENT ACTIVATION OF THE Na⁺/Ca²⁺ EXCHANGER
J. Arrus, J. Stabel and U. Heinemann

All excitatory amino acids produce dose-dependent decreases in [Ca²⁺]₀ and [Na⁺]₀. However the specific agonists quisqualate, kainate and AMPA induce in addition to the initial decrease a subsequent increase of [Ca²⁺]₀ above baseline which outlasts the application time. NMDA, DL-homospecific acid and the mixed agonists glutamate and aspartate do not produce an overshooting increase of [Ca²⁺]₀ response. The direction of Ca²⁺ flow across the cell membrane and therefore also the activation of the Na⁺/Ca²⁺ exchanger is determined by the electrochemical Na⁺- and Ca²⁺-gradients and the transmembrane potential. To test the hypothesis that activation of the Na⁺/Ca²⁺ exchange mechanism is involved in the production of the Ca²⁺-overshoots we studied the effects of low [Na⁺]₀ low [K⁺]₀. Oubain (5 μM) and lithium (5 mM) on quisqualato-induced [Ca²⁺]₀ changes. All these treatments enhanced the [Ca²⁺]₀ decreases and reduced the overshooting responses in a reversible manner. In some cases lithium could totally block the [Ca²⁺]₀-overshoots which may be explained by a direct blocking effect of lithium on the action of the Na⁺/Ca²⁺ exchanger. To clarify further the basic mechanisms which are involved in the generation of quisqualate-induced [Ca²⁺]₀-overshoots we tested the effect of dantrolene, ryanodine and caffeine; drugs which may influence the release of intracellular calcium. Dantrolene (20 μM) and ryanodine (20 μM) had no effect, whereas caffeine (5 mM) induced the [Ca²⁺]₀ decrease but did not affect the overshoot. The reduction of [Ca²⁺]₀ decreases by caffeine may be explained by a Ca²⁺ dependent inactivation of Ca²⁺ currents due to an enhanced intracellular [Ca²⁺].

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A PHYSIOLOGICAL ROLE FOR AMINO ACIDS IN THE GLUCOSE TRANSPORT REGULATION OF CARDIAC MYOCYTES
Y. Fischer, J. Thomas, H. Rose & H. Kammermeier

Glucose is one of the major substrates for the energy metabolism of the myocardium. Work (or contraction) and insulin are known to markedly increase glucose transport (GT) across the sarcolemmal membrane of cardiomyocytes, which is first and probably the rate-limiting step of the glucose metabolism in these cells. Whether other physiological factors might have a similar effect is still unclear. As we previously reported, the insulin-like, GT stimulating activity found in the partially purified yeast extract can be, at least in part, ascribed to the monomeric amino acids alanine and valine {also in a physiological range of concentrations, i.e. 30-300 μM). On the other hand, we found, in a parallel study, that L-cysteine strongly stimulates (2-4 fold) GT in isolated cardio-myocytes; L-cysteine was effective at concentrations as low as 10-100 μM, which, again, corresponds to physiological values measured in human (and rat) plasma.

We now further purified these fractions by gel filtration chromatography and could separate at least two active components: one major activity was eluted at an apparent molecular weight of ~100 D (peak A) and another less efficient component at ~500-600 D. Using cardiomyocytes obtained by a modified isolation procedure) that respond very strongly to insulin (0-20 fold stimulation of GT, as compared to control cells), we found that fractions from peak A evoked a 1.5 fold stimulation of 2-deoxy-D-glucose (2-DG) uptake. An amino acid analysis showed that alanine is, by far, present at the highest concentration in this peak. When tested in the range of concentrations found in peak A, alanine stimulated 2-DG transport ~1.5 fold, with an EC₅₀ of ~150 μM, which corresponds to a physiological plasma concentration. Among the other amino acids detected in lower concentrations, arginine was first and probably the rate-limiting step of the glucose metabolism in these cells. Whether other physiological factors might have a similar effect is still unclear. As we previously reported, the insulin-like, GT stimulating activity found in the partially purified yeast extract can be, at least in part, ascribed to the monomeric amino acids alanine and valine {also in a physiological range of concentrations, i.e. 30-300 μM). On the other hand, we found, in a parallel study, that L-cysteine strongly stimulates (2-4 fold) GT in isolated cardio-myocytes; L-cysteine was effective at concentrations as low as 10-100 μM, which, again, corresponds to physiological values measured in human (and rat) plasma.

We conclude (i) that the insulin-like, GT stimulating activity found in the partially purified yeast extract can be, at least in part, ascribed to the monomeric amino acids alanine and valine {also in a physiological range of concentrations, i.e. 30-300 μM). On the other hand, we found, in a parallel study, that L-cysteine strongly stimulates (2-4 fold) GT in isolated cardio-myocytes; L-cysteine was effective at concentrations as low as 10-100 μM, which, again, corresponds to physiological values measured in human (and rat) plasma.

We conclude (i) that the insulin-like, GT stimulating activity found in the partially purified yeast extract can be, at least in part, ascribed to the monomeric amino acids alanine and valine (ii) that some amino acids might play a physiological role in the regulation of GT and, consequently, of glucose metabolism in cardiac myocytes. (supported by the DFG, RO/755 1-1) Inst. f. Physiologie, Med. Fak. "RWTH, Pau Helsinki. D-5100 Aachen
THIOL/DISULFIDE EXCHANGE HAS OFTEN BEEN PROPOSED TO BE INVOLVED IN THE REGULATION OF GLUCOSE METABOLISM. IN PARTICULAR, THIOLS/THIOLATES WERE REPORTED TO BE CRUCIAL FOR THE ACTIVITY OF GLUCONEOGENIC ENZYMES AND THE INSULIN EFFECTS ON THESE ENZYMES.

1. INTRACELLULAR CL- ACTIVITIES IN NEURONES AND GLIAL CELLS OF THE LEECH CENTRAL NERVOUS SYSTEM AFTER PARTIAL CL- SUBSTITUTION

We have measured the intracellular Cl- activity, ACI, in identified neurones and neuritelike glial cells of the central nervous system of the leech Hirudo medicinalis, using double-barreled Cl-sensitive microelectrodes (Corning Electronic Products, Medford, Mass., U.S.A.). The ACI changed considerably in Retzius neurones and neuritelike glial cells, when the conventional, high-CI- and hypertonic leech saline (219 mmol/l; 110 mm Cl- concentration, corresponding to 85 m M Cl- activity, using an activity coefficient of 0.77) was exchanged for an isotonic saline (186 mmol/l), in which the Cl-concentration was reduced to 40 mM (31 m M activity) and substituted by 40 mM D-malate. This low Cl- saline appears to match the leech blood closer, since organic anions constitute a large fraction of the anions present in leech blood (A. Wenning, J. Exp. Biol. 143:103, 1988). In Retzius neurones ACI decreased from 8.5 ± 1.2 m M (± S.D., n = 5) in the high-CI- saline to 4.0 ± 1.7 m M (n = 13) in the new low-CI- saline. The Cl-equilibrium potential Ecl changed from -59 m V to -53 m V, while the membrane potential Em changed from -43 ± 5 m V to -47 ± 7 m V. In neuritelike glial cells ACI was 6.3 ± 1.6 m M (n = 8) in the high-CI- saline and 5.0 ± 6.6 m V at a mean E of -68 ± 5 m V; the ACI was 2.0 ± 1.1 m M (n = 20) in low-CI- saline, giving an Em of -70 m V at a mean of 7.2 ± 8.7 m V. In contrast to neurones, Cl- appeared to be in equilibrium across the basal cell membrane as suggested also by Ballyn & Schlue, 1990 (J. Physiol., in press). In the presence of CO2/HCO3 buffer (pH 7.4, 5% CO2) the CI-microelectrode reading was 1-5 m V higher in all cells, presumably due to some interference of HCO3- to the electrode.

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2. PHOTOMETRIC DETERMINATION OF NA+ H EXCHANGE IN PLATELETS

The purpose of the present study was to establish a routine assay for the determination of Na+/H+ exchange activity in different pathological states. When cells are incubated in a medium consisting of (in mM) Na+ 140, glucose 5, KCI 5, MgCl2 1, CaCl2 1, pH 6.7, the undissociated propionic acid permeates the cell membrane, acidifies the cytosol and thus activates the Na+/H+ exchanger. The internal pH after acidification in this standard medium was 5.7 as estimated from the fluorescence dye BCECF. The continuous uptake of Na+ then causes osmotic cell swelling. Prefixed aliquots (37°C) of platelet-rich plasma (PRP; 70 μl) were added to this medium (430 μl). Using a photometer (λ = 680 nm) a rapid decrease in absorbance of such suspensions was observed. The time course of the change in absorbance corresponded to a first order reaction (time constant 22 x 10-3 sec). Specific blockers of the exchanger also inhibited the change in absorbance (K+, amiloride, 11μM; K+ ethylisopropylamiloride, 0.1 μM). The change in absorbance occurred on the extracellular Na+ concentration (Km = 70 mM). The time constant decreased with increasing Na-proton exchange concentration. Maximum swelling was observed at an external pH of 6.7. A further investigation revealed an increased activity of Na+/H+ exchange in essential hypertensives (time constant 10 x 10-3 sec), corresponding to already published data established by more sophisticated and time-consuming methods.

Hence, our method provides a suitable test for routine screening of numerous specimens and for further attempts to clarify the connection between essential hypertension and Na+/H+ exchange.

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3. ULEX EUROPAEUS AGGLUTININ I INDUCES A DIDS-SENSITIVE CA INFLUX PATHWAY IN HUMAN ERYTHROCYTES

Of eleven lectins tested, only one, Ulex europaeus agglutinin I (UEA1), stimulated Na+ influx into quin-2 loaded erythrocytes (by about twofold) in a dose-dependent manner at subagglutinating concentrations. Na+ and Rb uptake resistant to ouabain plus furosemide was not altered by UEA1. The potency of the ABH-blood group specific lectin UEA1, to stimulate Ca influx decreased with HA,H2 A,B, e.g., in the same order as the lectins capacity to agglutinate the erythrocytes. UEA1 is thought to be an α-L-fucose specific lectin. Indeed, in the presence of 5 mM fucose, the UEA1 induced component of Ca influx was abolished. Since it is known that most of the protein linked ABH-antigens are attached to bands 3 and 4.5, the effect of inhibitors of the anion exchanger as well as of the glucose and nucleoside transporter was studied. Cytochalasin B and nitrobenzylthioinosine left the Ca influx induced by UEA1 unaffected, while DIDS caused a dose-dependent inhibition (complete inhibition at 10 μM, Kd at about 2 μM for A1 as well as A2 and H erythrocytes). In addition, also other inhibitors of the anion exchanger such as DNDS (10 μM) and diprydamide (20 μM) completely blocked the component of Ca influx elicited by the lectin. The Ca entry blockers verapamil (100 μM) and nifedipine (10 μM) did not affect the lectin induced Ca influx.

In conclusion, most probably by binding to ABH-blood group antigens, UEA1 induces a Ca influx pathway in human erythrocytes which is blocked by inhibitors of the anion exchanger.

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PLATELETS
The regulation of intracellular and extracellular pH in the leech central nervous system occurs via ion transport systems across cell membranes, such as Na⁺-H⁺ exchange, Cl⁻-HCO₃⁻ exchange and/or Na⁺-HCO₃⁻ cotransport. The latter has been shown to operate in glial cells, but not in neurons (Deitmer & Schlue, J. Physiol. 411:179, 1989). I have measured the pH and membrane potential in neuropile glial cells, and the pH in extracellular spaces of the leech c.n.s., using double-barreled pH-sensitive microelectrodes. Intra- and extracellular pH transients were evoked by addition and removal of weak acids or bases to estimate the apparent buffering power in cells and of the extracellular spaces. The experiments show that the CO₂/HCO₃⁻ and Na⁺-HCO₃⁻ dependence increase in the intracellular buffering power of glial cells is reversed by DIDS (4,4-dihisothiocyanostilbene-2,2'-disulfonic acid, 0.3-0.5 mM), which inhibits Na⁺-HCO₃⁻ cotransport. The buffering power of the extracellular spaces, which increased in CO₂/HCO₃⁻-buffered saline, decreased again after the addition of DIDS. This suggests that the presence of CO₂/HCO₃⁻ and Na⁺-HCO₃⁻ cotransport across the glial membrane, augments intra- and extracellular buffering power. In conclusion, the effects of CO₂/HCO₃⁻ and DIDS on pH regulation in neurons are relatively small. The results provide first evidence for the hypothesis that glial cells are directly involved in the regulation of H⁺ homeostasis in the nervous system.

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Measurement of intracellular free magnesium concentration in skeletal muscle fibers

D. Günzel and S. Galler

Numerous investigations exist on intracellular activities of different anorgamic ions but little is known about intracellular free magnesium concentration ([Mg⁺⁺]ᵢ) and its regulation. Since Mg⁰⁺ can compete with Ca⁰⁺ at protein binding sites, [Mg⁺⁺]ᵢ might have physiologically important functions in intracellular regulation processes. In fact, determination of [Mg⁺⁺]ᵢ was hardly possible because the selectivity of Mg⁺⁺ sensors was not sufficient to measure [Mg⁺⁺]ᵢ reliably in the intracellular medium with high potassium ion activity. The Mg⁺⁺ sensor based on the lanthone 1H TMB-8 (2. Wu et al. Anal Chem 61:874, 1989) is less sensitive to K⁺ interference. We measured this sensor, using micro glass pipettes with a tip diameter of about 0.8 μm. Mg⁺⁺ microelectrodes were calibrated in MgCl₂ solutions with ion-free background similar to that of the intracellular medium.

In fibers of sartorius muscle of the frog Xenopus laevis, [Mg⁺⁺]ᵢ was determined to be 1.81 ± 0.24 mM (S.E.) (n=21) in a Ringer solution containing 0.5 mM MgCl₂. As passive distribution of Mg⁺⁺ across the cell membrane with an electrical potential of -76.8 ± 2.6 mV (S.E.) (n=21) would lead to intracellular Mg⁺⁺ concentrations in the range of 0.3 M, active extrusion of Mg⁺⁺ from the muscle cell has to be possible. When sartorius muscles were incubated for up to ten hours in Ringer solutions ([MgCl₂]ᵢ = 0.5 mM) in which all Na⁺ was replaced by N-methyl-D-glucamine⁺ (NGM⁺), [Mg⁺⁺]ᵢ increased to 2.62 ± 0.34 mM (n=15). After readdition of Na⁺, [Mg⁺⁺]ᵢ decreased to 1.72 ± 0.31 mM (n=15). In Na⁺-free Ringer solutions containing 5 mM MgCl₂, [Mg⁺⁺]ᵢ increased to 3.52 ± 0.35 mM (n=15). In this case, readdition of Na⁺ lead to [Mg⁺⁺]ᵢ of 2.92 ± 0.42 mM (n=15). It can be concluded that a Na⁺-dependent Mg⁺⁺ transport system may partially be responsible for the regulation of intracellular [Mg⁺⁺].

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SODIUM-DEPENDENT AND SODIUM-INDEPENDENT SULFATE TRANSPORTERS FROM RAT INTESTINE ARE EXPRESSED BY mRNA'S OF DIFFERENT SIZE

J. Steffen, W. Schwarz and H. Koeppe

The oocytes of Xenopus laevis have been used as an expression system for various proteins. We have measured sodium-dependent and sodium-independent (DIDS-sensitive) sulfate uptake in non- or water injected oocytes and in oocytes injected with mRNA from rat intestine. About 50 ng of total mRNA were injected per oocyte (stage V or VI) and 2 or 3 days later uptake of 35SO₄²⁻ was measured in oocyte Ringer's solution. For determination of the sodium-dependent component, sodium was replaced by tetramethylammonium. For DIDS-sensitive transport, the fraction of sulfate uptake measured in the presence or absence of sodium which could be inhibited by 50 μM DIDS was determined. In non-injected oocytes about 35 % of sulfate uptake was sodium-dependent, about the same amount of uptake was inhibited by DIDS. Since the absolut amount of sulfate uptake varies between different animals, expression experiments were always performed in comparison to oocytes from the same animal. After injection of mRNA from rat intestine total sulfate uptake increased about 2.8 fold in comparison to non- or water-injected oocytes. About half of the increased sulfate uptake in the injected oocytes was sodium dependent and nearly half was DIDS-sensitive. We size-fractionated mRNA on 1 % agarose gel and electroeluted the mRNA. After injection of 10 ng/oocyte of one fraction (1.5-2 kb) the sodium-dependent sulfate uptake increased by about 18 fold. Injection of mRNA of higher molecular weight (3.7 kb) yielded a 8 fold increase of sulfate uptake which was not sodium-dependent but 45 % of this transport could be inhibited by DIDS. The data show, that sodium-dependent and sodium-independent sulfate transport can be expressed by mRNA fractions of different size.

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ALDOSTERONE REGULATES SODIUM TRANSPORT ACROSS ALVEOLAR EPITHELIIUM

H. Fischer and W. Claus

Intact lung tissues of the frog Xenopus laevis were investigated in Ussing chambers under voltage clamped conditions. Nonstimulated tissues exhibited a distinct Na⁺ current from the alveolar to the pleural side which was blockable by low doses of amiloride. The mineralocorticoid aldosterone (1 μmol/l) increased the amiloride-blockable transepithelial Na⁺ transport within 4 to 5 hours from 7.7 ± 0.7 μA/cm² to 17.9 ± 1.7 μA/cm² (means ±SE, n=7 and 15). Simultaneously the transepithelial resistance decreased from 754 ± 41 Ω·cm² to 646 ± 38 Ω·cm² significantly. This stimulatory effect of aldosterone on the transepithelial Na⁺ current was totally inhibited when the aldosterone-incubation was carried out together with the antimineralocorticoid spironolactone.

Transepithelial measurements were supplemented by analysis of the fluctuations in the short circuit current. In the presence of amiloride in the alveolar compartment we revealed a Lorentzian noise component in the power density spectra. This enabled us to calculate microscopic kinetic and channel characteristics. The single Na⁺ channel current (Iₙa=0.5 pA) and the blocker kinetics were unaffected by aldosterone-stimulation. The number of apical Na⁺ channels, however, increased from 0.85 ± 0.04 μm⁻² to 2.68 ± 0.054 μm⁻² in parallel to the transepithelial Na⁺ current. This shows that the lung epithelium is a physiological target tissue for aldosterone action.

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Chloride-related current fluctuation in frog skin

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The pathway for conductive transepithelial Cl- transport in amphibian skin, which can be activated by mucosal dCl potentials, has not unequivocally been identified. As possible routes, microvilli-rich (MR) cells or the tight junctions of the shunt have been proposed. To provide further information on the mode of passage, we have analyzed, on skins of Bufo viridis and Rana esculenta, the current fluctuation of transepithelial current related to this transport of Cl.

Lorenzini components in the power density spectrum (pds) were observed at serosa positive voltage perturbation, i.e. under conditions when Cl conductance (gCl) is activated. The corner frequency, which ranged between 44 and 100 Hz, was not related to the magnitude of the transepithelial clamp potential. The plateau value S1, in the order of 60-100 A/sec/cm², increased with the magnitude of ICl. Substitution of mucosal Cl by NO3 decreased ICl and led to disappearance of the Lorenzini components. Inhibitors of gCl (MX-196, DPC-analogs) had the same effect. After addition of gCl using theophylline or procaine, concommitant increases in ICl and S1 could be observed. The corner frequency was not systematically altered under any condition.

Assuming that the current fluctuation originates from channels with similar open and closed probability, we calculate individual channel currents in the order of 30 fA and channel densities between 20 and 300/m² of the 2/3 macroscopical area. If these channels are localized in the apical membrane of MR cells, the actual density must be much larger, since MR cells account for some 1-5% of the apical surface only. The magnitude of the individual channels current is much lower than reported for membrane channels. We propose that the fluctuation of gCl originates from specific transport sites in the tight junctions.

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LOCALIZATION OF ABSORPTIVE AND SECRETORY ION TRANSPORT IN RAT RECTAL COLUMN SURFACE CELLS AND CRYPTS BY VOLTAGE SCANNING

A. Köckerling, U. D. Schulze, E. Sorgenfrei and F. Schenker

Spatial distribution of conductive ion transport was investigated between surface cells and crypt openings. Totally stripped rat rectal column was mounted in a modified Ussing-chamber and clamped by an AC current of 30 Hz and ±50 µA/cm². The voltage drop between two positions (nominal distance zero from the epithelial surface and 45 µm apart) was sensed using a piezo-driven stepping electrode (Ling-Gerard type) which was positioned under microscopical control either above surface cells or above crypt openings. From these data and the morphometrically determined area contributions, the conductances per cm² gross tissue area of the two structures were calculated.

- Under control conditions the crypt openings, although covering only 5.5 ± 0.5% of the gross tissue area in the Ussing chamber, contribute 61 ± 7% to the total conductance.
- Theophylline (10-2 M) increased conductance of the crypt openings from 5.2 ± 0.5 to 8.8 ± 0.9 mS per cm² gross tissue area. Conductance of the surface cells was not significantly changed (n=14, paired t-test).
- After "acute" aldosterone (3-10-8 M, in vitro incubation for 6 h), amiloride (10-4 M) reduced surface cell conductance from 4.9 ± 0.7 to 2.2 ± 0.3 mS/cm². Conductance of the crypt openings was not significantly changed (n=12).
- After "chronic" aldosteronism (low Na+, high K+ diet for 2 weeks), amiloride again reduced surface cell conductance but not crypt conductance (n=13). However, conductances of both, surface cells and crypt openings, increased by a factor of 2 as compared to the conductances under acute aldosterone.

Our data demonstrate that theophylline-induced Cl- secretion is localized in crypts, but not in surface cells. On the other hand, acute as well as chronic aldosterone induces amiloride-sensitive conductive pathways in surface cells but not in crypts. In addition, chronic aldosterone induces as yet unidentified conductive pathways in both, surface cells and crypts.

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DEMONSTRATION OF A VACUOLAR TYPE ATP-DRIVEN H+ PUMP IN BRUSH-BORDER MEMBRANES OF THE HUMAN PLACENTA

B. Simon, V. Ganapathy, F. J. Leibach, and G. Burkhard

The brush-border membrane of the placental synctiotrophoblast forms the first barrier between maternal and fetal circulation systems. To investigate whether besides an Na+/H+ exchanger also an ATP-driven H+ pump is present in the brush-border membrane, vesicles were isolated from normal human term placenta by a Mg2+- precipitation technique. The vesicles were solubilized with 1.2% cholate and detergent was removed by overnight dialysis to reorient putative H+ pumps from the inside to the outside of the membrane. Addition of Mg2+ and ATP to these cholate-pretreated placental membranes resulted in a marked intravesicular acidification as visualized by acridine orange proving the presence of an ATP-driven H+ pump. H+ uptake was faster with ATP, much slower with GTP and IPT, and not detectable with UTP and CTP. ADP inhibited uptake with a Ki of 0.07 mM. The divalent cations Mg2+ and Mn2+ supported best, and Ca2+ partially, ATP-driven H+ uptake. ATP-driven H+ uptake into cholate-pretreated placental vesicles was weakly inhibited by vanadate, azide, and oligomycin. Complete inhibition, however, were observed with 0.01 mM N-ethylmaleimide (NEM) and 4-chloro-7-nitro-benzoxa-l,3-diazole (NBQI), and 0.2 mM Mn2+-disodium 4-chloro-6-methylbenzilidene (DCCD). This inhibitory pattern is typical for "vacuolar" type H+-ATPases. The stimulation of ATP-driven H+ uptake by iodide > chloride > nitrate >> sulfate, gluconate suggested that the H+ pump is electrogenic and requires uptake of permeant anions for charge compensation. Accordingly, ATP-driven H+ uptake was enhanced by the K+ ionophor, valinomycin, in the presence of K+ in the vesicles. In this case, each pumped proton is exchanged with K+ leaving the vesicles via its ionophor. The data provide the first evidence for the existence in human placental brush-border membranes of an ATP-driven H+ pump. The high sensitivity of this pump for NEM and NBQI reveals that this pump belongs to the class of "vacuolar" H+-ATPases.

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A CALCIUM-SENSITIVE CATION CHANNEL IN FROG COLON

K. Krattenmacher, Rosita Voigt, W. Claus

Electrogenic ion transport across the colon epithelium of the frog (Xenopus laevis) was investigated in an Ussing-chamber under voltage clamp conditions. With NaCl-Ringer on both sides of the tissue, the major part of the short circuit current (Isc) was caused by an electrogentic Na-transport (mucoza to serosa). This transport is insensitive to mucosal amiloride (10-4M/moll) and could be reversibly increased by removal of mucosal Ca-ions. The amount of this Ca-effect linearly increased with the mucosal pH. Half-maximal inhibition of the Ca-sensitive current was at about 1 µmol/moll mucosal Ca. Similar effects as with Ca were obtained with other bivalent cations (Mg, Ba). Noise analysis of the current fluctuations showed that Ca-removal induced a Ca-sensitive current that was inhibited by vanadate, azide, and oligomycin. Complete inhibition, however, were observed with 0.01 mM N-ethylmaleimide (NEM) and 4-chloro-7-nitro-benzoxa-l,3-diazole (NBQI), and 0.2 mM Mn2+-disodium 4-chloro-6-methylbenzilidene (DCCD). This inhibitory pattern is typical for "vacuolar" type H+-ATPases. The stimulation of ATP-driven H+ uptake by iodide > chloride > nitrate >> sulfate, gluconate suggested that the H+ pump is electrogenic and requires uptake of permeant anions for charge compensation. Accordingly, ATP-driven H+ uptake was enhanced by the K+ ionophor, valinomycin, in the presence of K+ in the vesicles. In this case, each pumped proton is exchanged with K+ leaving the vesicles via its ionophor. The data provide the first evidence for the existence in human placental brush-border membranes of an ATP-driven H+ pump. The high sensitivity of this pump for NEM and NBQI reveals that this pump belongs to the class of "vacuolar" H+-ATPases.

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TRANSPORT OF BILE SALTS IN RAT RENAL BASOLATERAL MEMBRANE VESICLES

S. Dietrich and G. Burckhardt

Bile salts, e.g. taurocholate, are actively reabsorbed in the proximal tubules of the rat kidney. They enter the tubule cell across the luminal membrane via a Na+-dependent transport system. The characteristics of the exit step across the basolateral membrane are unknown. To investigate the bile salt transporter we prepared basolateral membrane vesicles from rat kidney cortex by a Percoll density gradient centrifugation technique. The amount of [3H]taurocholate associated with the vesicles decreased with decreasing vesicle volume indicating uptake of this bile salt into the vesicle interior rather than mere binding to the membrane. Unlike bile salt transport in the brush-border membrane, [3H]taurocholate uptake into basolateral membrane vesicles was not stimulated by an out-in Na+ gradient. Unlabelled taurocholate and cholate in the incubation medium inhibited [3H]taurocholate uptake proving the presence of a saturable transporter for conjugated and unconjugated bile salts in the basolateral membrane. Substrates of the transport systems for sulfate, dicarboxylates, and p-aminohippurate (PAH) did not inhibit [3H]taurocholate uptake when added to the incubation medium. In contrast, unlabelled taurocholate and cholate inhibited strongly the uptake of [3H]PAH and, to a small extent, that of [3H]SCN-. These data indicate that bile salts are transported by a separate system in the basolateral membrane. In addition they interact with the transporters for PAH and sulfate without being measurably translocated by these systems. [3H]Taurocholate uptake into basolateral membrane vesicles was inhibited by bromosulfophthalein (BSP), the loop diuretics furosemide and bumetanide, by bilirubin, probenecid and 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS). With [3H]cholate comparable results were obtained although the degree of inhibition by organic anions, taurocholate and cholate in the medium was smaller than in experiments with [3H]taurocholate. In conclusion, our data provide evidence for the existence of a separate transport system for bile salts in the basolateral membrane not shared by sulfate, dicarboxylates and PAH.

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EFFECTS OF BARIUM ON POTASSIUM TRANSPORT IN ISOLATED MALPIGHIAN TUBULES OF FORMICA

A. Leyssens, S.L. Zhang, R. Weltens, P. Steels and E. Van Kerkhove.

In an attempt to elucidate the role of K-channels as a hypothetical pathway for the K-transport through the basolateral membrane of Malpighian tubules of Formica, the transepithelial and basolateral (Vb) potential, the specific transepithelial resistance (Rt), the relative value of the apical (Rap) over the basolateral (Rb) resistance and the fluid secretion rate were determined in the absence and presence of Ba2+ in the bath solution. Vb, measured in 3 different K-concentrations (5, 51 and 113 mM), revealed that the basolateral membrane behaved as an almost perfect K-electrode. In the presence of 6 mM Ba2+, this sensitivity was lost (the slope decreased from 49 to 7 mV/decade). Rap/Rb dropped from 39±5 (n=8) to 110±5 (n=20) while Rt increased between 9 and 25%. These results suggest the presence of basolateral K-channels.

Surprisingly, the addition of 6 mM Ba2+ resulted in a strong hyperpolarization of the apical membrane potential from -54±5 to -101±5, -96±6 and -94±6 mV (n=3) in 5, 51 and 113 mM K+, respectively. Ba2+ had also an effect on the urine formation; secretion rates fell with 67, 69 and 90% in 5, 51 and 113 mM K+, respectively. We conclude that the net transepithelial K-transport could be inhibited directly by a block of the basolateral K-channels and/or indirectly by a reduced activity of the apical electrogenic cation pump due to an increase of the apical membrane potential.

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SYNERGISTIC STIMULATION OF ALDOSTERONE AND THYROID ON LARGE INTESTINAL SODIUM ABSORPTION IN CHICKEN EMBRYOS

B. Hoffmann, R. Krattenmacher, M. Heinz, B. Habura and W. Claus

Stimulation of electrogenic Na+ transport (INa) across large intestinal epithelium (cropodermum) of 20 days old chicken embryos by thyroxine and aldosterone was studied in Ussing-chambers. Fluctuations in short-circuit current were measured with a low noise voltage-clamp for further noise analysis. With Na-Ringer's solution (supplemented with β-hydroxy-butyrate, glutamate and mannose) on both sides of the tissue INa was 4.7 ± 1.3 μA/cm2 (mean ± SEM, n=8). Neither thyroxine (10-9 mmol/l) nor aldosterone (10-8 mmol/l) induced in vitro a significant increase in INa. Interestingly, when both hormones were given INa was 11-fold stimulated to 53.1 ± 3.8 μA/cm2 within 4-6 hours. To determine the microscopic parameters of this stimulated Na-transport amiloride-induced current fluctuations were analysed. From the plateau values and the corner frequencies of the Lorentzian Na-channel density (M) of 16 Mio/cm2 and a single Na-channel current (iNa) of 2.2 pA was calculated. The Michaelis-Menten-constant (Km) for the amiloride-channel interaction was 0.8 μmol/l. Our results clearly show, that the electrogenic Na-transport system present in large intestine of adult hens already exits in embryonic chicken shortly before hatching. Noise analysis shows that microscopic parameters of apical sodium channels (INa and M) in this embryonic epithelium are similar to values found in sodium-conserving epithelia of adult animals. Furthermore we demonstrate that at this developmental stage sodium absorption can already be regulated by hormones. Obviously, aldosterone and thyroxine act in a synergistic way.

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The role of mitochondria-rich (MR) cells of frog skin in Na-transport

A. Dürge and W. Nagel

From electrophysiological and flux analyses it was proposed (Ehrenfeld et al. Pflügers Arch. 414, 59 (1989)) that MR-cells might be substantially involved in amiloride-sensitive Na transport under in vivo-like conditions (low [Na] on the apical side; open circuited). Particularly large was this flux after KCl-adaptation of the frogs. To test this possibility we determined on KCl-adapted Rana esculenta electrical parameters of principal cells, transepithelial Na net-fluxes and intracellular electrolyte concentrations along with Rb-uptake into principal and MR-cells across the basolateral membrane. Measurements were done under control conditions and after amiloride. With 2 mM mucosal Na and at open-circuit, the fractional resistance of the apical membrane was from 0.7 to 0.9. After amiloride, it increased virtually to 1.0. Amiloride-induced alterations of transepithelial clamp current at different holding potentials were associated with changes in intracellular potentials of proportionate magnitude. These data indicate that Na passes through principal cells under in vivo-like conditions. Transepithelial net Na transport, measured from the decrease in [Na] of the apical solution, was observed only at low transepithelial potentials. It was near-completely blocked by amiloride. The electrolyte composition of principal and MR-cells was essentially unchanged after amiloride. In MR-cells, large variation in [Na] and [Cl] was obtained as usually. Uptake of Rb across the basolateral membrane, which represents a measure of the transport activity of the Na/K-pump, was not different, on the average, in both cells types. It could be reduced by inhibition of net Na transport using amiloride only in principal cells. Our results indicate that under in vivo-like conditions as well as under "Ussing"-conditions, amiloride-sensitive Na transport occurs mainly, if not exclusively, through the principal cells.

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TRANSPORT OF SHORT-CHAIN FATTY ACIDS ACROSS DIFFERENT SEGMENTS OF THE GUINEA PIG LARGE INTESTINE.

G. Reckikemmer and W. von Engelhardt

Short-chain fatty acids (SCFA: acetate, propionate, butyrate) are produced in the large intestine by aerobic microbial fermentation of carbohydrates. SCFA are monocarboxylic acids with a pK around 4.8, SCFA constitute the major anions in large intestinal contents. Transport of SCFA across the large intestinal epithelium is not well characterized so far.

Unidirectional isotopic tracer fluxes of 14C-labelled SCFA were measured under short-circuit current conditions across the isolated epithelium of guinea pig caecum, proximal and distal colon. Flux from the mucosal to the serosal side of the preparation (ms-flux) and in the opposite direction (sm-flux) increased linearly with concentration for all three SCFA in all large intestinal segments at concentrations of 1, 10 and 25 mM SCFA. In the caecum and proximal colon net secretion of SCFA was observed, whereas in the distal colon SCFA were absorbed. Net secretion in the proximal colon decreased with SCFA-chain length, net absorption in the distal colon rose with chain length. Na transport in the proximal colon was stimulated by SCFA to a variable extent. Na transport in the proximal colon is accomplished by Na/H exchange. Na/H exchange can be inhibited by amiloride (0.1 mM). Amiloride significantly increased SCFA secretion in the proximal colon due to inhibition of the ms-flux. In the guinea pig distal colon an ouabain-sensitive K-ATPase has been demonstrated. Inhibition of the K-ATPase with ouabain (0.1 mM) abolished SCFA absorption in the distal colon and led to a significant SCFA secretion, similar in magnitude to SCFA secretion observed in the proximal colon. In conclusion SCFA transport across the large intestinal epithelium appears to be primarily passive but linked to hydrogen ion gradients established by cellular ion transport systems.

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COMPARISON OF AMILORIDE AND CDPC EFFECTS ON THE FROG SKIN ELECTROPHYSIOLOGY.

D.-G. Margineanu and W. Van Driessche

Recent studies (Helman and Baxendale, FASEB J. 2: A750, 1988; Krättenmacher et al., Pfuiigers Arch. 412:506, 1988) reported the use of the amiloride-analogue 6-chloro-3,5-diaminopyrazine-2-carboxamide (CDPC) as a blocker of sodium channels, suited for inducing Lorentzian noise in epithelia. Here we compare the effects of these two pyrazine derivatives on the short-circuit current (Isc), transepithelial impedance and blocker-induced noise in isolated skin of Rana temporaria.

From the linear dependence of the corner frequency (fC) of the Lorentzian noise on the amiloride concentration, a mean lifetime of 75 ms at an apparent dissociation constant of 0.8 µmol/l were calculated. The lifetime of CDPC complex with the channels is much shorter (4 ms) and the apparent dissociation constant higher (40 µmol/l), with either sulphate or chloride as major anion. The 50% inhibition of Isc was produced by about 0.5 µmol/l amiloride and by 150 µmol/l CDPC. In order to reveal the specific effects of the two blockers on the apical and basolateral membranes, we performed impedance measurements in hypotonic sulphate Ringer, in which the Nyquist plots consist in two distinct semicircles. These measurements do not reveal major capacitance changes. But, on the other hand, very significant resistance changes were recorded. The apical resistance is increased by both blockers, it rising to twice the control value in the presence of either 2 µmol/l amiloride or 150 µmol/l CDPC. The basolateral resistance continuously decreases up to highest CDPC concentration used, reaching 55% of the control value. At concentrations below 0.1 µmol/l, amiloride caused decreases (by about 25%) of the basolateral resistance, but above this concentration it continuously increases, reaching twice the control value at 2 µmol/l. The decrease of basolateral resistance might account for the scalling behaviour of Isc, commonly observed with CDPC as well as for the increase of fC above the control values produced by small amiloride concentrations.

In conclusion, the HT-29/B6 clone shows a cAMP-mediated Cl- secretion responding to physiological secretagogues. Under glucose-free culture conditions they differentiate. In this study transepithelial transport properties of differentiated HT-29 cells were described.

After prolonged glucose-free culture a clone (HT-29/B6) was selected. The cells were seeded onto filter membranes and the transmucosal resistance (Rm) was monitored daily. Rm exhibited a sigmoidal increase with a plateau value of 421 ± 9 ohm-cm2 after 7 days.

Light and electron microscopy revealed formation of monolayers consisting of cylindrical cells with tight junctions and a dense apical brush border. Filters were mounted in Ussing-type chambers and Isc, transepithelial voltage (Vt), and unidirectional Na+ and Cl- fluxes were measured. Control values were Vt = -1.1 ± 0.3 mV (mucosa negative), Isc = 0.1 ± 0.01, JNa = -0.3 ± 0.4 (ns), and JCl = 0.1 ± 0.2 µmol·h·cm-2·nmol (ns).

Serosal forskolin (FSK) 10-4 M increased Vt to -17.8 ± 1.5 mV, Isc to 1.9 ± 0.1, JNa to 1.7 ± 0.8 and JCl to 0.2 ± 0.4 µmol·h·cm-2·nmol (ns). The FSK-induced Isc was decreased to 7% by serosal bumetanide (BUM) 10-4 M and reversibly inhibited by the chloride-channel blockers DPC and NPPB with a respective IC50 of 10-5 M and 3·10-4 M.

Serosal dibutylr~AMP 10-4 M, VIP 10-8 M, or prostaglandin E1 10-8 M caused an increase of Isc and Vt which could be reversed by BUM and DPC.

In conclusion, the HT-29/B6 clone shows a cAMP-mediated Cl- secretion responding to physiological secretagogues. Thus glucose-free culture results in the formation of monolayers possessing attributes of colonic crypts. As the HT-29 cell line is derived, in contrast to other cellular models, from a primary tumor, the clone presented here is most suitable for the transepithelial investigation of intestinal chloride-secretion.

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LIPID PEROXIDATION AND DECREASE OF GLUTATHIONE (GSH) IN ACUTE SKIN GRAFTS
A.J. Augustin, R. Goldstein, E. Purucker and J. Lutz.

Oxygen free radicals are known to be involved in ischemia-reperfusion injury in skin flaps. The commonly used pedicle flap has to be subdivided at least into a lip area and a central zone, which includes the pedicle, because of different oxygen availability. Here, the free flap model, which shows no different areas, was used.

For experiments we used male wistar rats. A 2.5 x 2 cm abdominal wall skin flap was dissected, totally lifted and infolded immediately. The postischemic period lastend 12 hours. Control skin flaps were dissected in the same way. Lipid peroxide level of the skin was determined by modification of the method of Ohkawa et al. (Anal. Biochem. 95, 351, 1979). Glutathione level was determined by the method of Griffith (Anal. Biochem. 166, 207, 1980).

After 12 h skin tissue level of lipid peroxides increased from 185 ± 11 (SEM) mmol/g to 1871 ± 305 mmol/g (p<0.01). This elevation of lipid peroxides, indicating oxygen free radical damage, was accompanied by a decrease of tissue glutathione level from 541 ± 30 mmol/g in control animals to 77 ± 17 (p<0.05).

These data show free radical formation in the postischemic period, which can be compensated by protecting systems like peroxidases (extracellular) or superoxide dismutase and glutathione peroxidase. Glutathione (GSH, reduced form) could have served as electron donor for reduction of hydrogen peroxide:

\[ 2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2 \text{H}_2\text{O} \]

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CURRENT FLUCTUATION ANALYSIS OF BASOLATERAL K⁺ CHANNELS STIMULATED BY ALDOSTERONE IN ALVEOLAR EPITHELIUM OF XENOPUS LAEVIS
B. Illek, H. Fischer, and W. Claus.

Control and aldosterone-stimulated lung tissues were investigated in Ussing chambers under voltage clamp conditions using current fluctuation analysis. In theseays basolateral and alveolar-to-epithelial K⁺ current densities were induced by permeabilizing the apical membrane for K⁺ with the pore-forming antibiotic lidocaine and followed Michaelis-Menten kinetics. Halfmaximal lidocaine inhibition concentration was 302 ± 11 tzM (mean±SE, n=6).

The corner frequencies of the lidocaine-induced Lorentzians increased linearly with blocker concentration. Values for the association and dissociation rate constants were 1.3±0.03 M⁻¹s⁻¹ and 331±15 s⁻¹. The calculated mean single K⁺ current was 1.5±0.1 pA and did not change after aldosterone stimulation, corresponding to a single K⁺ conductance of 15 pS. After stimulating the tissues for 4 hours with 1 µM aldosterone, Ig was significantly increased to 66.9±6.8 pA/cm² (n=9), compared to 44.7±5.3 pA/cm² (n=7) in unstimulated tissues. Ig was in close correlation with the number of K⁺ channels (r=0.746, n=10). The regulation of basolateral K⁺ channel density permits changes in the rate of transcellular Na⁺ transport without modifying intracellular ionic content and volume. This is essential for cell homeostasis in Na⁺ transporting epithelia.

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INTRACELLULAR ELECTRODE MEASUREMENTS IN EPITHELIUM CULTURED CELLS DURING INDEPENDENT CHANGES OF APICAL AND BASOLATERAL FLUIDS
K-H. Thiele, J.S. Schwegler, A. Keuner and S. Silbernagl

We recently showed that monolayers of the cultured proximal tubular cell line OK are electrically extremely leaky (Pflügers Arch. 1989, 415:183ff). We now present a method to control both the apical and basolateral fluid of leaky epithelia during measurement of intracellular potential (PDin). To prove correct separation of both fluid spaces we superfused confluent OK cell monolayers grown on transparent filters with various K⁺ concentrations to measure apical (tPAp), basolateral (tPBp) and total (tPtot) K⁺ transference numbers. The sum of tPAp + tPBp is only 15 ± 5% higher than tPtot (n=5).

To follow a more direct approach for quantifying paracellular K⁺ leakage, we superfused the apical side with 5.4 mmol/l K⁺, the basolateral side with 20 mmol/l K⁺. K⁺ concentration on the apical side of an intercellular junction - measured by an extracellular K⁺ selective microelectrode - was 5.7 ± 0.1 mmol/l (n=4), indicating that a paracellular K⁺ leakage does not disturb measurements under our conditions. The ratio of apical to basolateral K⁺ conductance (tPAp/tPBp) was 0.45 ± 0.07 (n=5). Therefore, potassium conductance is located to two thirds on the basolateral and to one third on the apical membrane of OK monolayer cells.

We believe that this is a reliable method to study sidedness in leaky epithelia by an electrophysiological approach.

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ERYTHROPOIETIN REGULATION: A 50K PROTEIN WITH AN OXYGEN-DEPENDENT DNA-BINDING PROPERTY
Dittmer, J., and Rauer, C.

HepG2, a hepatoma cell line, produces erythropoietin (EPO) in a physiological manner, i.e. it shows basal and hypoxia-inducible EPO synthesis. In order to look for proteins involved in EPO regulation we analysed nuclear proteins of stimulated and nonstimulated HepG2 for their specific binding to a DNA-fragment of the 5’-flanking region of the EPO gene. We found a 50K protein whose DNA-binding activity could be modulated by metal ions. In presence of Fe³⁺ the DNA was bound to that protein from normoxic but not from hypoxic cells. In contrast, with Zn²⁺ and Co²⁺ no differences in DNA-binding could be seen between proteins from normoxic and hypoxic cells: Zn²⁺ promoted and Co²⁺ suppressed DNA-binding in both cases. The effects of Zn²⁺ and Co²⁺ on DNA-binding correspond to the action of these metal ions on the EPO synthesis in HepG2 cells. The addition of CoCl₂ yielded in a 2-3 fold higher EPO synthesis under normoxic conditions whereas in presence of ZnCl₂ hypoxia-induced production was lowered down to 60-70% of control value. We suppose that the 50K protein is an iron protein whose DNA-binding activity is regulated directly or indirectly by oxygen.

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115 THE EARLY DECLINE OF ERYTHROPOIETIN PRODUCTION AT CONTINUOUS HYPOXIA IS NOT DUE TO FEEDBACK INHIBITION
K-J. Eckardt, J. Dittmer, R. Neumann, C. Bauer and A. Kurz
Serum erythropoietin (EPO) levels in mice and rodents are known to increase within 1-2 hours following the onset of normobaric or hypobaric arterial hypoxia, but despite continuous hypoxia decline again prior to an increase in blood oxygen carrying capacity. In order to define the possible mechanisms underlying this phenomenon, we have investigated (i) how renal EPO mRNA content and EPO production rates underlying the early kinetics of serum EPO levels change under different degrees of normobaric hypoxia and (ii) if a feedback inhibition of either EPO formation or survival in the circulation exists by the hormone itself.

We found that serum immunoreactive EPO in rats peaked after 12 h exposure to 7.5% or 9% oxygen (2949 ± 600 mU/ml and 756 ± 108 mU/ml; mean ± SE) and declined to 29% and 64 % of peak levels, respectively, after 11.5 hours. Between 12 h and 36 h of hypoxia (122 ± 12 mU/ml and 182 ± 55 mU/ml, resp., mean ± SE), the decline in EPO levels under severe hypoxia (7.5% O2) was paralleled by a reduced in renal EPO mRNA content. Furthermore, the clearance rate of iodinated recombinant human EPO (rhEPO) in normoxic and hypoxic rats was unaffected by the exogenous application of EPO.

EPO formation in response to a subsequent hypoxic exposure of 12 hours was unaffected by the oxygenation history of EPO produced, the magnitude of the decline appears to be related to the degree of the preceding stimulation.

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116 RECEPTOR VERSUS ENZYME MEDIATED SPECIFITY OF MINERALOCORTICOID ACTION (Na+ TRANSPORT) IN RAT RECTAL COLON IN VITRO
M Fromm, JD Schulke, KH Kreusel, P Clausen, A Krohn, U Lempart, S Lüderitz, U Hegel and K Hierholzer

11-hydroxy-steroid-dehydrogenase (117HSD) catalyses the reactions corticosterone → 11-dehydro-corticosterone and cortisol → cortisol. We tested the possibility that local action of this enzyme is responsible for mineralocorticoid (MCC) action. This action is related neither to the classical concept of intracellular steroid receptors. Rat rectal colon was stripped of the muscularis mucosae, submucosa, and muscularis propria ("total strip") and mounted in a short circuit current (Isc) was measured over a time period of 8 hrs. In vitro added aldosterone (ALD, 10^-8 M) produced an increase of Isc which could be reversed by 10^-4 M amiloride (Aml).

- Addition of corticosterone (B) in a concentration of 10^-4 M resulted in no significant increase of JNa.
- Glycyrretinic acid (GLY, 10^-6 M), a component of liquorice, alone had no apparent effect on JNa.
- When GLY (10^-6 M) and B (10^-8 M) were added together a definite increase of JNa was observed which was not significantly different from that of ALD (10^-8 M).
- The metabolite of B, 11-dehydro-corticosterone, stimulated JNa at a pharmacologic concentration of 10^-5 M.

This effect was abolished in the presence of GLY which prevented the formation of B.

- Identical results were obtained with cortisol instead of B.

We conclude that specificity of MCC action in rat rectal colon is controlled by corticosteroid metabolism: 117HSD, localized within the target tissue, prevents corticosterone and cortisol from binding to the mineralocorticoid receptor by oxidation of the 11-OH group. It is directly demonstrated in a typical MCC target tissue that GLY inhibits the action of local 117HSD. This metabolic block plays a part in inducing symptoms of hyperaldosteronism at chronic liquorice consumption.

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117 INTERSTITIAL OSMOLARITY AND pH DETERMINE AXIAL DIFFERENTIATION IN RENAL COLLECTING DUCT.
L. Oertel, L. Wojnowski and M. Niederhoffer

The renal (1) inducing cell differentiation in renal collecting duct are still unknown. We used cultured canine kidney (MDCK) cells as a differentiation model for collecting duct epithelium. The cells were grown to confluence and then incubated in different media: pH 6.4 pH 7.7 (control); 0 mosmol/l (osmop); 200 mosmol/l (osm6). Four days later we tested the capacity of the apical cell membrane to bind peanut lectin (PNA). PNA binds in vivo exclusively at the apical membrane of bicarbonate secreting (D-type) intercalated cells. Furthermore, we measured pH in vasa recta formation. These blisters underneath the MDCK epithelium are formed due to CI- reabsorption in exchange for HCO3-. We measured in rat kidney in vivo pH (ion-selective microelectrodes) and osmolality in vasa recta of the papilla in antidiuresis and diuresis.

| MDCK cell (MEAN ± SEM) | PNA (%) | rat kidney (vasa recta) |
|------------------------|---------|------------------------|
| osmol 74 mOsm/l | 7.29 ± 0.08 | 356 |
| pH 7.40 ± 0.05 | 1352 |
| mosmol 90 mOsm/l | 0 |

PNA binding capacity of MDCK cells is near zero shortly after cell splitting. The typical PNA binding pattern in a young confluent layer is found in both wild-type and cloned MDCK cells. In alkaline or hypotonic medium PNA binding is enhanced and dome formation is stimulated. PNA positive cells are cuboidal, possess a dark cytoplasm with small vesicles and intercellular spaces (intercalated cell type). In acid environment or in hypertonic medium MDCK cells lose their PNA binding capacity and become flat and possess a light cytoplasm with large vesicles (principal cell type). In rat, high osmolarity is coupled to a high H+ activity in the interstitial space of the inner medulla. We postulate: After cell splitting MDCK cells differentiate dependent on the metabolic conditions. Osmolality and pH may force collecting duct cells to differentiate either to principal cells or intercalated cells. B-type intercalated cells (CI- reabsorption, HCO3- secretion, PNA binding) are expressed only in conditions found in renal cortex.

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118 ELECTRICAL ACTIVITY OF MAGNOCELLULAR NEURONES IN THE NUCLEUS PARAVENTRICALISIS IS INCREASED AFTER OSMOTIC CHALLENGE IN THE NEWBORN CHICKEN.
R. Größmann, Xu Bin and F. Ellendorff

Neurohypophysial arginine-vasotocin (AVT), an analogue to mammalian arginine-vasopressin, plays a major osmoregulatory role in birds. A close relationship between electrical firing discharge and hormonal activity has been demonstrated in the mammalian hypothalamo-neurohypophysial system. In order to examine whether this feature exists in avian species as well and, if so, is established already during development, single unit recordings from antidromically identified magnocellular neurones in the hypothalamic nucleus paraventricularis (PVN) were performed.

Within 24 hours after hatching chicks were anaesthetized with urethane (3 mg/g.i.p.) and heads fixed in a stereotaxic frame. In each of nine chicks one neurone was studied. Mean spontaneous firing rate, averaged over at least 5 min before any manipulation of plasma osmolality was 0.8 spikes/sec (range 0.3-3 spikes/sec). In four cells tested with 0.1 m physiological saline (<i>s</i>)(<i>i</i>) firing frequency was unaffected for up to 30 min. After injection of 0.1 ml 1 M NaCl i.p., an osmotic stimulus which elicited significant amounts of AVT in the newborn chick (Klemp, Ellendorff, Großmann, this meeting), in one cell mean firing discharge was increased by more than 1 spike/sec during the period 25 to 30 min after injection compared to basic frequency. In one cell mean firing discharge increased by more than 4 spikes/sec. Two neurones were unaffected and one neurone inhibited.

Our results show that magnocellular PVN neurones in the newborn chick are osmosensitive and respond to osmotic changes with increasing firing activity, which in turn may lead to secretion of AVT.

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EFFECT OF SECOND MESSENGERS ON RENIN SYNTHESIS AND RENIN SECRETION IN PRIMARY CULTURES OF MOUSE RENAL JUXTAGLOMERULAR CELLS

R. dalla Bruna and A. Kurz

Utilizing primary cultures of mouse renal juxtaglomerular cells we have examined the effects of the second messengers CAMP, cGMP and protein kinase C on the synthesis and the secretion of renin. Rates of renin synthesis were estimated from the cellular release of renin activity, those of synthesis by specific immunoadsorption of protein produced by cells pulsed with 3S-methionine.

An increase of cAMP induced by forskolin (1μM) or isoproterenol (10μM) stimulated both the synthesis and the secretion of renin. Stimulation of secretion by cAMP occurred within minutes, stimulation of synthesis was observable with a delay of around eight hours.

An increase of cGMP by 8-bromo-cGMP (100μM) inhibited only basal renin secretion and was without effect on synthesis. Activation of protein kinase C by the phorbol ester PMA (10μM) had no significant effect on basal and stimulated secretion nor on basal synthesis but blunted the increase of renin synthesis induced by cAMP.

Our findings suggest that cAMP is an important regulator of both synthesis and secretion of renin in renal juxtaglomerular cells. The inhibitory effect of C-kinase activation on cAMP-induced stimulation of renin synthesis could provide a novel explanation for the inhibitory effect of angiotensin II on renin synthesis in vivo.

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CONTROL OF PRESSURE NATRIURESIS IN CONSCIOUS DOGS: ROLE OF THE SYMPATHETIC NERVOUS SYSTEM

H. Ehnike, P.B. Persson, M. Seyfarth and H.R. Kirchheim

The relationship between renal artery pressure (RAP) and urinary sodium output (UNaV) was investigated during (1) control conditions, (2) common carotid occlusion (CCO), (3) CCO with intrarenal propranolol infusion, and (4) low-dose intrarenal methoxamine infusion. Pressure natriuresis curves (PNC) were determined in 12 conscious dogs on a normal-salt diet. RAP was reduced in 10 mmHg steps by inf lation of a cuff placed around the renal artery. (1) Under control conditions, a reduction in RAP resulted in a strong decrease in urine output and UNaV. In all dogs, the PNC was found to be closely related to the individual resting blood pressure; urine flow rate reached zero 30-40 mmHg below resting blood pressure. (2) A baroreflex activation of the sympathetic nervous system by CCO shifted the PNC by 11.1 ± 3.1 mmHg (P < 0.01; n = 8) to the right. The sensitivity of pressure natriuresis was not affected by CCO. (3) The shift was blocked, when the selective alpha1-adrenoceptor antagonist prazosin was infused intrarenally during CCO (n = 9). Without CCO, prazosin did not alter UNaV at the control RAP. (4) Similar to CCO, intrarenal infusion of the selective alpha1-adrenoceptor agonist methoxamine shifted the PNC by 19.3 ± 7.7 mmHg to the right without altering the slope of the PNC. Without CCO, a marked decrease in renal blood flow nor glomerular filtration rate were changed by the methoxamine infusion. These results indicate that the sympathetic nervous system regulates UNaV by shifting the PNC through intrarenal alpha1-adrenoceptors without altering its slope.

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INTERACTION OF ANGIOTENSIN II AND ADENOSINE IN RENAL MICROCIRCULATION
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It is still a subject of controversy to what extent an inhibition of angiotensin II (A II) can block the renal vasoconstriction due to adenosine. The purpose of the present investigation was to analyse the interaction of these two vasoconstrictory substances by means of in vivo microscopy in the split hydronephrotic rat kidney (cf. for the technique: Kidney Int 35: 1151-1160, 1989). In agreement with Spielmann and Osewald (Am J Physiol 237: F 463-467, 1979), we found in a first series of experiments that local application of the A II-antagonist saralasin (10^-5M) abolished the vasoconstriction and the reduction of glomerular blood flow induced by the A I-adenosine receptor agonist N6-cyclohexyladenosine (CHA, local concentration 10^-4M). Without saralasin, CHA reduced glomerular blood flow and decreased vessel diameters as previously reported (Holz and Steinhausen, Renal Physiol 10: 272-282, 1987). In a second in vivo experiment, we found that the blockade of CHA by the selective A I-adenosine receptor antagonist 1,3-diglypropyl-8-cyclogentyl-gantheme (DPCPX, 10^-5M) did not abolish the vasoconstrictory action of A II (10^-7M). In separate series, we confirmed the inhibitory action of DPCPX on the adenosine-induced vasoconstriction and the stability of our preparation.

We suppose that adenosine needs a functioning A II-receptor system for its vasoconstrictory effect. The receptors on MCs as opposed to VSMCs and JGECs. Cells from TBs did not react to the application of any vasoactive substance.

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Electrophysiological and pharmacological differences between mesenchymal cells of the renal cortex

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Little is known about the comparative electrical membrane properties of mesenchymal cells within the kidney cortex owing to difficulties of their microtopographical localization. The microvessels and glomeruli embedded in a mass of tubular epithelia (TBs). Three types of preparations have been developed to overcome this difficulty: the mouse hydronephrotic mouse kidney, the mouse kidney slice, and cultured renal cortical cells. We investigated, whether vascular smooth muscle cells (VSMCs), juxtaglomerular epitheliocells (JGECs), mesangial cells (MCS) and tubular epithelia differ sufficiently in their electrical membrane properties or in their responses to vasoactive agents, as to permit an electrophysiological discrimination when visual identification is impossible. Within the experimental scatter, membrane potential and cellular input resistance do not permit to discriminate between JGECs, VSMCs and MCS in all preparations. Only tubular epithelia are easily discernible owing to their high membrane potential and low input resistance. In all preparations, JGECs, VSMCs and MCS reacted with depolarizations in response to the vasoactive peptides AVP and ANG II. The only difference consists in the absence of alpha 1-receptors on MCS as opposed to VSMCs and JGECs. Cells from TBs did not react to the application of any vasoactive substance.

INTERACTION OF STEROID HORMONES WITH THE CONTRALUMINAL ANION TRANSPORTERS IN THE PROXIMAL RENAL TUBULE
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The prevailing hypothesis is that steroid hormones cross the cell membrane by simple diffusion. Our specificity scheme of the contraluminal anion transporters (Am J Physiol 254:F453, 1988) would predict that steroid hormones are also transported by the contraluminal para-aminobipurate (PAH) transporter. To test this we applied the stop flow microperfusion technique of the peritubular capillaries and studied the interaction of steroid hormones with the contraluminal uptake of 3^H-PAH and 35^S-sulfate: Pregnenolone (5-pregnen-3B-ol-20-one) and progesterone (4-pregnen-3,20-dione) do not interact with the PAH transporter. If, however, progesterone has one additional OH-group in position 6, 17x or 21, or two additional OH-groups either in position 11 and 21 (corticosterone), or in position 17a and 21 (11-deoxycorticisol), the compounds exert a high to moderate inhibitory potency against contraluminal PAH-transport (app. K^P AH 0.13-0.38 mmol/l). If the pregnenolone or progesterone molecule has 3 additional OH-groups to yield cortisol or uro cortisol interaction with the PAH transporter decreases. If cortisone or cortisol has an additional OH-group in position 6 interaction with the PAH transporter vanishes. 21-sulfatation or acetylation of corticosterone does not change its K^P AH (0.2 mmol/l). Sulfatation, however, exerts additional interaction with the sulfate transporter (app. K^SO_4 3.2 mmol/l). 1x influx of 3^H-cortisol was inhibited by 29% with 10 mmol/l probenecid when the starting concentration of cortisol was 0.1 mmol/l, and by 18% when it was 1 mmol/l. The data show that steroid hormones are indeed transported by the PAH transporter, probably in addition to diffusion through the lipid bilayer.

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WHOLE CELL RECORDING OF SODIUM-COUPLED ALANINE TRANSPORT IN SINGLE PROXIMAL TUBULE CELLS
J. Hoyer and H. Gögelein

Single cells from proximal convoluted tubules (PCT) were isolated from cortical slices of New Zealand White rabbits (800-1200g). Alanine driven sodium current was measured with the tight-seal whole-cell recording method. Addition of L-alanine to the extracellular side induced an inward-directed sodium current and a cell depolarization. The cotransport current was sodium- and voltage-dependent, stereospecific, and independent on extracellular pH (pH 6.4 to 8.4). It followed simple Michaelis-Menten kinetics with an apparent K_m of 6.6 mmol/l and an I_max of 0.98 pA/pF at -60 mV (bath solution (in mmol/l): 140 Na-cyclamate, 1.3 Ca^2+, 0 - 40 alanine; pipette: 140 Tris-cyclamate, 10^-7 Ca^2+, 0 alanine).

Transport rate at physiological alanine concentration was in the same order of magnitude as the estimated cotransport current required for 90% alanine reabsorption in PCT. A coupling stoichiometry of 1 Na^+ and 1 alanine was estimated by Hill plots of cotransport current data. Apparent K_m for Na^+ and apparent I_max were potential dependent, whereas apparent K_m for alanine was potential independent in absence or presence of a large inward-directed Na^+ gradient. Apparent K_m for alanine increased when inward-directed sodium gradient was decreased. From these kinetic data and from additional theoretical treatment a cotransport model with a simultaneous transport mechanism, a potential dependent binding or unbinding of sodium, and a negatively charged empty carrier is derived.

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MERCURY IONS DISSIPATE THE TRANSEPIHELIAL POTENTIAL DIFFERENCE IN DISTAL CONVOLUTED TUBULES OF THE RAT KIDNEY.

A. Jungwirth, M. Ritter, F. Lang

The kidney is the main target organ for mercury toxicity. Acute administration of mercury ions is followed by marked alterations of renal electrolyte excretion. The present study has been designed to elucidate the acute effects of mercury ions on electrolyte transport systems in distal convoluted tubules. To this end, male Munich Wistar rats have been prepared for micropuncture in the usual way. Distal tubules have been identified by injection of lissamine green and perfused utilizing a microperfusion pipette allowing multiple fluid exchange. Transepithelial potential difference (Vte) amounts to -19 ± 3 mV. Na2HPO4, 0.2 NaH2PO4, 5.0 NaHCO3 Vte amounts to -19 ± 3 mV. Na+ stimulated [3H]PAH uptake in the presence, but not in the absence of unlabeled PAH, probenecid, and 2-oxoglutarate in the medium proving that the same transporter was operative under both experimental conditions. Benzylpenicillin inhibited [3H]PAH uptake with an apparent Ki of 1.5 mM. Cephalexin inhibited also, but with a weaker potency (app. Ki > 5 mM). In conclusion, mercury ions depolarize the epithelium approaching -9.3 ± 2.7 mV within 8 minutes. For significant depolarization, 0.1 /~mol/1 mercury ions are required, half maximal depolarization is observed at approx. 3/~mol/1. A linear correlation occurs between Vte before application of mercury ions and the depolarization caused by mercury ions. Further analysis reveals that the depolarization is mainly due to a decline of the amiloride sensitive portion of Vte, whereas the potassium sensitive portion of Vte is not significantly altered by mercury ions.

Further analysis reveals that the depolarization is at least in part due to an inhibition of the amiloride sensitive sodium channels at the luminal cell membrane of principal cells.

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Parathyroid hormone increases thiol-proteinase activity by activation of protein kinase C in cultured kidney tubule cells (OK)

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Intracellular proteases were measured in cultured opossum kidney cells during chronic (24h) exposure to parathyroid hormone (PTH) by assaying the rate of breakdown of azocasein at pH 5.4. PTH increases the total proteolytic activity from a control of 6.4 ± 0.1 U/mg protein/45min in a saturable, dose-dependent manner to a maximum of 14.2 U/mg/45min (n=4). Saturation occurs at as low as 0.1 U/mg/45min (E64 (1 mmol/l), an unspecific inhibitor of thiol proteases, markedly reduces this PTH-mediated increase (21 ± 0.3 U/mg/45min vs. control 5.7 ± 0.1 U/mg/45min at 10−10 mol/l PTH). Application of the phorbol ester TPA (10−9 mol/l, 24h) mimics the effects of PTH on total proteolytic activity (6.2 ± 0.1 U/mg/45min vs. control 5.6 ± 0.2 U/mg/45min; n=4). TPA-induced increase is also blocked by 1 mmol/l E64. Staurosporin (10−7 mol/l), a potent inhibitor of protein kinase C, is equally effective in blocking the TPA- and PTH-induced protease activity increase (TPA + staurosporin: 4.9 ± 0.1 U/mg/45min vs. control 6.4 ± 0.2 U/mg/45min; PTH + staurosporin: 5.2 ± 0.2 U/mg/45min vs. control 6.2 ± 0.0 U/mg/45min; n=4). Staurosporin on its own has no significant effect on protease activity. Both the calcium ionophore A23187 (5·10−7 mol/l, 24h) and dibutyryl cAMP (10−4 mol/l, 24h) significantly reduce azocasein breakdown (p<0.05).

The data are highly suggestive of the notion that PTH increases the intracellular protease activity by an activation of the protein kinase C.

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Sympathetic modulation of renal autoregulation

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The sympathetic nervous system may exert an important influence on renal autoregulation (AR). This study was designed to investigate the mechanisms by which a moderate sympathetic stimulus influences AR of renal blood flow (RBF) and glomerular filtration rate (GFR) in 39 experiments in 7 conscious dogs. Common carotid occlusion (CCO) increases renal nerve activity by some 60%, which a moderate sympathetic stimulus influences AR of renal blood flow (RBF) of the contralateral kidney. Common carotid occlusion (CCO) increases renal nerve activity by some 60%., which a moderate sympathetic stimulus influences AR of renal blood flow (RBF) of the contralateral kidney. The sympathetic nervous system may exert an important influence on renal autoregulation (AR). This study was designed to investigate the mechanisms by which a moderate sympathetic stimulus influences AR of renal blood flow (RBF) and glomerular filtration rate (GFR) in 39 experiments in 7 conscious dogs.

Common carotid occlusion (CCO) increases renal nerve activity by some 60%, which a moderate sympathetic stimulus influences AR of renal blood flow (RBF) of the contralateral kidney. The impairment of AR was reversed by an intrarenal infusion of a Ca II antagonist (Prazosin, fig. 1). An intrarenal infusion of the Ca~cadrenoceptor agonist methoxamine induced a similar effect as CCO. In another group it was shown, that a combination of CCO with an intrarenal AII-blockade (saralasin) did not significantly alter the response to CCO.

Atrial natriuretic factor (ANF) antagonizes systemic and central effects of the osmoregulatory hormones vasopressin (VADH) and angiotensin II (ANG-II) and aldosterone (ALDO) by (1) lowering their circulating levels and (2) antagonistic actions at the same target organ. To study receptor-mediated alterations in circulating ADH and ALDO, ANF was infused i.v. at 15 pmol/min/kg b.w. (15 min) resulting in increased plasma levels of 270 ± 55 compared to 63 ± 14 pg/ml at slightly diminished arterial blood pressure (~3 ± 3 mm Hg). Plasma osmolality, oncotnic pressure, sodium concentration and the hematocrit remained unchanged. In animals either slightly dehydrated or pre-injected with 10 pmol/min/kg of ANF, neither ADH and ALDO secretion were suppressed by 20-50 % at unchanged corticosterone levels. Receptor-autoradiography using 125I-labelled ANF revealed high affinity ANF-specific binding sites in the zona glomerulosa of the rabbits' adrenal and renal structures also endowed with receptors for ANG-II. In the hypothalamo-neurohypophyseal axis, the choroid plexus, the heavily vascularized periventricular region of the IIIrd ventricle, the median eminence and the neural lobe - all structures accessible to blood-borne ANF were densely labelled by both radioiodinated ANF and brain natriuretic factor (BNF). Displacement studies support the specificity of the binding sites for ANF/BNF. Endogenous ANF may therefore be of regulatory importance in controlling the release of ADH and ALDO as well as in inhibiting angiotensinergic actions.

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STRUCTURE-ACTIVITY RELATION OF SULFONYLUREA COMPOUNDS (SUC) ON NaCl-TRANSPORT IN ISOLATED PERFUSED RABBIT CORTICAL THICK ASCENDING LIMBS OF HEINE'S LOOP

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Antidiabetic SUCs inhibit ATP-dependent K⁺-channels in pancreatic β-cells; diuretic SUCs inhibit the Na⁺/Cl⁻ K⁺-cotransporter and Cl⁻-channels in the cTAL. This study examines the structure-activity relation of SUCs on equivalent short circuit current (Isc) in cTAL segments. Diuretic SUCs were derivatives of tosarsamide (TOR), and the meta- tolalol- (R1) and the iso-propyl-prop (R2) replaced by cyclo-alkyl residues (6-8). Half maximal inhibition of isc after luminal (IC₅₀ Tor) and after basolateral addition (IC₅₀ BL) and lipid solubility as log of octanol/water-distribution (P) were determined.

![Diagram of tosarsamide and glibenclamide with R₁/R₂ = 6/6, 6/7, 6/8, 7/6, 8/6, 8/7, 8/8]

The introduction of cyclo-alkyl residues increases lipid solubility and preserves the affinity to the Na⁺/Cl⁻ K⁺-cotransporter (e.g. 8/8). Antidiabetic SUCs (glibenclamide, glipizide, glucilamide, glioxoxide, tolbutamide) had no effect from the lumen and basolateral side except for glibenclamide which inhibited ic with an ic of 80 μmol/l. We conclude that antidiabetic SUCs do not inhibit the luminal cotransporter and K⁺-channel nor the basolateral Cl⁻-channel in cTAL. Supported by DFG Gr 480/9.

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ROLE OF LIPOPHILITY IN THE RENAL EXCRETION OF MIDDLE-WEIGHT PROTEINS IN THE RAT

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In order to clarify the role of lipophility in the tubular reabsorption of partially filtered middle-weight proteins, the excretion rate of enzymes of similar size (46 - 57 kDa) but of different isoelectric points (pI) and lipophility was studied in the rat 1.) in vivo after a bolus injection, 2.) in vitro on isolated rat kidney tubules and renal cortical slices. The following enzymes were used: rat pancreatic lipase (BL, 48 kDa, pI: 7.0 ± 6.5 ± 6.3), human pancreatic lipase (BL, 46 kDa, pI: 5.8 ± 5.85), rat pancreatic amylase (RPA, 54 kDa, pI: 4.5 ± 4.5), rat salivary amylase (RSA, 57 kDa, pI: 5.6 ± 4.7 ± 4.5). The injected activity was excreted according to the following pattern (the best filtered protein showing the smallest degree of excretion and vice versa):

| Protein | half life | sieving | extent | protein-bound | coefficient |
|---------|-----------|---------|--------|---------------|-------------|
| BL      | 17.9      | 0.129   | 6.8    | 20.9          |
| RL      | 17.1      | 0.126   | 13.0   | 16.4          |
| RPA     | 20.0      | 0.114   | 15.9   | 60.2          |
| RSA     | 65.0      | 0.023   | 16.2   | 72.9          |

ad 2.) In vitro experiments carried out on renal cortical slices and on isolated kidney tubules show a.) faster reabsorption of 125I-lipases (BL = EL) than of -amylases (RPA ≥ RSA ≥ BPA) for 125I-di-iodo-tyrosine from lipases than from amylases (RPA ≥ RSA). - Extraction of highly purified preparations with neutral paraffin-oil resulted in the decrease of activity by: BL = 99 ± 10; EL = 98 ± 3; BPA = 73 ± 3; RSA = 9 ± 0.1. We conclude that 1.) reabsorption of middle-weight proteins does not occur strictly according to their pI; 2.) lipophility of filtered proteins seems to be of importance for their tubular reabsorption.

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Kinetics of intracellular Ca⁺⁺-changes in single glomerular mesangial cells

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Measurements of intracellular free calcium in individual glomerular mesangial cells (MCs) were done using a microscope fluorimeter with dual wavelength excitation, the Ca⁺⁺-sensitive dyes fura-2, and extremely sensitive, photon counting-based light detection. Fast (up to 200 x s⁻¹) filter changes allow quasi-continuous measurements at different wavelengths (Nobiling and Bührle, J. Micr. Nov 1989:159:149-161).

Cultured MCs are frequently used as models for the closely related vascular smooth muscle cells of the kidney vessels. The important role of Ca⁺⁺ for cellular stimulation, e.g. in renin secretion and contraction, is still widely accepted. Many details, however, are still unclear and subject to extensive work. We report here certain characteristics of the Ca⁺⁺ transients that occur in MCs after the application of the vasoactive peptides AVP and ANG II: Generally, a delayed cellular reaction with respect to the onset of the Ca⁺⁺ increase is observed. This delay is dependent on the concentration of the agonists, but also on the temperature of the superfusion medium. Analysis of the kinetics demonstrates that, obviously, two processes are involved in the generation of these transients. The identification and correlation to cellular processes such as transmembrane currents and or Ca⁺⁺-liberation from intracellular stores may contribute to a better understanding of the processing of stimuli in these cells.
The dependency of the production of the renal glycoprotein hormone erythropoietin (Epo) on the renal O2 supply was studied in the isolated perfused rat kidney (IPRK). The kidneys were perfused at constant perfusion pressure (100 mmHg) in a recirculation system with a substrate enriched Krebs-Henseleit solution containing 60 g/l bovine serum albumin and freshly drawn human erythrocytes. When the kidneys were perfused at an arterial pO2 of 720 or 150 mmHg (hematocrit 5%), Epo production measured by RIA was very low (0.1-0.2 U/g kidney after 3 h of perfusion). At a pO2 of 20 mmHg, Epo production increased significantly to 0.9 U/g kidney. However, the production of Epo was little affected by changes in the hematocrit – i.e. the O2 carrying capacity of the perfusion medium – in a range between 40 and 0%. These results indicate that the production of Epo in the IPRK is mainly under the control of the arterial pO2.

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GSH AND GSSG CHANGES IN KIDNEY, LIVER AND GUT AFTER ACUTE OCCLUSION AND REPERFUSION
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Reduced glutathione (GSH) as specific substrate for GSH-peroxidase plays a critical role in detoxification of hydrogen peroxide and other reactive oxygen species. The relationship between GSH and its oxidized disulfide form (GSSG) is thought to have an important effect on oxidation-reduction state of protein-thiols, resulting in enhanced or diminished activity of, e.g., catalytic enzymes.

In male wistar rats we investigated the tissue concentration of GSH and GSSG in controls. After 45 min of subdiaphragmatic aortic occlusion, and after reperfusion of 45 min, GSH and GSSG were determined by a modification of the method of Griffith (Anal. Biochem. 105, 207, 1980). Tissue was homogenized in 5% 5-sulphosalicylic acid immediately after dissection to prevent GSH-loss and conversion of GSH to GSSG. GSH in controls amounted to (x ± SEM in µM/g wet weight): kidney 2.0 ± 0.1, liver 6.1 ± 0.6, gut 2.2 ± 0.0. After 45 min of occlusion the values decreased by 29.7%, 21.8% and 17.1% resp. (p < 0.05). 45 min after reperfusion the concentrations no longer differed significantly from controls. GSSG levels (x ± SEM in µM/g ww) in controls were: kidney 35.9 ± 4.4, liver 195.0 ± 18.6, and gut 9.2 ± 4. After the occlusion period the values decreased by 51.5 % in the kidney and 38.1 % in the liver. They rose by 114.1 % in the gut. (p < 0.01). After reperfusion in the kidney a slight increase by 23.7 % of control occurred, but without statistical significance; in liver the decrease continued by 60.6% of control, whereas in the gut a large increase by 279.3% of control took place (both values significant, p < 0.05).

Using the GSSG/GSH ratio as a measure of recovery from occlusion - reperfusion stress, we interpret a decrease in this ratio, as in the liver, as a good response, a nonsignificant increase, as in the kidney, as a fair response, and an increase, as in the gut, as a progressive injury during reperfusion.

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The role of the interstitial fluid volume (ISVF) in body fluid homeostasis is heavily debated. Using the salt-acclimated Pekin duck as a model, we attempted to elucidate the influence of ISVF on efferent and afferent control of renal function including the contribution of the osmoregulatory hormone ArgVal Vasotocin (AVT) and ValValAngiotensin (AII). During steady-state diuresis driven by systemic infusion of 11 ml/min of isotonic Krebs-Ringer solution (KR), dextran-70 (5 %) was added for 30 min inducing directed fluid shifts between the two extracellular fluid compartments. Hematocrit decreased from 36.4 to 32.3 % with concomitant rise in plasma colloidosmotic pressure from 9.5 to 12.9 mmHg at constant plasma osmolality and electrolyte concentrations. Depletion of ISVF caused a marked antidiuresis to < 50% of control values accompanied by reduced osmolar excretion. Effective renal plasma flow dropped from 27.3 to 21.7 ml/min/kg and glomerular filtration rate decreased from 3.3 to 1.7 ml/min/kg of kidney. All these effects were induced by infusing increasing amounts of ISVF, which remained unchanged excluding their involvement in the observed reactions. Cardiovase side-effects of the dextran application can be ruled out due to the constancy of mean arterial pressure and central venous pressure. Thus, our results suggest a contribution of the ISVF to renal function in the salt-acclimated Pekin duck.

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Functional ANF systems are involved in body fluid homeostasis of vertebrates regulating electrolyte and water transport. In addition to the kidneys as osmoregulatory organs, marine birds possess supraorbital salt glands to excrete a strongly hypertonic salt solution (NaCl). To elucidate the role of chicken ANF (cANF) in the control of salt gland function, conscious salt-acclimated Pekin ducks received 15 pSs/min/kg b.w. cANF for 10 min at two states of salt gland activity. (1) During steady-state diuresis and salt gland activity induced by systemic infusion of 1.1 ml/min isotonic Krebs-Ringer solution, cANF applied i.v. enhanced secretion rate from 0.21 to 0.29 ml/min and osmolality of secretion from 870 to 910 mOsm/kg. At threshold conditions of salt gland activity, cANF infused i.c. stimulated secretion rate from 0.07 to 0.15 ml/min at elevated osmolality of 760 compared to 480 mOsm/kg. Employing receptor autoradiography with 125I-BH labelled cANF as ligand, specific binding sites could be demonstrated throughout the salt and tissue of both fresh-water (FD) and salt-acclimated (SD) animals. Scatchard analysis using an enriched membrane fraction revealed high affinity (Kd = 0.9 nM) binding sites of 270 fmol/mg protein density by radioreceptor assay. Displacement studies with unlabelled chicken and human ANF showed comparable Kd values for both peptides in FD as well as SD, suggestive of the same receptor structure to be recognized by the receptor. Our results indicate an important role for ANF in salt and water homeostasis of birds.

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The ISVF and its contribution to the regulation of renal function including the afferent and efferent control of the salt and water metabolism of birds are heavily discussed. To elucidate the role of the ISVF in renal function, conscious salt-acclimated Pekin ducks were used. ANF was infused i.v. and i.c. and the response on the salt gland was measured by determining the sodium and potassium content of the secretions. The results showed that ANF significantly increased the secretion rate and osmolality of the secretions. Furthermore, the hemocrit decreased significantly after ANF infusion, indicating a fall in filtration fraction. Therefore, the role of the ISVF in the regulation of renal function was evaluated using conscious salt-acclimated Pekin ducks. The results suggest a contribution of the ISVF to renal function in the salt-acclimated Pekin duck.
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INFLUENCE OF MAXIMAL AND SUBMAXIMAL EXERCISE UNDER NORMOXIC AND OXYGEN DEFICIENCY CONDITIONS ON PLASMA ATRIAL NATRIURETIC PEPTIDE AND ALDOSTERONE LEVEL.

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The present study was designed to investigate the influence of exercise intensity and duration as well as of inspiratory oxygen content on plasma atrial natriuretic peptide (ANP) concentration and to compare the behavior of plasma aldosterone. Nine untrained male subjects performed a vita maxima test (KB) on a bicycle ergometer and a 60 min lasting submaximal test with 60% of maximal performance (SE) under normoxia (N) and normobaric hypoxia (H), (P02 92 mmHg). Five subjects were exposed to resting hypoxia for 90 min. [ANP] was mostly affected by exercise intensity (5 min after ME-N: +29.8 ± 11.7% and less by exercise duration (at the end of SE-N: +22.9 ± 6.8%). Hypoxia has no effect at rest and reduces the exercise response (ME-H: +184.3 ± 76.8%, SE-H: +172.4 ± 41.5%). In contrast to ANP, Aldo response was more affected by duration at submaximal level (+230.1 ± 89.9%) than by short maximal exercise (+235.7 ± 62.7%). Hypoxia exposure rapidly decreased [Aldo] (-28.5 ± 7.4% after 30 min., 2p < 0.01) but did not influence the exercise effects (ME-H: +206.2 ± 69.7%, SE-H: +321.6 ± 126.3%). [ANP] increase was faster than [Aldo] during the maximal tests and not different during submaximal exercise. Changes in plasma volume, sodium, and osmolality were most pronounced during maximal exercise (for ME-H: Fv = -13.1 ± 3.6%, sodium +5.9 ± 2.7 mmol/l, 0.01 < p < 0.05, 3.6 ± 6.5 mmolosmol/kg). Regression analysis yields higher correlations between changes in [ANP] and osmolality (ME-N: r=0.64) than for changes in sodium (r=0.51), heart rate (r=0.41), blood pressure (r=0.56), and changes in plasma volume (r=0.51). It is concluded that besides other mechanisms increased osmolality might be involved in the exercise dependent increase of plasma [ANP].

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FURTHER STUDIES ON REGULATORY VOLUME DECREASE (RVD) IN MADIN DARBY CANINE KIDNEY CELLS.

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As shown previously, Madin Darby Canine Kidney (MDCK)-cells are able to regulate their cell volume in hypotonic extracellular fluid. If exposed to hypotonic media (removal of 80 mmol/l mannitol), they initially swell but then gradually shrink close to their volume in isotonic extracellular fluid. In the present study cellular cable analysis has been applied to determine the potential difference across the cell membrane (PD), the cell membrane resistance (Ron) and the intracellular coupling resistance (Rvd). Furthermore, fluorescence measurements have been performed to determine intracellular calcium (Cai) and hydrogen ion (pHi) concentration. Exposure of MDCK cells to hypotonic perfusate leads to a transient hyperpolarization of the cell membrane, followed by a sustained depolarization, to a transient increase of Re and a sustained depolarization of the cell membrane. Cai approaches 67 ± 8 nmol/l and sodium approaches 144 ± 7 mmol/l. Decrease of sodium and water movement into the cell is paralleled by an increase of chloride concentration (from 0.02 to 0.34 mmol/l) and leads to a significant acidification (pH 7.05 ± 0.04) of the cellular fluid. In conclusion, RVD in MDCK cells is not only in part accomplished by the activation of an anion channel, and is paralleled by intracellular acidification. In the unstimulated MDCK cells does apparently not require a substantial increase of Cai.

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UPTAKE OF SO435 IN RAT PAPILLARY COLLECTING DUCTS (PCD) STUDIED IN THE ISOLATED PAPILLA *

A. Heuner (with the techn. assist. of M. Speier and M. Dass)

Microperfusion studies were performed with PCD in situ in the isolated papilla of the rat kidney to study the uptake of SO435 in this final segment of the nephron. Male Wistar rats (about 250g b.w.) were anesthetized with Inactin intraperitoneally and the kidneys removed by an abdominal approach. The papilla was prepared according to the method of Morgan et al. (Am. J. Physiol. 214, 574-581, 1968) as modified by Hafferle et al. (Renal Physiology 9, 54, 1986). The exercised papilla was placed in a special chamber containing a bathing medium: Urea 700 mm, NaCl 150 mm, NaHCO3 25 mM, sodium acetate 20 mm, Glucose 33 mm, KCl 10 mm. Na2SO4 2.4 mm, MgSO4 2.4 mm, CaCl2 2 mm (Marck). The ducts were microperfused at 30 ml/min with the following solution: Urea 700 mm, NaCl 150 mm, NaHCO3 25 mM, KCl 10 mm, K2HPO4 5 mm, MgSO4 2.4 mm, CaCl2 2 mm and containing Inulin-H3 and SO35 (Amersham Buchler). The bathing medium was also used to perfuse the vasa recta. ADH (10-8 M Sigma) was added to the bathing medium in some experiments. Only results with Inulin-H3 ratios - collected fluid (CF)/ perfused fluid (PF) - of 0.95 to 1.0 were accepted. Although the results obtained are from different lengths of perfused segments of PCD (from 0.2 to 1.5 mm) the pooled results are reported here. Under these conditions the (CF/PF)SO35 ratios were 0.87 ± 0.07 (57) (mean ± SD, n), a value significantly less than 1. Addition of ADH did not change these SO35 ratios (n=48) significantly. It is concluded that SO35 disappears from the perfusion fluid. This may represent either binding to the apical membrane or transport across this membrane into the cellular fluid. Further experiments are necessary to elucidate the transport of this divalent anion in these cells.

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* These experiments were performed in the laboratory of Dr. D.A. Hafferle.

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MERCAPTURIC ACID FORMATION IN CULTURED OPOSSUM KIDNEY CELLS (OK)

N. Golenhofen, A. Heuner, S. Mildenberger, J. S. Schwegler, S. Silbernagl

The kidney is known to participate in mercapturic acid formation. We investigated the last step of this formation, the N-acetylation of cysteine S-conjugates, in the established OK kidney cell line, which shows characteristics of the proximal tubule. We used S-benzyl-L-cysteine (BC) as model substance for such a cysteine S-conjugate. Methods: OK cells were grown on plastic petri dishes of 3.5 cm diameter. The intracellular concentration of BC was measured in confluent monolayers incubated with medium containing 1 mmol/l BC, in some experiments 1 mmol/l BC and 30 mmol/l L-phenylalanin. The concentration of the mercapturate N-acetyl-S-benzyl-L-cysteine (AcBC) in the medium, i.e. in the extracellular space, was measured during incubation with various BC-concentrations (0.75mm, 1.0mm, 1.25mm ). BC and AcBC were quantified by reversed phase HPLC. Results: BC accumulated in the intracellular compartment to twenty times more than in the extracellular one, this accumulation being significantly reduced by the addition of 30 mmol/l L-phenylalanin. After uptake into the cells BC was acetylated and the acetylated product occurred at a rate of 20 mmol/h/dish in the extracellular space. This rate was independent from the extracellular BC-concentration. Conclusions: OK cells reabsorbed BC via the neutral amino acid carrier. In the intracellular space BC is transformed to AcBC which is secreted into the extracellular Acetylating and/or secretion proceeds to a maximum rate of 20nmol/h/dish.

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EFFECTS OF ALTERATION OF MIXED VENOUS PO2 ON THE BLOOD VOLUME REGULATION
B. Flemming, D. Roloff, T. Wróński, C. Bauer, and W. Jelkmann

An extracorporeal veno-venous bypass applying an oxygenator to alter mixed venous pO2 (pO2mv) nearly about 1.3 kPa was used in experiments on anesthetized cats. Comparing the alterations of pO2mv in cats in osmotic diuresis the higher level of pO2mv was connected with an increase of the renal sodium excretion (60%) (B. Flemming et al., Biomed. Biochim. Acta 44:1687, 1985) if the tip of the reperfusion catheter was located in the inferior vena cava. The same effect was found in rats after administration of plasma or its different fractions (low molecular weight fraction (MW700), lipid soluble extract) from cats with high pO2mv (D. Roloff et al., Physiol. Bohemoslov. 37:83, 1988). The renal sodium retention of cats with low pO2mv in chloralose-urathana anesthesia was coupled with a significant increase of erythropoietin blood level (25 mmol/l after 2 h) without significant changes of clearances of PAH and Inulin.

Our results seem to suggest a possible role of decreased mixed venous pO2 on renal sodium retention and the release of erythropoietin in the blood volume regulation, i.e. in anemia, heart failure, heavy exercise and so on.

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ROLE OF AN INCREASE IN K+-PERMEABILITY (P) OF THE PANCREATIC B-CELL MEMBRANE FOR THE INHIBITION OF INSULIN RELEASE BY ADRENALINE AND GALANIN
G. Drews, A. Debuyser, J.C. Henquin

The mechanisms by which adrenaline and the neuropeptide galanin affect pancreatic B-cell function were studied with mouse islets. In B-cells stimulated by 15 mM glucose, adrenaline (100 nM) and galanin (50 nM) caused a transient hyperpolarization followed by a sustained partial inhibition of electrical activity. These changes were accompanied by a biphasic decrease in V-K efflux from islet cells and nearly complete (90%) inhibition of insulin release. Diazoxide (10-15 mM), a selective activator of ATP-sensitive K channels largely mimicked these effects. When P was markedly increased by 100 μM diazoxide and the hyperpolarization reversed by high K+, adrenaline and galanin were without effect on the membrane potential and V-K efflux but still partially inhibited insulin release. Tolbutamide (which blocks K-ATP channels) or arginine (which depolarizes because of its transport in a positively charged form) largely prevented the ability of adrenaline and galanin to affect the membrane potential of B-cells. They also decreased the inhibitory potency of galanin on galanin release. In conclusion, inhibition of insulin release by adrenaline and galanin involves at least two mechanisms: a partial repolarization of the B-cell membrane through an increase in P, and a mechanism independent of changes in membrane potential.

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CALCIUM CURRENTS IN SECRETORY CELLS OF RAT ANTERIOR PITUITARY THIN SLICES.
M.B. Jackson, S.A. DeRiemer and A. Konnerth

Thin slices prepared from the pituitaries of 15 to 25 day-old rats were studied with the patch clamp technique under Nomarski optics to allow visual identification of cells (Edwards et al, Pfliigers Archiv 414, 1989). In the presence of tetrodotoxin to block Na+ currents, and with intracellular Cs and TEA to block K+ currents, depolarizing voltage steps from negative holding potentials activated Ca2+ currents in all cells tested. In recordings made from the most frequently observed (80-90%), "small" cell type (diameter 6 to 9 μm), a high voltage-activated, non-inactivating Ca2+ current was observed, which was partially blocked by either nimodipine or omega-conotoxin, and enhanced by BayK 8644. A complete block was observed following application of 20 μM Cd2+. In contrast, 100 μM Ni2+ was ineffective. The "large" secretory cells of the anterior pituitary (diameter 9 to 15 μm) had Ca2+ currents with an inactivating and a non-inactivating component. The transient component could only be activated from holding potentials equal to or more negative than -80 mV. The non-inactivating Ca2+ current resembled the current observed in the small cell type in terms of its activation properties. This study shows that cells in the intact pituitary can be distinguished on the basis of both size and membrane characteristics. By combining this approach with immunocytochemical techniques, we hope to elucidate and distinguish different membrane mechanisms in the regulation of secretion of different pituitary hormones.

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SINGLE CHANNEL RECORDINGS OF VOLTAGE-DEPENDENT Ca2+ CHANNELS IN FUSED ENDOPLASMIC RETICULUM VESICLES FROM RAT EXOCRINE PANCREAS.
A. Schmid, I. Schütz and H. Gögelein

The endoplasmic reticulum (ER) is known to play a central role in regulation of cytoplasmic free Ca2+ concentration. With the deactivation/rehydration method we fused isolated ER vesicles from rat exocrine pancreas and investigated the fused vesicles by means of the patch-clamp technique. With K+-solution (75 mmol/l KCl, 280 mmol/l HEPES, 10 μmol/l BaCl2) in the pipette and Ba2+-solution (50 mmol/l Ba(OH)2, 280 mmol/l HEPES) in the bath single channels with a mean conductance of 47±4 pS (n=15) were recorded. The channel activity was markedly voltage regulated. At positive clamp potentials (0 to +30 mV, sign referred to the bath) the channel was most of the time in its open state, whereas, small negative voltages (-10 mV) caused channel inactivation. The extrapolated reversal potential is more negative than -30 mV indicating a P Ba2+/P K+ ratio of at least 5:1.

Replacement of Ba2+ by Ca2+ demonstrated that the channel is also permeable to Ca2+. Caffeine (10 mmol/l) in the bath or in the pipette activated the channel in part of the experiments. Ruthenium red (100 μmol/l) in the pipette caused complete channel inhibition. Ryanodine (0.1-200 μmol/l) and nifedipine (10-100 μmol/l) on either side of the membrane patch had no effect on channel activity. The channel was not dependent on the free Ca2+ concentration (<10-5 to 10-3 mol/l) on both sides. We conclude that the voltage-dependent Ca2+ channel mediates Ca2+ release from a caffeine and ruthenium red sensitive but IP3-insensitive intracellular Ca2+ pool.

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CHOLECYSTOKININ (CCK) ACTIVATES DIFFERENT GTP-BINDING PROTEINS (G-PROTEINS) IN RAT Pancreatic ACINAR CELLS

S. Schnefel, A. Pröfrock, K.-D. Hirsch and J. Schulz

CCK activates phospholipase C (PLC) via 40 kDa G-protein(s) (S. Schnefel et al., FEBS Lett. 230:125, 1988). Following ADP-ribosylation of pancreatic plasma membranes with cholera toxin (CT) or with pertussis toxin (PT), three 40/41 kDa proteins with pI-values of 5.30, 5.60 and 5.75 were detected by two-dimensional gel electrophoresis. In addition, CT ADP-ribosylated five 45 and five 48 kDa proteins with pI-values of 5.60, 5.80, 5.95, 6.10, 6.30 and 5.35, 5.50, 5.65, 5.70 and 5.80, respectively, presumably corresponding to the low and high molecular forms of Gα subunits of the adenylyl cyclase. CCK enhanced CT-induced ADP-ribosylation of one 45 and of all 48 kDa proteins and decreased CT- and PT-induced ADP-ribosylation of all 40/41 kDa proteins. Incorporation of the photoaffinity analogue [32P]GTPγ-s-azidoanilide into the three 40/41 kDa proteins and into one 48 kDa protein was stimulated by CCK. Using affinity purified antipeptide antibodies raised against specific sequences of aminoisobutyric α-subunits of Gα1, Gα2 and Gα3 we identified the three 40/41 kDa proteins as Gα1, Gα2 and Gα3. The data indicate that CCK-receptors functionally interact with Gα1 and with three Gα-proteins: Gα1, Gα2 and Gα3. We assume that one, two or all of the three Gα-proteins are involved in regulation of phospholipase C activity in pancreatic acinar cells.

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MEASUREMENT OF MAGNETIC RELAXATION ABOVE THE LIVER BY MEANS OF NEEDLE-SHAPED GAMMA Fe3O4 PARTICLES

J. Lutz, A.J. Augustin and J. Milz

The alignment of magnetic particles, phagocytosed in the liver of laboratory animals can be sensed magnetically after application of a strong external magnetic field (Gehr et al., Nature 302: 336 (1983); Weinstock et al., Cells of the Hepatic Sinusoid J. Acad. Press NY p. 51 (1986)). By this method the capacity of the macrophage system under different loads can be examined. In contrast to formerly used Fe3O4 (magnetite) particles or Fe2O3 particles, gained by coagulation of iron paste on carboxyly, we used a suspension (50 mg/ml) of needle-shaped Fe3O4 particles of ca. 0.3 μm length with a diameter of less than 0.05 μm, as used for production of tape material (BASF, Ludwigshafen, FRG).

Mala Wistar rats were injected iv. with 2.5 to 5 mg/kg bwt. of the iron oxide and were magnetized after different time intervals in a magnetic field of 0.26 Tesla (2600 Gauss) for 30 s. Immediately afterwards the animals were put into a magnetically shielded chamber. The depleted skin area above the right lower rib cage corner was held in close contact to a double FOERSTER probe (Magnetoscorp 1.068, Inst. Dr. Foerster, Reutlingen, FRG) in a gradiometric mode of field detection. From the curves of declinamagnetism the relaxation constant, κ, was calculated according to:

κ = ln (cN - cO) / (tN - tO)

κ and the correlation coefficient, r, were determined from curves fitted by the method of least-squares to 12 values obtained between 0.3 and 10 min after the end of magnetization. 95% of the curves gave a correlation coefficient r > 0.940.

By these experiments it could be shown that needle-shaped magnetic particles rapidly change their alignment in vivo and that this change is markedly slowed by substances that are taken up by sessile macrophages.

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WILHELM SANDER-STIFTUNG 88.034.1

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pH-RECOVERY AND H+ -EXTRUSION IN ISOLATED RABBIT PARIELAL CELLS IN THE ABSENCE AND PRESENCE OF CO2/HCO3: Na+/H+ EXCHANGE, Na+/HCO3- COTRANSPORT, OR BOTH

G. Lamprecht and U. Seidler

Recovery of pH1 after an intracellular acid load requires active H+ - extrusion mechanisms. In some cell types, the predominant acid extrusion mechanisms in the absence of CO2/HCO3: is the Na+/H+ exchanger, whereas in the presence of CO2/HCO3: a Na+/HCO3- cotransporter becomes the predominant acid extruder. Controversy exists as to the situation in the parietal cell. To clarify the issue we measured intracellular pH-recovery capacity (β), pH1-recovery (ΔpH1/dt) and H+ -extrusion rates (dpH1/dt x β) in isolated rabbit parietal cells in the presence and absence of HCO3: (required for Na+/HCO3- cotransport), of 1 mM amiloride (Na+/H+ exchange inhibitor), and of external Na+ (Na+/K+ exchange inhibitor). All experiments were performed at an extracellular pH (pH1) of 7.4 in a free buffer (to suppress CI/HCO3- exchange activity). pH1 was measured fluorometrically after loading isolated CI-depleted rabbit parietal cells with the pH-sensitive dye BCECF. β1 was determined over the pH1 range from 6.4 - 7.4 in the absence and presence of 20% CO2/86 mM HCO3-.

Results; β1 in the absence of CO2/HCO3: decreased from 54±6 mM/mph1 unit at pH1 6.4 to 21±4 mM/mph1 unit at pH1 7.4. In CO2/HCO3- β1 increased from 72±2 mM/mph1 unit at pH1 6.4 to 229±4 at pH1 7.4. Due to this difference in β1 a higher intracellular acid load was required to acidify the cytoplasm to the same pH1 (6.7) in the presence than the absence of CO2/HCO3:.

The initial H+ -efflux rate in the absence of CO2/HCO3: was 9±1:3 mM/min and dropped very rapidly (t1/2=15 min) as pH1 approached the steady-state levels (7.4). In CO2/HCO3-, the initial H+ -efflux rate was the same (8.2±0.9 mM/min), but the efflux rate remained high for a much longer time (t1/2=4.5 min). The dependency of H+ -efflux rate on pH1 showed a strong inverse correlation (demonstrating the steep pH1-dependency of the Na+/H+ exchange rate on the pH1), but was identical with or without CO2/HCO3:, arguing against an additional acid extrusion mechanism in the presence of CO2/HCO3:.

In the presence of 1 mM amiloride, maximal H+ -efflux rates were inhibited to the same degree (78±4 and 77±7) in the absence and presence of CO2/HCO3:. efflux was abolished in the absence of Na+ with or without CO2/HCO3:. Summary and conclusions: During recovery from an identical acid load, the parietal cells had to extrude a higher acid load in the presence than in the absence of CO2/HCO3:, resulting in higher overall H+ -extrusion rates during pH1-recovery. For a given pH1 the H+ -efflux rates were identical with and without CO2/HCO3:, arguing against a role of the Na+/HCO3- cotransporter in acid extrusion from isolated rabbit parietal cells. The only identifiable acid exchanger in this experimental setting was the Na+/H+ exchanger.

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STEADY-STATE pH (pH1) IN GASTRIC PARIELAL AND SURFACE, BUT NOT CHIEF CELLS, IS MORE ACIDIC IN THE PRESENCE THAN THE ABSENCE OF HCO3-: IS THIS DUE TO CI-/HCO3- EXCHANGE, CI-DEPENDENT HCO3- TRANSPORT, OR BOTH?

U. Seidler and W. Silen

Cellular pH-regulation systems have usually been studied after the application of acute intracellular acid load and base loads. What actually determines the steady-state pH1 has remained largely speculative. We have found that isolated and highly purified rabbit parietal and surface cells, but not chief cells, have a consistently more acidic steady-state pH1 in the presence than in the absence of CO2/HCO3- (Talseth et al., Am. J. Physiol. 257: G101-107). This effect could be due to HCO3- efflux in exchange for CI- or for electrogenic HCO3- efflux, possibly coupled to Na+.

Other known HCO3- dependent ion transport mechanisms alkalinize the cytosol and therefore cannot explain the observed effect. Methods: To clarify this issue, pH1 was measured fluorometrically in highly purified rabbit parietal, chief and surface cells after loading with the pH-sensitive dye BCECF and incubation for 30 min, 1, 2, and 3 h in buffer that contained either CI- and HCO3-, only CI- or HCO3- for each of the four conditions at buffer pH-values for the pH1-range from 6.2 to 7.8. Results: All three cell types in- and decreased steady-state pH1 with in- and decreasing pHb, but the difference between pH1 and pHb also increased both a high and low pHb, demonstrating a remarkable capability of the cells to maintain a pH-gradient for very long time periods. In chief cells, steady-state pH1 was not significantly different in the presence or absence of either HCO3- or CI for any pHb tested. In surface cells, the presence of CI- resulted in a more acidic steady-state pH1 (at equivalent pHb), which was not influenced by the absence or presence of CI-. In parietal cells, the presence of CO2/HCO3: resulted in a more acidic pH1 both in the presence and absence of CI-, but more so in its presence.

Conclusions: 1) All three gastric cell types were able to maintain a considerable pH-gradient between cell interior and surrounding medium. 2) Maintenance of chief cell pHb does not involve CI- or HCO3- dependent ion transporters. 3) In surface cells, CI-dependent and CI- independent acid extrusion mechanisms contribute. 4) In parietal cells, the more acidic steady-state pH1 in the presence of CO2/HCO3: is predominantly caused by CI- independent HCO3- efflux, and partly by CI/HCO3- exchange.

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9.3, 9.0
EFFECTS OF ENTERAL AND PARENTERAL APPLICATIONS OF DEXTROSE SOLUTIONS ON THE MOTILITY OF THE GUT IN CONSCIOUS RATS

D. Asiz, W. Reschke and G. Fischer

Chronic catheters were implanted in the antrum, duodenum, jejunum, v. cava, A. carotis, A. mesenterica of rats. Solutions of 5.2 % (isotonic) or 30 % (as used for total parenteral nutrition) of dextrose were infused at a rate of 100-500 pl/min for 2.5 - 10 min in the various sites and manometric measurements of motility performed in one or more sites in the gut. The aim was to study the ability of the nutrient to increase motility regardless of luminal load, b) to disperse phase III of the migrating motor complex (MMC), c) to induce retrograde inhibition of motility (brake effect).

Results: Both enteral and parenteral 5 % dextrose increases motility in a variable manner compared to isotonic NaCl. Intracarotid infusions to the CNS do not enhance the effect. Infusions 3 - 6 cm distal to Oddi’s sphincter inhibit motility in the prox. duodenum. 30 % dextrose into the antrum changes phase I immediately to phase II-like activity in the duodenum and proximal jejunum and disrupts the MMC. Into the A. mesenterica sup., an enhancement of the next 2 phases II is observed, with subsequent abolishment of MMCs, particularly in the distal jejunum. It is concluded that dextrose can affect motility from the blood side.

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INTESTINAL AND HEPATIC LIPID PEROXIDATION AFTER AORTIC OCCLUSION AND REPERFUSION

A.J. Augustine, E. Purucker, J.S. Schwegler and J.Lutz

Ischemia induced oxygen free radical damage can be initiated in different ways, e.g. the xanthine oxidase mechanism or activated neutrophils. The initiating system, coming to the fore, seems to be dependent on the respective ischemic tissue. This reveals to the different ischemic tolerance of the organs.

Experiments were done with 12 male wistar rats by reversibly occluding the aorta subdiaphragmatically for 45 min. In one group the aorta was reopened for 45 min (reperfusion time); a second group without reflow served as controls. The tissue level of lipid peroxides (LPO) was determined by a modification of the method of Ohkawa et al., Anal. Biochem. 95, 351 (1979).

The intestinal tissue level increased during the ischemic period from 68 ± 29 nmol/g (basic LPO - content ± SEM) to 641 ± 40 (p<0.001), whereas the liver LPO-level remained nearly constant (127 ± 17 vs. 125 ± 13). In the reperfusion period the intestinal lipid peroxidation went up to 1029 ± 155. LPO-content of the liver increased to 480 ± 59 (p<0.05) after this time.

These data indicate intestinal oxygen free radical damage already in the ischemic period, whereas hepatic tissue sustains damage only during reperfusion. This between tissue ischemic tolerance of the liver is explicable by the higher oxygen extraction ratio (Lutz et al., Pflüg. Arch. 260, 7, 1975) and - concerning free radical damage - by the different sensitivity of the initiating radicals in intestine and liver. Neutrophils should play a major role in the intestine, whereas liver tissue can be primarily altered by the xanthine oxidase mechanism.

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DIURESIS IN FORMICA POLYCTENA AND ACHETA DOMESTICA: EFFECT OF CRUDE EXTRACTS AND PARTIAL PURIFIED FACTORS.

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When studying the Malpighian tubules of Formica throughout the year, ants were found with inactive tubules and others with tubules secreting at various rates (8-800 pl/min.). This could be due to the presence of different levels of a hormonally regulated activity. Crude head extracts were made and tested in 2 diuretic bioassays, which measure the volume of the secreted droplet of the Malpighian tubule, in a given time interval. In a slow Formica assay (Van Kerkhove et al.,1989), the crude extract gave 2 successive stimulations of the fluid secretion, one when the extract was added in the bath solution and a second when the extract was washed out. In a quick Acheata assay (Coast,1988), a single diuretic effect of the crude extract was observed. Purification of the crude extract was performed by means of RP-HPLC. It resolves the activity into 2 major fractions, which were active in the Acheata bioassay. On a Bio-rad Hi-pore semi-prep. column, their retention times were 29-34 min. and 34-39 min. The average effect of the stimulation increased fluid secretion with 65 % for the 29-34 fraction and with 45 % for the 34-39 fraction. Further purification of these active fractions is accomplished by addition of RP-HPLC.

These studies provide evidence for the presence of diuretic factors in Formica and a sensitive and quick bioassay for these factors.

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Van Kerkhove E., Weitens R., Roinel N. and De Decker N., (1989), J. Insect Physiol.(in press.)

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GTP-β-S INDUCES CALCIUM OSCILLATIONS BUT NOT EXOCYTOSIS IN MAST CELLS.

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Several G proteins are involved in stimulus-secretion coupling in rat mast cells. GTP-γ-S has been found to induce a transient rise in intracellular calcium as well as mast cell degranulation. In an attempt to further differentiate the roles of various G proteins in this response, the thiosylated GTP-analogue GTP-α-S and GTP-β-S (R- and S- isomers) were introduced into rat peritoneal mast cells via a patch pipette. Degranulation and intracellular calcium were monitored by cell capacitance and Fura-2 measurements.

R-GTP-α-2 has effects similar to, but somewhat weaker than, GTP-γ-S (the S-isomer was largely ineffective). GTP-β-S (R-S-isoener more so than the S-form) was found to induce repetitive large calcium spikes which were not regularly accompanied by degranulation. These calcium oscillations appeared with an average latency of 230 s and continued for up to 15 min before damping out. They were thus distinct from the rapid and short calcium changes mediated by GTP-γ-S and R-GTP-α-S.

The oscillations were independent of extracellular calcium. They were abolished by high concentrations of IP3 (10 μM) as well as kainin (500 μg/ml) and GDP-β-S (300 μM), implicating G protein-mediated PI turnover in their generation. Their frequency could be modulated by changing the intracellular ATP concentration.

These results provide further evidence that calcium transients are not necessarily linked to exocytosis. They suggest that GTP-β-S selectively activates a G protein related to intracellular calcium signalling. The system could moreover provide a valuable model for studying the generation of calcium oscillations.

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EPITHELIAL ION TRANSPORT IN THE SHORT BOWEL SYNDROME

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Epithelial ion transport and morphology in the short bowel syndrome was characterized in vitro using rat ileum 2 months after 70% and 80% small intestinal resection.

Subepithelial (Rbasol) and epithelial resistance (Re) was determined by impedance analysis. Re decreased from 2.5 ± 0.1 to 1.9 ± 0.1 Ω cm² (P < 0.01) in the short bowel. Due to variable Rbasol at different stages of intestinal adaptation, comparison of active transport rates requires correction for the ratio (Rbasol/Re) which was 1.6 ± 0.1 in control and 2.5 ± 0.2 (P < 0.01) in short bowel. These rates were measured in HCO3-containing medium. Fluxes were corrected for bath and subepithelial resistance.

In control ileum, net Na- and Cl- absorption were of same magnitude compatible with electroneutral NaCl-absorption. I sc was due to the residual flux and assigned to HCO3-secretion. Neither NaCl-absorption nor HCO3-secretion was significantly changed in the short bowel. However, Na/glucose-cotransport (measured as the glucose-induced change in I sc) increased in Vmax from 2.0 ± 0.3 to 5.0 ± 1.0 μA cm² cm⁻² (P < 0.01) in short bowel, while K remained unchanged.

In freeze fracture EM of tight junction structure showed no change in strand number, but a slight increase in tight junction density in short bowel. This, however, can not explain the decrease in epithelial resistance and may be due to the higher mitotic index in short bowel. In contrast, microdissection morphometry showed an increase in villus height from 121 ± 5 μm (n = 17) to 168 ± 6 μm (P < 0.05) in short bowel.

We conclude that the mucosa in the short bowel syndrome is characterized by decreased epithelial resistance per cm² of gross area due to mucosal surface amplification. HCO3-secretion through electroneutral NaCl-absorption is unchanged, while glucose-coupled Na-absorption increases to 250% in the short bowel syndrome. This can be considered as an adaptive response to the reduced absorptive area of the remaining intestine.

Depot: Gastroenterology and Clinical Physiology, Klinikum Steglitz, FU Berlin, FRG; 2Dept. Medicine, East Carolina University, Greenville, NC, USA. DFG Schu 559/2-1

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NONSELECTIVE CATION-CHANNELS IN THE BASOLATERAL MEMBRANE OF CRYPT CELLS FROM RAT DISTAL COLON

C. Slener and H. Gégout

Intact crypts from rat distal colon were isolated and investigated with the patch-clamp technique. The isolated distal colon was inverted and filled with a Ca²⁺-free NaCl-solution containing 1.25 mM CaCl₂ (in mM: 127 NaCl, 5 KCl, 1 MgCl₂, 5 glucose, 5 Na-pyruvate, 10 HEPES, 1% albumin, pH 7.4). After incubation in Ca²⁺-free NaCl-solution at 37°C for 15 minutes, entire crypts as well as single cells were obtained by rapid shaking. The crypts were twice centrifuged at 500 g for 1 minute and resuspended in a NaCl-solution containing 1.25 mM CaCl₂ (in mM: 127 NaCl, 5 KCl, 1 MgCl₂, 5 glucose, 5 Na-pyruvate, 10 HEPES, 1% albumin, pH 7.4). The isolated crypts were stored on ice until use.

We investigated single channel currents in the basolateral membrane of cells from the bottom of the crypt. All experiments were performed at 35±1°C. In cell attached and cell excised patches nonselective cation-channels with a conductance of 37 ± 0.6 pS (n = 87) were found, which did not discriminate between potassium and sodium ions and which were impermeable to chloride ions. In some cell attached experiments the channel could be evoked by carbachol (100 μM, n = 6) or by the calcium-ionophore A23187 (1 μM, n = 2) added to the bath-medium. The channel was inhibited, when free Ca²⁺ was decreased at the cytosolic side (n = 5).

Previous investigations (Gégout et al., Pflügers Arch. 411, R107) have shown that the Na transport across the rumen epithelium can be stimulated by weak acids like short chain fatty acids (SCFA). The stimulation was mainly due to activation of a Na⁺/H⁺ exchange on the mucosal side of the tissue. It was demonstrated that the permeability for Na⁺ increased in the opposite direction, i.e. whether Na may influence the permeation of weak acids. For that purpose unidirectional flux rates of Na⁺ and D-benzoic acid were measured under short circuit conditions across isolated, stripped rumen mucosa of sheep in Ussing-chamber experiments. D-benzoic acid was chosen as a representative for weak acids instead of labelled SCFA since it is metabolized to a much smaller extent in the tissue.

In a HCO₃ buffered solution the mucosal to serosal flux of benzoic acid (Jb) was significantly larger than the corresponding serosal to mucosal flux (Jm). Replacement of Na by choline on the mucosal side or on both sides of the tissue decreased Jm leading to a reduction of Jb. Serial addition of 0.1 mM ouabain diminished Jb. Jm only in Na containing solutions.

Elevation of the pCO₂ from 5 kPa to 19 kPa (HCO₃⁻ constant; pH decrease: 7.3 to 6.7) on both sides of the tissue led to similar relative increases of the Na and benzoic acid fluxes: Jn increased by 70%, Jm by 80%, Jb by 108% and Jm decreased by 108%. The PHi-co induced increase of the unidirectional and net fluxes of both Na and benzoic acid could almost be abolished by mucosal addition of 1 mM amiloride. Elevation of the pCO₂ on the mucosal side increased the amiloride sensitive Na and benzoic acid flux to a similar extent as the elevation of the pCO₂ on both sides of the tissue.

Our results suggest that the transport of weak acids like benzoic acid across the rumen epithelium may partly depend on the activity of the Na⁺/H⁺ exchange on the mucosal side and in protonation of dissociated acid anions thus facilitating the uptake of undissociated acids. On the other hand the elevation of the intracellular pH by the Na⁺/H⁺-exchange lead to an increase of the transmembranal gradient of the undissociated acid.

This work was supported by the Deutsche Forschungsgemeinschaft. Ga 329/2-2.

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THE INFLUENCE OF Na ON THE TRANSPORT OF BENZOIC ACID ACROSS THE ISOLATED RUMEN EPITHELIUM OF SHEEP

G. Gélée, P. Rothenbinder, E. Smith and H. Marien

The influence of Na on the transport of benzoic acid across the isolated rumen epithelium of sheep was investigated using the Ussing-chamber technique. The isolated rumen epithelium was stimulated by weak acids like short chain fatty acids (SCFA). The stimulation was mainly due to activation of a Na⁺/H⁺ exchange on the mucosal side of the tissue.

It was demonstrated that the permeability for Na⁺ increased in the opposite direction, i.e. whether Na may influence the permeation of weak acids. For that purpose unidirectional flux rates of Na⁺ and D-benzoic acid were measured under short circuit conditions across isolated, stripped rumen mucosa of sheep in Ussing-chamber experiments. D-benzoic acid was chosen as a representative for weak acids instead of labelled SCFA since it is metabolized to a much smaller extent in the tissue.

In a HCO₃ buffered solution the mucosal to serosal flux of benzoic acid (Jb) was significantly larger than the corresponding serosal to mucosal flux (Jm). Replacement of Na by choline on the mucosal side or on both sides of the tissue decreased Jm leading to a reduction of Jb. Serial addition of 0.1 mM ouabain diminished Jb. Jm only in Na containing solutions.

Elevation of the pCO₂ from 5 kPa to 19 kPa (HCO₃⁻ constant; pH decrease: 7.3 to 6.7) on both sides of the tissue led to similar relative increases of the Na and benzoic acid fluxes: Jn increased by 70%, Jm by 80%, Jb by 108% and Jm decreased by 108%. The PHi-co induced increase of the unidirectional and net fluxes of both Na and benzoic acid could almost be abolished by mucosal addition of 1 mM amiloride. Elevation of the pCO₂ on the mucosal side increased the amiloride sensitive Na and benzoic acid flux to a similar extent as the elevation of the pCO₂ on both sides of the tissue.

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In avian embryos the switch from embryonic to adult hemoglobin production is cell lineage specific and caused by the substitution of primitive red cells with embryonic hemoglobin by definitive red cells with adult hemoglobin. The mechanisms controlling the timing of the switch are not understood. We have previously observed that incubation of chick embryos at reduced PO2 causes premature appearance of adult hemoglobin. Using specific antibodies against primitive and definitive red cells we have investigated the time course of the switch and assessed the contribution of the yolk sac and intraembryonic hemopoiesis to the production of the first population of definitive red cells. The experiments were carried out with embryos incubated for 4 to 5 days in air, 13.5% O2 or 100% O2. The results show that the rate of early embryonic erythropoiesis is independent of ambient PO2. The maximum size of the first red cell population produced in the yolk sac is about 170 million cells. Depending on the incubation PO2 this population consists entirely of primitive red cells (100% O2) or contains up to 50% definitive red cells (13.5% O2). Circulating definitive red cells are already found at day 4.5 to 5 in hypoxia but only at day 7 in normoxia. The development of intraembryonic erythropoietic sites is unaffected by PO2; they store only definitive red cells and cannot be labelled for adult hemoglobin prior to day 6. Thus in hypoxia and normoxia the first definitive red cells arise in the yolk sac, whereas in hyperoxia definitive red cells appear only when intraembryonic erythropoiesis has started.

Zentrum Physiologie, Medizinische Hochschule Hannover, D-3000 Hannover 61, supported by DFG Ba 691/3

THE Haldane-EFFECT ON RABBIT BLOOD DURING RESPIRATORY AND METABOLIC ACID-BASE DISTURBANCES
F. Wierlmeister, B. Kiwull-Schöne and P. Kiwull

Detailed data concerning the Haldane effect (HE) in a wide range of respiratory and metabolic acid-base conditions are available for human blood. Within the physiologial range of acid-base disturbances, the HE, e.g. the difference in pH (ΔpH) of oxygenated and deoxygenated hemoglobin (Hb) was shown by (1) to be linearly related to the logarithm of the bicarbonate concentration (log HCO3-). Since there are differences in the binding affinities of Hb in different species, the question arises, whether the empirical relationship derived for humans (1) can also be used to estimate the HE in rabbits, as common laboratory animals. Therefore, arterial blood was taken from anesthetized rabbits, and samples were treated by different concentrations of lactic acid or NaHCO3, in order to achieve different metabolic acid-base conditions in the pH-range between 7.1 and 7.5. Subsequently, the samples were equilibrated in random sequence by 4% and 8% CO2 in oxygen or nitrogen. For regression analysis, 90 pairs of ΔpH (y) and log HCO3- (x) were calculated from 540 measured pH-values. Considering the mean Hb-concentration (±SEM) of 11.4 ±0.25 g/dl, the following linear relationship (r = 0.96) resulted for rabbit blood: y = (2.0 - log x) Hb/200 compared to humans (1) y = (1.9 - log x) Hb/225 to and to dogs, calculated from (2) y = (1.8 - log x) Hb/150. By using the formula for human blood (1), the HE would have been underestimated by about 20% in rabbits and by about 50% in dogs. This is of special importance during hypoxia, when determining the PCO2 by the equilibration (Astrup) method. If the appropriate HE for the species is not considered, the resulting PCO2 may be erroneous by up to several 100 Pa. (1) V. Mengden et al. (1966) Birlehe Physiol. 6, 151-159 (2) Reeves et al., (1982) J. Appl. Physiol. 53, 87-95

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166 TRANSCUTANEOUS TRANSPORT OF LMW-HEPARIN
J. Vahle, M. Ott and R.E. Zimmermann

The question as to whether heparin is able to permesate human skin arouses controversy among physiologival and dermatological experts. Some scientists claim that heparin enhances the resorption of subcutaneous hematoma, improve endothelial proliferation by including capillary growth and, therefore, enhancing blood supply to tissues and organs. Research is about to determine the antiphlogistic effect of heparin on cutaneous inflammation. To investigate the later effects we used a low molecular weight heparin (average molecular weight 3200 dalton) supplied as an ointment with 30 000 units/100 g and were able to show at first that heparin permeates human skin in vitro as well as in vivo. Applying heparin topically in vivo we found an increase of systemic anti-factor IIa-activity in the test persons plasma. In a second experiment we produced hematoma by injecting blood subcutaneously in order to test the effects of heparin on the resorption of subcutaneous hematoma by measuring the skin colour with a specific reflexion photometer. Finally we induced erythema by applying UV-A radiation and compared the visible chances of inflammation between the placebo and heparin treated samples. The results of our experimental studies led us to the conclusion that topicaly applied heparin has significant and evident local effects although its systemic potency turned out to be poor.

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A COMPARISON OF DIFFERENT FIBRINOLYTIC AGENTS BY A FLUORESCENCE MEASUREMENT METHOD (IN VITRO)

J.C. Elsner, R.F. Zimmermann

In order to measure fibrinolytic activities the most common methods are the fibrin plate assay (Kassup and Mullertz, 1952), the standard hanging clot method (von Kaulla and Taylor, 1961) and the lysis of radioactive labelled clots. We tried to develop a new method because the former techniques have some disadvantages. Human fibrinogen was labelled with fluorescein isothiocyanate (FITC), purified on Sepharose 4 B-columns, dialysed, lyophylised and stored at -20°C. FITC-labelled clots were preformed in a flexible tube by adding thrombin to the fibrinogen which led to clots with defined size. The lysis of the labelled clots was continuously recorded at 522 nm after exitation of the supernatant at 498 nm. The obtained results showed a variation coefficient (VD) of 4.3-5.2 % determined with 30 and 50 U streptokinase per 2 ml plasminogen phosphate-buffer after 3 to 4 hours of lysis. According to the producers specification of lytic activity or the molar basis equivalent the fibrinolytic agents streptokinase, APSAC, t-pa, urokinase were compared with one another. The lytic activity of plasmin was also determined. At low concentrations Streptokinase and APSAC seemed to be more efficient than urokinase and t-pa, but at higher concentrations of the lytic agents they presented similar activities. Fibrin specificity was measured after clot incubation with the fibrinolytic agent, washing and measuring the residual lytic activity of the clot. t-pa presented the highest fibrin specificity of all agents investigated.

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REGULATION OF CARBONIC ANHYDRASE EXPRESSION IN EMBRYONIC RED CELLS

D. Million, F. Willner and R. Baumann

During chick embryonic development carbonic anhydrase (C.A.) expression of the erythrocytes is kept at a very low level until the last week of incubation. The standard hanging clot method (Von Kaulla and Taylor, 1961) and the lysis of radioactive labelled clots. We tried to develop a new method because the former techniques have some disadvantages. Human fibrinogen was labelled with fluorescein isothiocyanate (FITC), purified on Sepharose 4 B-columns, dialysed, lyophylised and stored at -20°C. FITC-labelled clots were preformed in a flexible tube by adding thrombin to the fibrinogen which led to clots with defined size. The lysis of the labelled clots was continuously recorded at 522 nm after exitation of the supernatant at 498 nm. The obtained results showed a variation coefficient (VD) of 4.3-5.2 % determined with 30 and 50 U streptokinase per 2 ml plasminogen phosphate-buffer after 3 to 4 hours of lysis. According to the producers specification of lytic activity or the molar basis equivalent the fibrinolytic agents streptokinase, APSAC, t-pa, urokinase were compared with one another. The lytic activity of plasmin was also determined. At low concentrations Streptokinase and APSAC seemed to be more efficient than urokinase and t-pa, but at higher concentrations of the lytic agents they presented similar activities. Fibrin specificity was measured after clot incubation with the fibrinolytic agent, washing and measuring the residual lytic activity of the clot. t-pa presented the highest fibrin specificity of all agents investigated.

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CYTOCHROME P450 IN THE CONTROL OF THE PRODUCTION OF ERYTHROPOIETIN IN HEPATOMA CELL CULTURES

*W. Jelkmann, T. Fandrey and J. Siegers

The mechanism by which hypoxia stimulates the production of erythropoietin (Epo) in the kidneys and the liver is still poorly understood. Recently, Goldberg et al. (Science 242: 1412, 1988) have reported that hypoxia triggers Epo gene expression in hepatoma cells of the line Hep3B and that this gene expression requires the formation of a hepatoma specific promoter. To further elucidate the biochemical nature of the O2 sensitive hemo- protein controlling the synthesis of Epo, we have investigated the effects of agents interfering with microsomal mixed-functional oxidases (cyto- chrome P450 and its reductase) on the production of Epo in hepatoma cell cultures of the line Hep2D.

Radioimmunoassay measurements showed that the production of Epo is increased when agents known to induce P450 microsomal hemoproteins, in particular cytochrome P450 reductase, were added to the cultures, namely phenobarbitol, 3-methylcholanthrene, cobaltous chloride and thyroid hormones. On the other hand, the production of Epo was suppressed in the presence of compounds that inhibit microsomal mixed-functional oxidases. Diethyldithiocarbamate and cysteamine chloride were found to act this way.

Based on these findings it is proposed that O2 sensitive hemoproteins of the microsomal mixed-functional oxidases are critically involved in the control of the synthesis of Epo.

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METABOLIC EFFECTS ON SARCOPLASMATIC RETICULUM AND MYOSIN EXPRESSION IN THE HEART OF DIABETIC AND FASTED RATS

M. Lazzeri, Y. Elimbah, N.S. Dhalla

In the diabetic heart, the molecular structure of the myocyte is markedly changed involving sarcoplasmatic reticulum (SR) and myosin. In order to describe the remodeling induced by the subcellular changes, the effect of the physiological load of intermittent fasting (1 d fasting, 1 d ad libitum feeding; 6 wk total) was studied. During fasting plasma insulin was reduced to 9 uU/ml vs. 17 uU/ml of controls (C) and 4 uU/ml of diabetic rats (65 mg/kg streptozotocin). Intermittent fasting induced changes in Ca\textsuperscript{2+}-stimulated ATPase of SR (95 vs. 130 nmol P/mg/min of C) and in myosin V1 (38% vs. 53% of C) which were equidirectional to diabetic rats (75 nmol P/mg/min; 15% V1). Treatment of intermittently fasted rats with sucrose (0.5% in drinking water) prevented the changes in SR and myosin. Plasma glucose levels were reduced by 4020 Halle/Saale

w~ich increased daily calorie intake by only 1%.

The effect of cGMP on Ca current (I\textsubscript{Ca}) was investigated in isolated guinea-pig ventricular cells using whole-cell patch clamp method combined with intracellular perfusion technique. When I\textsubscript{Ca} was increased by isoprenaline (0.1 M) or by intracellular perfusion with cAMP (50-100 M), cGMP (50 M) induced an additional increase of I\textsubscript{Ca}. This effect was reversible and could be repeated in the same cell. However, cGMP (1-10 M) had no effect when I\textsubscript{Ca} was maximally stimulated by the nonhydrolyzable 4-chloro-phenyl-thio cAMP (5-100 M). On the contrary, in the presence of nonselective phosphodiesterase inhibitor, IBMX (40 M), I\textsubscript{Ca} stimulated by isoprenaline (0.1 M) was reduced by cGMP (10 M). This effect was partially reversible.

Furthermore, high concentration of cGMP (100 M) also inhibited I\textsubscript{Ca} which was elevated by intracellular perfusion with cAMP (50 M). 5'-GMP, the metabolite of cGMP, had no effect on I\textsubscript{Ca}. 8-bromo cGMP, a potent activator of cGMP dependent protein kinase, strongly reduced I\textsubscript{Ca} which was stimulated by isoprenaline (0.1 M) or cAMP (50 M). To test whether cGMP dependent protein kinase affects I\textsubscript{Ca}, we perfused the pipettes with the active fragment of cGMP dependent protein kinase (67 kDa). In some experiments I\textsubscript{Ca} was reduced when cGMP (0.3-1 M) was intracellularly applied. It is concluded that cGMP regulates I\textsubscript{Ca} in two ways. Firstly, cGMP increases I\textsubscript{Ca} by inhibiting hydrolysis of cAMP. Secondly, cGMP inhibits I\textsubscript{Ca}, probably due to an activation of active cGMP dependent protein kinase.

CHANGE OF MYOSIN ISOENZYME EXPRESSION IN THE VENTRICLE OF COLD ADAPTED RATS AND HIBERNATING EUROPEAN HAMSTERS

I. Morano, **M. Agostini, **M. Mühleisen, and **W.F.H.M. Mommserts

Adult Sprague Dawley-rats kept for 6 weeks at +4°C for cold adaptation were killed at the age of 20 weeks. Adult European Hamsters hibernating (HH) in a cold and dark chamber at +4°C starting in November were killed in January. Summer active hamsters (SH) were killed in July. Left ventricular myosin isoenzymes (MI) of rats and hamsters were studied by the pyrophosphate gelelectrophoresis technique displaying three components with increasing mobility V3, V2, and V1 identically in both species investigated. Values are given as means. SD were <10%.

Summer active hamsters (SH) expressed mainly V3, while during hibernation (HH) the V1-form dominated; MI patterns (XVI/XVIII) were 15/86 and 70/10 in SH and HH respectively. Ca2+-dependent ATPase activity of ventricular myofibrils (MF) was higher in HH than in SH; at maximal Ca2+-activation (10 M Ca2+) MF ATPase-activity (nmol Pi/mg/min) was 86.4 in HH and 28.6 in SH. MI patterns of control rats kept at 25°C were 65/15 and changed in favour of the V1-form to 85/5 in the cold adapted rats. At maximal Ca2+-activation MF ATPase activity was 85 and 80 for control and cold-adapted rats respectively.

The shift of myosin isoenzyme expression to the highly active V1-form during hibernation and cold exposure could be related to an enhanced activity of the thyroid gland.

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The relative contribution of the calcium influx to the myofilament activation was shown to increase the post rest recovery of atrial and ventricular cardiac sarcomeres in the manner of a positive staircase (C.F. Hamill, A. Marty, E. Neher, B. Sakman and F.J. Sigworth, Pflügers Arch 391:85, 1981) and laser diffractometry (W. Wesseling, W. Schenk and B. Milius, J Mol Cell Cardiol 19:897, 1987).

When regularly paced after a rest period of at least three minutes we observed in rat cardiac myocytes a negative staircase of the peak slow inward current (depolarization steps from -45 to +40 mV, step duration 300 ms) as well as of the positive one of the sarcomere shortening. The magnitude of such staircase was dependent on the stimulation rate. Guinea pig cardiac myocytes showed a negative staircase of the slow inward current, too, but a positive one of the sarcomere shortening. In rat cardiac myocytes the slow inward current was small compared to guinea pig heart cells whereas the extent of the steady state sarcomere shortening amounted to about 200 nm at a stimulus frequency of 50/min in all cells investigated.

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TWO COMPONENTS OF Ca\textsuperscript{2+} RELEASE FROM SARCOPLASMIC RETICULUM IN GUINEA-PIG ATRIAL MYOCYTES

P. Lipp and L. Port

An intracellular Ca\textsuperscript{2+}-transient due to Ca\textsuperscript{2+}-release from the sarcoplasmic reticulum causes a transient inward current (I\textsubscript{Na}) at negative membrane potentials which is carried by electrogenic Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange (Lipp & Port J. Physiol. 397: 601, 1986). I\textsubscript{Na}-measurements were used to study properties of spontaneous and Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+}-release from the SR of myocytes dialyzed via patch-clamp pipettes with solutions containing (mM) CsCl (4-95), MgCl\textsubscript{2} (10), EGTA (10), Na\textsubscript{2}ATP (5). Ca\textsuperscript{2+} was continuously monitored by the calcium-sensitive dye Fluo-3-AM. A late Ca\textsuperscript{2+}-release component (I\textsubscript{L}) was identified by increasing the membrane potential during test pulses ("whole-cell clamp technique") over the range -30 to +70 mV. Late Ca\textsuperscript{2+}-release was found to be increased when the transmembrane Na\textsuperscript{+} gradient was moderately reduced. The evidence suggests that the late contraction isolated either pharmacologically or kinetically is substantially activated by Ca entering the cell via the sarcosomial Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange.

LATE CONTRACTION IN VENTRICULAR MYOCYTES ACTIVATED BY THE NA-Ca EXCHANGE

K. Schütz and G. Szymanski

When single ventricular myocytes of guinea-pigs were incubated for 90 min in 10 \mu M ryanodine a late contraction (measured by an optical technique) was obtained. This late contraction was found to be increased when the transmembrane Na\textsuperscript{+} gradient was reduced either by reducing Na\textsubscript{Cl} or by experimental manoeuvres expected to increase Na\textsuperscript{+}/I. The late contraction increased with membrane potential during test pulses. The latter technique over the range +10 to +70 mV. Late contraction continued to develop over the whole duration of test pulse or of action potential. In another approach a late contraction could be kinetically isolated: When a train of normal contractions (0.2 Hz) was interrupted by a period of rest of 10 to 15 min the first post-rest contraction was found to be a late contraction. This so-called rested-state contraction was found to be increased as well when the transmembrane Na\textsuperscript{+} gradient was moderately reduced. The evidence suggests that the late contraction isolated either pharmacologically or kinetically is substantially activated by Ca entering the cell via the sarcosomial Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange. In dependence on the stimulation pattern the following properties of the ryanodine-isolated component were found in guinea-pig papillary muscles: (1) no postextrasystolic potentiation, (2) faster restitution, (3) nearly complete properties of the ryanodine-isolated component were found in guinea-pig papillary muscles: (I) no postextrasystolic potentiation, (2) faster restitution, (3) nearly complete properties of the ryanodine-isolated component were found in guinea-pig papillary muscles: (I) no postextrasystolic potentiation, (2) faster restitution, (3) nearly complete.
CONTRACTION AND ACTIVE NA/K TRANSPORT OF SHEEP CARDIAC PURKINJE FIBRES AS AFFECTED BY 22, 23-DIHYDROBUTAFIL, A NEW CARDIOACTIVE STEROID
H.G. Glitsch, H. Pusch and Ch. Zylka
Physiologisches Institut II, Universität Tübingen, Gmelin-Wards structural dilatation due to discontinuation of u- or Na/K-ATPase for the lactone grouping of cardioactive steroids the lactone moiety carries a residue to which various reactive described a general synthetic pathway to bufenolides where ~-adrenergic stimuli can, with certainty, be excluded for differences were found within the AS groups. Likewise, the was about 50% as compared to the SO group. No significant significant differences, except when compared to the SO group. Conclusions: A change in the ventricular configuration to ventricular radii-thickness ratio could be enhanced by inhibition of a catecholamine induced stimulation of protein synthesis. Thus the pumping function of the heart could be affected event the basis of geometric factors alone. The investigations were made in Wistar rats with experimen-

DOES A LONGTERM BLOCKADE OF THE α- OR β-ADRENERGIC RECEPTORS HAVE INFLUENCE ON THE DEGREE OF LEFT VENTRICULAR HYPERTROPHY, CONFIGURATION AND PURGING PERFORMANCE?
W. Brandle and E. Jacob
As recent investigations (Lake & Moraday 1976; Ostman-Smith 1979, 81) have revealed, the catecholamines and their recep-
tor subclass play an essential role as a mediator of cardiac growth in the adaptation of the heart to increased load. Furthermore, it is known that in terminal cardiac insufficiency the number of adrenergic receptors decreases in relation to the severity of the disease (Apoe & Tarazi 83). It was the aim of the present study also in view of the frequent application of sympatholytics in the clinic to investigate the effect of the removal of adrenergic stimuli on ventricular configuration under unchanged loading condi-
tions. It would be conceivable that the ventricular radius-

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Leukefomyocytes maintain their sarcolemmal integrity (no enzyme release) and re-establish a nearly normal phosphorylation potential within 15 min (Legrand et al., 1981). In the same model it was now investigated whether a temporary contractile blockade by 20 mM 2,3-butanediol monoxide (BDM) can prevent reoxygenation-

Supported by the Deutsche Forschungsgemeinschaft (PG Konzelli)

**CONTRACTILE BLOCKADE PREVENTS HYPERCONTRACTION IN ANOXIC-REOXYGENATED CARDIOMYOCYTES**
B. Siegmund, T. Klettz, P. Schwartz, H.M. Piper
Reoxygenation after 120 min substrate-free anoxia causes sudden hypercontraction in isolated rat cardiomyocytes. Reoxygenated hypercontracted cardiomyocytes maintain their sarcolemmal integrity (no enzyme release) and re-establish a nearly normal phosphorylation potential within 15 min (Legrand et al., 1981). In the same model it was now investigated whether a temporary contractile blockade by 20 mM 2,3-butanediol monoxide (BDM) can prevent reoxygenation-induced hypercontraction. When BDM was present during 120 min anoxia and removed immediately before reoxygenation, it had no effect, but when it was present during 15 min reoxygenation hypercontraction was prevented in 85% of the cells. The anoxic changes of high-energy phosphate contents, the phosphorylation potential and the ultrastructure remained unaffected by the presence of BDM. When BDM was applied anoxically immediately prior to reoxygenation, it also prevented hypercontraction. When it was washed out after the first 15 min of reoxygenation, contraction still remained absent but the cells could be electrically stimulated to contract. Conclusions: The results demonstrate that a temporary contractile blockade (15 min) at the onset of reoxygenation prevents hypercontraction in anoxic-reoxygenated cardiomyocytes. This result, the energetic recovery and sarcolemmal integrity of cardiomyocytes in anoxia-reoxygenation, demonstrate that reoxygenation-induced hypercontraction is not based on an already irreversible cell damage. (Supported by the DFG)

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XAMOTEROL RECRUITS AN INOTROPIC RESERVE IN REPERFUSED MYOCARDIUM WITHOUT DETRIMENTAL EFFECTS ON ITS SUBSEQUENT RECOVERY.

Christa Linder, Stefan Schäfer, and Gerd Heusch

The β₂-adrenoceptor partial agonist xamoterol (X) is characterized by the combination of its negative chronotropic, anti-ischemic effect during sympathetic active and its positive inotropic effect at a low resting cardiac sympathetic tone. X may therefore offer a new therapeutic approach in patients with exercise-induced myocardial ischemia and subsequent post-ischemic dysfunction. In 8 anesthetized dogs, we tested the effect of X on regional myocardial function (sonomicrometry and during 8 h reperfusion (R) after a 15 min LCX-occlusion. During occlusion, mean systolic thickening velocity (V) decreased from 9.31 ± 0.91 (SD) mm/s to -1.35 ± 0.72 mm/s. X (100 μg/kg i.v., infused at 10 min R) increased V from 1.47 ± 2.34 mm/s (10 min R) to 7.13 ± 0.35 mm/s (30 min R, p<0.05), whereas in a placebo-group (P, n=8) V remained unchanged. (3.14 ± 3.30 mm/s at 10 min R vs. 2.96 ± 3.74 mm/s at 30 min R). At 8 h R, V was not different in both groups (X: 7.97 ± 4.23 mm/s vs. P: 6.87 ± 4.00 mm/s). Histological examination revealed no difference in the extent of necrosis between the two groups.

Conclusion: Xamoterol recruits an inotropic reserve in reperfused myocardium and this recruitment of a reversible degree surprisingly will not alter this behaviour.

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LEFT VENTRICULAR ASYNCHRONY IS AN INDICATOR OF REGIONAL MYOCARDIAL DYSFUNCTION.

Stefan Schäfer and Gerd Heusch

There is a marked heterogeneity of myocardial wall thickening within the left ventricle and between different individuals. It is therefore difficult to detect regional myocardial dysfunction from absolute values of systolic wall thickening (WT). We now tested whether the extent of left ventricular asynchrony can be used to quantify the severity of regional myocardial dysfunction. In 6 open-chest dogs, regional myocardial wall thickness (sonomicrometry) was measured under control conditions (C), at three degrees of ischemic dysfunction produced by at least 4 min steady state stenoses on the left circumflex coronary artery (1a-L) and after release of a 15 min LCX-occlusion, when two matched degrees of reperfusion dysfunction (R=1 and R=2) were present. Two indexes of left ventricular asynchrony were calculated: (1) post-extrasystolic thickening (PET) and (2) the phase difference of the first Fourier harmonic of posterior versus anterior wall motion (PD). WT was decreased from 15.32 ± 5.1 (SD) % (C) to 9.72 ± 4.1 % (1a-L), 4.28 ± 1.5 % (1a), and -3.75 ± 1.1 % (1a). Conversely, PET increased from 0.02 ± 0.4 mm (C) to 0.19 ± 0.22 mm (1a-L), 0.19 ± 0.15 mm (1a), and 0.50 ± 0.26 mm (1a). PD increased from 94 ± 28 degrees (C) to 221 ± 19 degrees (1a-L), 54 ± 18 degrees (1a), and 107 ± 21 degrees (1a). During reperfusion, PET and PD recovered to 0.34 ± 0.19 mm and 0.25 ± 0.30 mm (1a-L) and 1.9 ± 1.47 mm and 2.9 ± 2.68 mm (1a). There were inverse linear relationships between WT and PET (r=-0.82, p=0.001) and between WT and PD (r=-0.86, p=0.001). Inotropic stimulation by postextrasystolic potentiation or norepinephrine (0.5-1.0 μg/kg min i.v.) increased posterior and anterior WT but did not alter the extent of left ventricular asynchrony. Thus, the severity of regional myocardial dysfunction at a given inotropic state can be determined by analysis of left ventricular asynchrony using PET or PD.

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NYOCARDIAL RECOVERY AFTER LOW-FLOW ISCHEMIA: PHARMACOLOGICAL INTERVENTIONS AND EFFECTS OF GRANULOCYTES

H.F. Becker, R. Reichenolz, P. Rachke, B. Leipertz and E. Gerlach

The attenuated recovery of function in ischemic myocardium subsequent to a restoration of flow has been ascribed to both the coronary no-reflow phenomenon caused by myocardial stunning, oxygen radicals and cytokines, formed especially during reflow by polymorphonuclear granulocytes (PMN), are deemed to be a cause. To better differentiate between damage resulting directly from hypoxia and that suffered upon reflow, isolated guinea pig hearts were subjected to low-flow ischemia (1 ml/min, 37°C, 30 min) and reperfusion in the absence and presence of a) various radical scavengers and antioxidants, and b) autologous PMN (infusion rate: 106 cells/min). Myocardial function was assessed from the performance of pressure-volume work prior to and after ischemia.

Results: 1) External heart work recovered to about 35% of the pre-ischemic value (control). Supplementation of the Krebs-Henseleit perfusate with superoxide dismutase + catalase, uric acid, captopril or allopurinol enhanced recovery to between 55% and 63%. However, the non-scavenger substances bradykinin and iloprost were similarly effective. 2) For all conditions functional recovery was directly albeit rather loosely, related to the myocardial ATP content (r=0.61) and to the total loss of purine compounds during the low-flow phase (r=0.4-0.5 μmol/g, r=0.52), but not to purine loss during reperfusion (0.11-0.16 μmol/l), lactate release or post-ischemic coronary flow. 3) Perfusion with unstimulated PMN, either before, during or after the low-flow phase, did not noticeably alter myocardial function or coronary flow.

Conclusions: The level of adenosine nucleotides remaining in the myocardium after a period of low-flow ischemia is a major determinant of functional recovery. As evidenced by the purine losses, the ischemic and not the reflow phase is decisive for this aspect. The large scatter of performance for hearts with similar ATP values suggests, however, that in some cases work performance is under additional restraints (stunned myocardium). The beneficial effects of the various additives tested probably arise from influences on this phenomenon, though the modes of action are obviously multiple. As to be expected, passage of unstimulated PMN through the coronary system does not lead to acute vascular or heart muscle dysfunction. Ischemia of a reversible degree surprisingly will not alter this behaviour.

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QUANTITATIVE ANALYSIS OF CARDIAC DYNAMICS BASED ON FRANK'S DIAGRAM AND COMPUTER SIMULATION. SIGNIFICANCE OF MATHEMATICAL MODELS

B. Dierberger, R.W. Gohlch and R. Jacob

A methodological approach for analysing the determinants of stroke volume under the conditions of altered ventricular geometrical configuration and presentation of left ventricular dynamics of rats with experimental aortic stenosis and spontaneous hypertension. The analysis is based on Frank's pressure-volume (P-V) diagram and on model calculations describing relationships between stroke volume as a function of end-diastolic inner radius or inner volume, respectively, is calculated after transforming P-V relations into stress-length (circumference) relations. Changes of this relation permit a quantitative evaluation of the significance of the following factors for stroke volume: Ventricular inner dimensions, wall thickness, "myocardial contractility" and distensibility, preload (end-diastolic wall stress) and systolic pressure load (end-systolic pressure).

An ascending branch of the relationship between stroke volume and end-diastolic ventricular size can be demonstrated using the model of a thickwalled sphere as well as some more sophisticated models such as an ellipsoid of revolution with uniform or non-uniform wall thickness. The calculated value of stroke volume is only slightly affected by the choice of the model used up to a three-fold increase in inner volume. - It is shown in the present examples that, as a rule, myocardial alterations (reduced contractility and distensibility) are the decisive factors for impaired cardiac pumping function. The methodological approach presumes a certain indispensable completion of Frank's P-V diagram. Presupposing exact measurements this concept can also be applied to clinical cases. Current investigations should clarify whether further improvement of analysis of ventricular function is possible based on the finite element theory.

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VENTRICULAR MYOCYTES: INACTIVATION CURVES OF FORCE AND CA-CURRENT DISSOCIATE AT NEGATIVE POTENTIALS

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Isometric contractions were measured in single myocytes isolated from guinea-pig ventricles. By means of poly-L-lysine, one cell was attached to the beveled ends of a pair of thin glass rods. Contraction disabled the rods by 1.3 μm, with the calibrated compliance (0.72 mM) the displacement could be transformed into a force signal.

By means of the whole cell patch-clamp technique, the cells were depolarized at 1 Hz with 160 ms long clamp-pulses from -45 mV to +5 mV. At 35°C and 1.8 mM [Ca⁺], force peaked within 9 ± 20 ms to 3.2 ± 1.6 mN/mm² (mean ± S.D., n=9). More positive holding potentials decreased peak force and peak calcium current in parallel, the inactivation curves of current and force had similar slopes (-9 mV) and 50% inactivation potentials (20 mV).

Holding potentials more negative than -45 mV potentiated peak force but they did not significantly change the calcium current. Contraction was maximal at a holding potential of -80 mV; pulses from -80 to +5 mV evoked forcewitches 6.4 ± 0.5 times larger than those from -45 to +5 mV. Between -80 and -45 mV, more positive holding potentials reduced twitch force along an S-shaped inactivation curve that had a slope of -20 mV and a midpoint at -57 mV. For testing the possibility, inactivation of contraction being related to inactivation of the fast sodium current, the experiments were repeated in the presence of 30 μM TTX. TTX did not significantly change the inactivation of force, the slope (-18 mV) and shape of half maximal inactivation (-60 mV) were similar in the presence and absence of TTX.

We discuss the results to suggest that activator-Ca derives not exclusively from Ca-induced SR-Ca-release. It may be provided also by depolarization-induced SR-Ca-release, the contribution of which decreasing with less negative holding potentials.

[1] Shepherd, N, Vornmann, M, Isenberg, G, Am J Physiol 258, in press

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Mg²⁺ - Ca²⁺ - INTERACTION IN CARDIAC MUSCLE

U. Pohl, S.Y. Wang, R. Meyer, H.G. Haas

Membrane currents of isolated ventricular myocytes of the guinea-pig were investigated by patch clamp in the whole cell recording mode. External Mg²⁺-concentration was varied and its effect on membrane currents was tested. Emphasis was placed on activation and inactivation of current flow through Ca²⁺ channels of the L-type. Using Tyrode solution with normal concentrations of Na⁺, K⁺, and Ca²⁺, an increase in [Mg²⁺], from 1 to 2.5; 5; 10 or 20 mM caused a shift of the voltage for the half activation (Vₐₐ₃) of Iₛ from -20 to -40 mV, respectively, to more positive potentials. Shape and steepness of the activation curve were almost unaffected by Mg²⁺. The effect of Mg²⁺ on activation gating was found to be superimposable with the effect of other agents. When external Na⁺ was replaced by choline and Mg²⁺ was increased subsequently, either change was followed by a shift in activation gating to more positive potentials and the effect of increased [Mg²⁺] was simply added to that of choline. The effects of Mg²⁺ on activation of Ca²⁺ channels may be interpreted as due to a decrease of membrane surface potential by binding of Mg ions to fixed negative surface charges.

Unlike activation, influence of [Mg²⁺] on steady inactivation of Iₛ was less pronounced and not clear. In some experiments increasing [Mg²⁺] from 1 to 10 mM had almost no effect on the availability of Iₛ while in others a shift of the steady inactivation curve to more negative potentials was observed. The shift of the inactivation curve was always smaller than the shift of the activation curve observed from the same cell. The apparent discrepancy between the effects of [Mg²⁺] on Iₛ activation and inactivation could be explained in several ways: (i) Inactivation gates may be located deeper in the membrane so that they are less affected by changes in the surface potential or (ii) Iₛ inactivation is a Ca²⁺-dependent process rather than a voltage dependent process.

In a Ca²⁺ - free, Mg²⁺-free solution with normal [Na⁺] and 2 mM EGTA a long lasting inward current developed which is carried by a flow of Na ions through the L-type channels. This current was reversibly blocked by addition of Mg²⁺.
Efficiency of the Heart Under Adrenaline

G. Kissling

The investigations were performed on a modified heart-lung preparation of the rat. After opening the chest under urethane anaesthesia (1.2 g/kg b.w.) the aortic root as well as both caval veins were ligated and the aortic root connected to the vena cava inferior via a shunt with a starting resistance. Via this resistance left ventricular afterload could be adjusted arbitrarily. The following haemodynamic parameters were measured: right and left ventricular pressure, central aortic pressure, pulmonary flow and the flow in the shunt circuit. The oxygen consumption of the preparation was determined by means of the difference in oxygen concentration between the inspired and expired air and the respiratory volume per minute. The efficiency was calculated from the external work and the oxygen consumption under control conditions as well as under adrenaline (bolus: 60 μg/kg, continuous infusion: 10 μg/kg/min).

Our investigations have confirmed that the efficiency of the heart depends on the external mechanical conditions: compared to high pressure loads, at low pressure loading a certain amount of work is performed with a significantly increased efficiency. Under the chosen experimental conditions, a given work is performed under adrenaline with a reduced pressure development and a higher volume shift compared to the control conditions. Simultaneously, the efficiency increases significantly. However, the improvement in efficiency under adrenaline cannot be attributed to the more favourable mechanical conditions alone. Even in cases where a given work is performed under identical pressure and volume loading, adrenaline significantly improves efficiency.

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Ca-Synergistic and Ca-Antagonistic Effects on Net 45Ca Uptake into Neonatal Rat Myocytes - A Model to Study Modulation of Ca Influx in Cell Cultures

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Transmembrane Calcium (Ca) influx triggers cascades of enzymatic reactions in a variety of cell types in both, physiological and pathophysiological processes. Consequently, modulation of transmembrane Ca entry by Ca promoters or Ca-antagonistic inhibitors is effectively used for pharmacological interventions. In order to investigate various mechanisms of influencing transmembrane Ca influx on a cellular level, net 45Ca uptake was measured on monolayers of neonatal rat myocytes, cultured by standard methods. 45Ca (0.02 μCi/l) was added for 5 min. Subsequently, the monolayers were washed with ice-cold, Ca-free, EGTa (2.5 mM/ml)-containing solution, detached and lysed. From the cell lysate samples were taken for fluorescent protein determination (excitation 286 nm, emission 335 nm) and for measurement of 45Ca activity by liquid scintillation.

Under control conditions (37°C, pH 7.4, Ca2+ 1 mM/ml) net 45Ca uptake was 131.1 ± 10.13 ng Ca/mg protein. Additional Ca uptake above control values - amounting to 35%, 34%, or 74% respectively - was evoked by three different mechanisms: (1) Depolarization (n=10) with K+ 65 mM/ml; (2) β-receptor stimulation (n=10) with isoproterenol or norepinephrine; (3) depolarization of the opening state of L-type Ca channels (n=4) with 50 μM/ml Bay K 8644. Conversely, highly specific Ca antagonists (1 μM/ml nitrendipine) reduced transmembrane Ca entry below normal: Verapamil by 43.2 ± 5.6% (n=6), diltiazem by 26.3 ± 4.5% (n=3) and nifedipine by 20.5 ± 3.4% (n=5). The data suggest, that determination of net 45Ca uptake into neonatal myocytes offers an adequate model for studies on modulation of transmembrane Ca influx in cell cultures.

Hemodynamic Effects of a Bradycardic Agent on the Canine Heart

Jochen D. Schipke*, Yasuhiko Harasawa, Seiryo Sugiuara, Joe Alexander Jr., and Daniel Burkhoff

Bradycardic agents are supposed to improve the oxygen demand/supply ratio during ischemia both by bradycardia and by the increased diastolic coronary flow. We tested whether the benzazepin UL-FS49 acts exclusively on sinus node cells or additionally affects myocardial contractile state to avoid interference with the peripheral system, we performed experiments on isolated, blood-perfused canine hearts and determined HR (1/min), duration of systole and diastole (Tsyst, Tdia, ms), ventricular contractile state (paw isovolumic systolic pressure: LVPW, mmHg), myocardial oxygen consumption (MVO2, ml/min/100g), and cardiac output (CO, l/min) during control and after injection of UL-FS49 (1 μg/kg i.c.).

| HR | Tsyst | Tdia | LVPW | MVO2 | CO |
|----|-------|------|------|------|----|
| control | 104±7 | 200±16 | 324±51 | 72±6.5 | 9.1±1.1 |
| UL-FS49 | 93±7* | 313±19* | 427±44* | 72±6.0±4.1 | 12±2.1 |

*p < 0.05 control vs. UL-FS49; data are mean ± SD

We conclude that UL-FS49 decreases MVO2 by bradycardia but not by reduced contractility. Hence, the bradycardia does not further depress ischemic ventricular function. Myocardial blood flow will, additionally, be improved via prolonged diastole. Moreover, peripheral blood flow is maintained at the dosage used. Thus, usage of bradycardic agents could become an alternative strategy in treating ischemic myocardial disease.

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Novel Cardiotonic Drugs - A Comparative Study on Their Ca2+-Sensitizing Effect on the Contractile Proteins of Guinea-Pig Papillary Muscle

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A novel approach for the positive inotropic therapy of heart failure may be to alter the response of the myocardium to Ca2+ and by so doing to achieve an increased contractile state with little change in the free Ca2+ transient during the beat (SOLANX, NARAE Suppl. 31, 1989). Ca2+-responsiveness of the contractile proteins in skinned fibres was investigated by studying the relation between force and Ca2+ concentration in skinned fibres (RUESCH and NARANG, J. Cardiovasc. Pharmacol. Suppl. S1, 1989). We now investigated the influence of the PDE III-inhibiting positive inotropic drugs adi- and UD-CG 212 (U), on the Ca2+-responsiveness of skinned fibres of guinea-pig papillary muscles. Guinea-pig papillary muscles were chemically skinned using 50 g glycerol and 1% Triton X100. Subsequently, Ca2+-concentration response curves (1.8 - 4.5 μM) were performed in the absence and presence of various concentrations of the test compounds and the EC50 were calculated. The Ca2+-responsiveness at the skinned fibres in the presence of the drugs was calculated as the shift of the EC50 expressed as its negative logarithm (decadic logarithm of the molar concentration (EC50). Values higher than 0 (result obtained in control experiments) represent a Ca2+-sensitization, those smaller than -0.84° characterize a Ca2+-desensitization of the contractile proteins.

Results (means, n = 4-6):

| Drug | pCa shift in the presence of ... |
|------|--------------------------------|
|      | 10 | 102 | 500 |
| A    | -0.04 | -0.03 | +0.06 |
| P    | -0.04 | -0.06 | +0.04 |
| U    | -0.04 | +0.12 | n.d. |
| U    | -0.06 | -0.25 | -0.70 |

a = p < 0.05 for Ca2+-sensitization
b = p < 0.05 for Ca2+-desensitization
n.d. = not determined due to limited solubility of the compound

An increase of the Ca2+-responsiveness was observed in the rundown of PDE III at concentrations higher than 10 μM. However, U decreased the Ca2+-responsiveness at concentrations higher than 10 μM. The relative contribution of the Ca2+-sensitization to the overall inotropic activity of A, P, and U in vivo remains to be determined since the PDE III inhibiting action of these drugs also induce an increase in myocardial contractility. Nevertheless, the Ca2+-sensitization does not contribute to the inotropic effect of U in vivo, in particular in guinea pigs.

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Previous studies have shown that an increase in coronary perfusion pressure (CPP) with aortic pressure (PAO) kept constant resulted in a rise of left ventricular performance, while left ventricular end-diastolic pressure (LVEDP) decreased, thus demonstrating the garden-hose-effect (GHE).

In perfused, and line perfused guinea pig hearts we studied the coronary flow induced changes in CPP in a range between 30 and 70 mmHg during norepinephrine (N) and verapamil (V) infusions at a constant heart rate. To adjust drug concentration equivalently to coronary flow, the latter could be varied independently. We measured left ventricular pressure (LVP), LVEDP and myocardial contractility (DP/dt max) during control (C) and infusion of the drugs. The differences of the left ventricular parameters were calculated between the data shown for example at a CPP of 40 and 60 mmHg:

| Parameter | ΔLVP | ΔLVEDP | ΔDP/dt max | Type |
|-----------|------|--------|------------|------|
| ΔCPP 50 | 5.0 ± 0.5 | 6.5 ± 0.5 | 15 ± 5 | C vs. V |
| ΔCPP 60 | 5.0 ± 0.5 | 6.5 ± 0.5 | 15 ± 5 | C vs. V |

During N we observed a decrease of GHE, but no significant change under V. Corresponding data were obtained at different CPP. We conclude that the decrease of GHE under N is the result of a higher performance level of the heart. The influence of V might be explained by an inhibition of excitation-contraction-coupling resulting in no increase of GHE.

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CALCIAL CURRENTS IN ISOLATED RAT ATRIAL MYOCYTES.
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Atrial calcium currents (ICa) have been studied in many species including guinea-pig, dog, rabbit, and human, and notable interspecies differences have been found. Surprisingly, little work has been done on rat atrial myocytes and therefore this study was aimed at quantifying ICa in these cells. Atrial myocytes were isolated using a technique similar to that described by Isenberg and Kockner (Pflügers Arch. 395, 6-18, 1982) and kept in a modified "KB" medium for up to 3 days. Cells were suspended in Tyrode solution (in mM: NaCl 150, KCl 5.4, Glucose 10, HEPEs 5, MgCl2 1.2, CaCl2 2.5, TTX 0.02mM, pH 7.4). The electrode solution contained in mM: KCl 140, MgCl2 3.5, CaCl2 2, BETA 11, NaATP 0 or 5, HEPEs 10 pH 7.1). Whole cell recordings (WCR) were obtained with cells held at -80mv. At 2-5 mins after WCR, IMax was 26.03 ± 2.53 pA/pF (n=9,3SE), activated at about -55 mv, and peaked at -20 mv. Presence or absence of ATP in the patch electrode did not appear to influence ICa. Rundown of ICa varied from cell to cell, being reduced in magnitude by 50% often after 5-10 mins, but in some cells was unchanged after 30-40 mins. The time to peak current varied between 4 and 20 milliseconds. Interestingly, ICa in Na' and K' free solutions (TEA and Cs substituted respectively) was only 6.31 ± 0.74 pA/pF (n=16,SE), with a shift in activation voltage to -15 mv, reaching peak values at 10 mv. Again, the presence or absence of ATP with or without 0.1mM Cyanide did not influence ICa. Rundown occurred at a similar rate compared with cells in normal solutions. Furthermore, there was no correlation between tip size (or series resistance) and the rate of rundown. Supported by NHI (Aust.) and CSIRO/URG to RHF and MLR.

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METABOLIC EFFECTS ON SARCOPLASMIC RETICULUM AND MYOSIN EXPRESSION IN CARDIAC HYPERTROPHY
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Pressure overload of rat heart leads to an increase in the proportion of myosin V3 and a reduced Ca2+-stimulated ATPase activity of sarcoplasmic reticulum (SR). Because a longstanding overload is likely to result in cardiac failure, it was attempted to prevent the subcellular changes by interventions which preferably do not provide an additional load for the heart. Administration of sucrose in the drinking water in a low dose (0.8%) proved to be the most efficient means. The sucrose intake corresponded to approx. 1% of the daily consumed calories and did neither affect ventricular weight nor other growth characteristics. In rats with abdominal aortic stenosis (AS); the sucrose treatment prevented the reduction in V1 (54% control, 56% AS, 54% AS + sucrose) and the reduction in SR ATPase activity given in nmol Pg/min (145 control; 89 AS, 113 AS + sucrose). The proportion of V1 can also be increased by a fat-rich diet (20% mackerel oil). In the case of SHR, V1 increased from 21% to 30% and the rate of Ca2+ - uptake of SR increased from 55 to 105 nmol Ca2+/mg/mg. This treatment which was associated with an increased daily calorie intake led, however, to increased growth parameters. Taken together, the data show that a restructering of the myocyte from pressure loaded ventricles can be achieved by interventions which most probably act via an altered fuel metabolism of the heart.

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MATHEMATICAL ANALYSIS OF THE RELAXATION CHARACTERISTICS OF PAPILLARY MUSCLE UNDER THE INFLUENCE OF ISOPRENAINE AND PHENYLEPHRINE IN SWIM-TRAINED RATS
Ch. Ross and R. Jacob

A mathematical analysis is presented permitting quantitative description of myocardial muscle relaxation using only one function with the form:

\[ \sigma = \sigma_{\max} e^{-\gamma^2} \]

\[ \sigma_{\max} = \text{Max. stress developed during an isometric contraction} \]

\[ \gamma = \text{Stress at the inflection point} \]

\[ \lambda = \text{Constant} \]

\[ \tau = \text{Time elapsed after } \sigma_{\max} \text{ was reached} \]

A double logarithmic plot of this function yields a straight line of the form \[ y = \alpha x + c \] where the gradient \( \alpha = \lambda \) and the \( y \)-intercept \( c \) can be used to evaluate the second constant \( \gamma \). To test the sensitivity of this mathematical tool, the effect of a swim-training programme, \( \beta \)-receptor stimulation with phenylephrine and \( \beta \)-receptor stimulation with isoprenaline was investigated.

The relaxation characteristics of muscles from swim-trained rats are similar to those of muscles under the influence of isoprenaline. This is clearly visible in the rectified plot which showed a parallel shift to the left, representing a speeding up of muscle relaxation. Phenylephrine, on the other hand, showed an increase in gradient in the rectified plot which represents an initial increase in the velocity of relaxation with a subsequent decrease.

The results reveal that such a rectification is very sensitive to variations in the form of the mechanogram and makes very small differences in the muscle relaxation characteristics very conspicuous. Furthermore, it permits description of relaxation simply with only the two constants \( \lambda \) and \( \gamma \).

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Coronary autoregulation or vasoactive substances can cause transient changes in vascular resistance. The quantitative evaluation of these effects requires the constancy and reproducibility of one major determinant of coronary blood flow, i.e., coronary perfusion pressure. A constant perfusion pressure also avoids a pressure dependent transmural flow redistribution. Due to the transient nature of changes in coronary vasomotor tone, such a perfusion system must have a fast regulatory response. A roller-pump, combined with an air chamber of 40 ml, provides blood at a pressure of up to 100 mmHg and a maximal flow of 350 ml/min. The pressure in the air chamber is measured and the pump speed is controlled by the first servo-loop to maintain this pressure. To reduce the high pressure of the windkessel to the selected coronary perfusion pressure, a variable flow-regulator using a feedback-loop to the pressure signal measured at the tip of the perfusion cannula by a 8Am 4-327-I transducer. The dead volume of the whole system is about 60 ml.

The dynamic performance was tested in vitro and in acute experiments. Pressure steps with a simulated load are fully regulated within 300 ms and the overshoot is less than 10 mmHg. In vivo, pressure is stabilized within less than two cardiac cycles during postocclusive reactive hyperemia with a peak flow of about 300 ml/min. During five hours of perfusion in vitro and in vivo hemolysis was less than 5%. Conclusion: This perfusion system is suitable for experiments with constant perfusion pressure and permits the investigation of transient changes in coronary vessels. It combines fast regulatory performance with a low dead volume.

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CALIBRATION METHODS FOR INTRACELLULAR ION MEASUREMENTS WITH FLUORESCENT DYES
F. Gollnick, R. Mayer, S.Y. Wang

The new generation of dual wavelength fluorescent dyes for ion measurement and localization allows absolute quantitation. By microspectrofluorimetry and/or by image analysis intracellular ion-concentrations can be measured in single cells, fluorescent dyes make use of these facilities reliable calibration procedures are necessary. Different in vivo or in vitro calibration procedures have been developed in the last years. We have compared some of these with respect to their applicability to two cell types: 1. isolated heart muscle cells, 2. freshly isolated neutrophils (Amoeba proteus). Three in vitro calibration procedures and one in vivo procedure were tested. Two of these work in vitro with pure ionic mixtures and can be carried out easily. The composed solutions resemble the ionic and osmotic composition of cytoplasmic. Solutions were measured as thin layer (1 mm thick) in a special chamber or in microcapillaries with a rectangular profile of 50 μm height (viewing pathlength) and 500 μm width. The third in vitro method was to bring a cold methanol extracted cell model in a small droplet (cell volume) of calibration solution. This led to less effects and drying artifacts. In vivo calibration was carried out using the Rmin/Rmax-technique (G. Grynkiewicz et al., Biophys. J. 49:1404-1414, 1986). Rmin was evaluated in fur-2 loaded heart cells by internal perfusion from a patch clamp pipette with 10 mM EGTA less than 1 mM Ca²⁺, Rmax (1 mM Ca²⁺) was measured by permealisation of the cells with digitonin. All tested methods reveal potential sources of artifacts, e.g. ratios of R(340/380 nm) of Rmax varied from 3.7 ± 0.8 (in vivo calibration), 6.2 ± 0.7 (calibration with capillaries), and 11.8 ± 1.5 (calibration in thin layers); Rmin was always around 0.5 independent of the calibration method (± S.D.). Kd as calculated from measurements with capillaries was 480 ± 79 mM. The great variety of R(340/380 nm) which are relevant for the determination of Kd can be explained by the small signal to noise ratio at excitation with 380 nm. This leads to artificially elevated ratios in the case of 1 mm layers. At in vivo calibrations loss of dye due to digitonin treatment may suppress the ratio before the real maximum is reached. The biggest quantitation errors occur at high Ca²⁺-levels beyond the physiological range. Thus absolute quantitation seems to be useful although intracellular Kd-values are still uncertain.

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A NEW THEORY OF THE MONOPHASIC ACTION POTENTIAL
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In the past the Monophasic Action Potential (MAP), derived by means of extracellular suction or pressure electrodes, was generally explained in terms of the "injury theory". This postulates that the cells, "injured" by suction or pressure, are depolarized and serve as a reference electrode.

We offer a new explanation ("coupling theory"), which postulates an increase in conductance of the membranes under suction or pressure. Thus, the extracellular MAP electrode becomes able to register the intracellular potential which is clamped to the neighbouring cell potentials by the low resistance of the gap junctions. This means that the MAP consists of two signals: the local extracellular potential and the intracellular potential, diminished in amplitude, due to the resistance of the gap junctions and the membranes under suction or pressure.

The following findings support the "coupling theory": (1) Within the MAP signal the local extracellular potential can be identified. (2) Inside the cells under suction or pressure action potentials can be recorded by means of microelectrodes. (3) The typical potential distribution of the MAP with 1/3 positive and 2/3 negative can be explained. (4) Deformations of the MAP-plateau are the result of excitory phenomena occurring at the membranes under suction or pressure. (5) The time course of the decrease in MAP-amplitude corresponds to the electrical decoupling of the gap junctions (healing over).

The "coupling theory" allows a stringent interpretation of the MAP and offers a more precise evaluation of the intracellular action potential.

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HEMODYNAMIC DEPENDENCY OF MYOCARDIAL OXYGEN CONSUMPTION INDICES
J.D. Schipke, D. Burkhoff, and J. Alexander Jr.

Myocardial oxygen consumption (MVO₂) is difficult to measure in situ. Accordingly, multiple indices exist to predict MVO₂ from more easily measurable physiological signals. We tested the dependency of Bretschneider's total energy requirement (Et), Roosk and Feigl's pressure work index (FWI), and Sugar's product volume area (PVA) on afterload, contractile conditions and contractile state. MVO₂ was measured according to Fick's principle in 7 isolated, canine hearts at 5 end-diasstolic volumes at each of 4 settings of afterload resistance: 1.5, 3.0 and 6.0 mmHg/g/ml, and the heart contracting isovolumically. The measured values of MVO₂ (MMVO₂) were compared to values predicted (PMVO₂) by the three indices. There was no consistent influence of afterload resistance on the MMVO₂/PMVO₂ relations. In 5 of the hearts we also tested the indices when contractility and heart rate were varied simultaneously. In this case, there was a larger influence on the MMVO₂/PMVO₂ relation for the three indices. While there was always a high degree of correlation between MMVO₂ and PMVO₂ for each of the parameters under the conditions tested, there was significant variability in the regression coefficients from one heart to another. As a result, linear regression applied to the data pooled from all hearts and conditions provided regression coefficients that were different than those obtained in the individual hearts. We conclude that there is a type of influence of afterload and larger influence of contractile state on the three indices of MVO₂ which we tested.

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In the dog heart in situ measurement of the accumulation of S-adenosylhomocysteine (SAH) can serve as a sensitive index of regional free adenosine (Deussen et al., Circ. Res. 63: 250-261: 1988). This method is based on the enzymatic properties of SAH-hydrolase, a cysteysys enzyme homogenously distributed in canine myocardium. In order to investigate whether the “SAH-method” of measuring adenosine is limited to the dog heart, the activity of the SAH-hydrolase was determined in hearts of various species (dog, rat, guinea pig, sheep, rabbit, bovine) including human tissue samples taken from explanted hearts of patients suffering from dilative cardiomyopathy. Cytosolic extracts were prepared from tissue of the left and right ventricular wall, the septum and the atria. Homogenates were assayed for SAH-hydrolase activity in direction of SAH-synthesis using HPLC-techniques.

In each species analyzed a substantial SAH-hydrolase activity was found: bovine myocardium exhibited the highest activity (4.54 ± 0.16 nmol/min/mg protein) followed by guinea pig (2.27 ± 0.40), sheep (1.77 ± 0.10), pig (0.96 ± 0.14), rat (0.94 ± 0.06), rabbit (0.90 ± 0.04) and dog (0.88 ± 0.18). Human SAH-hydrolase activity amounted to 0.78 ± 0.09 nmol/min/mg protein. Enzym activity in most species was homogenously distributed over the different regions of the heart. Only the atria of pig and dog exhibited activities 70% above those of the other myocardial regions. In wistar rats aged 1 month, 4 months and 2 years mean hydrolase activity was 0.87, 0.90 and 0.97 nmol/min/mg protein, respectively.

Conclusions: The cardiac activity and distribution of SAH-hydrolase in various species including man is adequate to assess the regional adenosine metabolism by means of the “SAH-method”.

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204 ENERGETIC ASPECTS OF ISOLATED CARDIOMYOCYTES

H. Rose, S. Pipping, H. Kammermeier

A new technique was developed to measure simultaneously oxygen consumption and shortening of isolated ventricular myocytes of rat hearts during stimulation. The length-time integral (Iₜ) was taken as a measure of the “work” performed by the contracting cells and correlated to the oxygen consumption per beat (Vᵧₒ₂). Stepwise inhibition of the actin-myosin interaction by addition of 2,3-butanedione monoxime (BDM) was characterized by a linear relationship between Iₜ and Vᵧₒ₂. Extrapolation to the point of complete inhibition of the actin-myosin ATP-ase led to an oxygen consumption due only to the cycling of ions (Ca²⁺,Na⁺,K⁺), related to contraction.

Basal oxygen consumption was 215 ± 14 nl/min/mg protein. Vᵧₒ₂ amounted to 0.722 nl/min/mg protein (with 0.5 mM [Ca²⁺]), 21% of which was used for lncycling. This value increased to about 40% of Vᵧₒ₂ of the unloaded cells when [Ca²⁺] was 1.8 mM. The influence of inotropic drugs on calcium cycling should be detectable with this new method.

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205 COMPUTER ASSISTED MEASUREMENT OF CONTRACTIONS AND OXYGEN CONSUMPTION OF ISOLATED CARDIOMYOCYTES

H. Rose, K. H. Stratmann & H. Kammermeier

The course of contraction of isolated cardiomyocytes is difficult to measure mechanically and most optical methods have a poor time resolution. Thus we developed a technique with a resolution of less than 1 ms. The myocytes (some of which stick to a cover slip) were stimulated electrically by biphasic pulses in a stimulation chamber, observed through a microscope and CCD camera, and the images were recorded and digitized. 36 frames illuminated by a stroboscope were taken at increasing time intervals between stimuli and snap. The difference between these frames and a reference frame of the cells in the relaxed state gave the information to quantify the contractions. This system was coupled with a pO₂-electrode, thus oxygen consumption and contraction could be measured simultaneously.

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206 EVALUATION OF INOTROPIC REACTIONS OF ISOLATED CARDIOMYOCYTES

H. Rose, B. Lukaszek & H. Kammermeier

Isolated Myocytes of adult rats were fixed on petridishes and stimulated by alternating series of biphasic electrical pulses (1 Hz, 30 s) and quiescence (30 s). Inverted microscope, CCD camera and dimension analyser enabled us to measure the amplitude (ΔL) shortening- and relaxation-velocity (+/-dL/dt) both, from (i) the first contraction of a serie and (ii) for the steady state contractions. The “Treppe” phenomenon could thus be quantified.

From these data the contractionrate r⁺ (ΔL/dt_max)/L and relaxationrate r⁻ (ΔL/dt_min)/L were calculated. Reduction of temperature (from 37 to 21°C) led to a strong positive inotropic response, which was accompanied by a reduction in r⁺ and r⁻ (Eₐ = 48 kJ/mol). 2,3 butanedionesmonoxime (BDM) significantly reduced ΔL, dL/dt, while r⁺ and r⁻ remained unchanged. Reduction of temperature as well as 10 mM caffeine reduced the BDM efficiency. Caffeine alone abolished the negativ treppe and reduced r⁻. Oscillations occurred during the relaxation, they were enhanced if Na⁺ was replaced by Li⁺. With 50 % Na⁺ exchange r⁻ was reduced by 65 % and time of relaxation was extremely prolonged. With the setup the role of SR and Na⁺/Ca⁺-exchange can be assessed.

(supported by DFG Ro 755/1-1)

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DETERMINATION OF EFFICACY OF SOME VASOACTIVE AGENTS ON TRANSMURAL DISTRIBUTION OF CORONARY FLOW IN ISOLATED RABBIT HEARTS BY THE MODIFIED THERMODIEMETRIC TECHNIQUE

K. Haverkampf, M. Guhlmann, H. Antoni

Clinical finding that the subendocardial layer of the heart is more susceptible to ischemic damage than the other ventricular regions has focussed the experimental research on the analysis of transmural distribution of myocardial blood flow. In order to more simplify the assessment of flow distribution we have modified the conventional thermocouple method. Bolus injection of cold Tyrode solution (500 µl) into the aorta of thermally isolated Langendorff-perfused rabbit hearts induced typical epicardial (EPI) resp. endocardial (ENDO) time courses of temperature, which were recorded thermocouples (diameter: 0.5 mm) imbedded at the top of every branch of a special constructed forceps (length: 15 mm), whereby temperature measurements of corresponding epicardial and endocardial areas of the left ventricular wall were enabled. The study was undertaken to evaluate this indirect method of analysing transient temperature courses as function of transmural flow distribution and to elucidate if adenosine and diltiazem increase blood flow proportionately in subendocardium and subendocardium of nonischemic regions. Vasocostriction produced by norrenephrin (0.02 IE) showed a reduction of the minimal temperature value (Tmin: −3 ± 5°C) in comparison to control; that means that the subepicardial flow is more reduced than the subendocardial flow. Vasodilatation caused by diltiazem (1 µM) induced proportionate distribution of coronary flow to subepicardial and subendocardial layers (Tmin ø + 39 ± 3°C: + 39 ± 1°C EPI). In contrast, adenosine (0.2 µM) produced a greater increase in subepicardial flow (Tmin ø + 25 ± 6°C EPI) than in subendocardial flow (Tmin ø + 25 ± 6°C EPI). These results suggest that the developed method is appropriate to determine the transmural distribution of coronary flow in isolated hearts.

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RHYTHM PHENOMENA OF NEONATAL HEART CELL CULTURES DUE TO EXTERNAL STIMULATION

K. Haverkampf, M. Guhlmann, H. Antoni

Action potentials and coupling phenomena derived from cell patch clamp recordings in cell cultures of neonatal rat heart were evaluated and the development of spontaneous beating rhythms was analyzed.

Morphologically, the cells in culture are first single and uncoupled, then they grow to pairs, afterwards to clusters of many cells up to a monolayer covering the whole culture dish. The electrical and mechanical activity of the culture varies during growth due to the varying interaction between the cells. This leads to more or less complicated patterns of spontaneous beating, which can be classified into distinct groups.

To investigate the cell layer under controlled conditions, we stimulated the whole culture via field application in the frequency range between 2 and 20 Hz. By this means we were able to compare the behaviour of our strongly coupled cell monolayer of simple geometry with the response of a single cell and of the much more complicated structure of the whole heart. The cell layer shows a similar behaviour as a single cell in that entrainment regions can be found as well as irregular responses which are characteristic in nature. A chaotic behaviour is also observed in the spontaneous bursting mode of the culture. From this it can be concluded that during this bursting mode the sodium system must be often inactivated by partial voltage clamp mode to strong coupling between cells in the culture.

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Distal a severe stenosis sympathetic activation can induce ischemic myocardial dysfunction due to an adrenergic coronary constrictive effect. We tested the effect of the new α-adrenoceptor-antagonist BDF 8933, that isought to have a higher affinity to post- than to presynaptic α-adrenoceptors, on this mechanism. In anesthetized mongrel dogs sympathetic activation was performed by electrical stimulation of the left n. cervicallis ventrolateralis before and after production of a severe stenosis on the ramus circumflexus of the left coronary artery and after injection of 150 μg/kg of BDF 8933. In one group (n = 7) enddiastolic coronary resistance and in an other group (n = 6) systolic myocardial wall thickening (sonomicrometry) was calculated.

**Conclusion:** The new α-adrenoceptor-antagonist BDF 8933 prevents sympathetic-induced myocardial dysfunction in poststenotonic myocardium and increases peak left ventricular pressure.

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Mean systolic wall thickening (WT) is often used for the description of myocardial function. However, WT does not account for early systolic wall thinning and post-systolic wall thickening, which can both be observed in ischemic and reperfused myocardium.

In 10 open-chest dogs the myocardial function of the posterior wall was investigated after 14 min occlusion of one or two side branches of the left circumflex coronary artery. In the ischemic myocardium the function was assessed using sonomicrometry. Mean systolic wall thickening expressed as a percentage of end-diastolic wall thickening was calculated, as well as systolic wall thickening during the contraction cycle (GT) expressed as a percentage of the minimal systolic wall thickness. Results:

|               | without stenosis | stenosis | stenosis+BDF 8933 |
|---------------|------------------|----------|-------------------|
| R             | 1.43±0.7         | 1.25±0.7 | 1.43±0.1          |
| WTP           | 12.7±3.6         | 16.8±5.4 | 14.2±2.9          |
| WTC           | 15.2±2.9         | 19.2±5.9 | 15.6±2.7          |
| LVP           | 131±21           | 161±24   | 124±28            | 124±28       |
| * p < 0.05 SNS vs control |     | 152±51   | 177±25           |

**Conclusion:** The extent of post-systolic myocardial wall thickening (WT, [%]) has been described as a marker of myocardial dysfunction during ischemia and reperfusion. However, the dependency of post-systolic myocardial wall thickening on heart rate (HR [1/min]) is unknown. To investigate this dependency experiments were performed in 6 open-chest dogs. One or two side branches of the left circumflex coronary artery were occluded for 15 min to induce reperfusion dysfunction in a posterior myocardial area. 30 min after onset of reperfusion, global ventricular function was evaluated by measuring peak left ventricular pressure (LVP [mmHg]). Both regional myocardial function in the reperfused (WT, [%]) and in an anterior control area (WT, [%]) was assessed using sonomicrometry. HR was varied by left atrial pacing. Results:

|                | HR | LVP | WT | WT |
|----------------|----|-----|----|----|
|                | 142±5 | 98±2 | 16 | 6.2±1.4 |
|                | 179±6 | 98±2 | 16 | 6.2±3.4 |
| * (p < 0.05; Mean ± SD) |     |   |    |    |

These data demonstrate that global ventricular function was unaffected by changes in HR. In contrast to a significant decrease in WT, posterior myocardial function was unchanged. This might in part be due to the recruitment of a increasing portion of WT into systolic function with increasing HR. We conclude that the description of myocardial dysfunction with the help of WT is limited if HR changes.

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The extent of post-systolic myocardial wall thickening (WT, [%]) has been described as a marker of myocardial dysfunction during ischemia and reperfusion. However, the dependency of post-systolic myocardial wall thickening on heart rate (HR [1/min]) is unknown. To investigate this dependency experiments were performed in 6 open-chest dogs. One or two side branches of the left circumflex coronary artery were occluded for 15 min to induce reperfusion dysfunction in a posterior myocardial area. 30 min after onset of reperfusion, global ventricular function was evaluated by measuring peak left ventricular pressure (LVP [mmHg]). Both regional myocardial function in the reperfused (WT, [%]) and in an anterior control area (WT, [%]) was assessed using sonomicrometry. HR was varied by left atrial pacing. Results:

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In the presence of a severe coronary stenosis oxygen supply in the poststenotic myocardium is reduced. In a situation which exists in severe coronary stenosis coronary blood flow and decrease myocardial performance could have a beneficial effect on the poststenotic myocardium. The structurally novel benzopyran K-agonist BRL 34915 relieves vascular smooth muscle cells. Additionally, cardio-depressant properties have been reported about this drug. We therefore investigated in 6 open-chest dogs, if cumulative intracoronary doses (1 μg, 4 μg, 14 μg) of BRL 34915 could enhance coronary blood flow and simultaneously reduce myocardial function in poststenotic myocardium, thereby increasing oxygen supply and lowering oxygen demand. This substance increased mean left circumflex coronary blood flow [ml/min×100g] dose-dependently from 59 ± 12 (mean ± SEM) (no BRL) to 227 ± 44 (14 μg BRL) (p < 0.05) in intact coronary arteries and from 36 ± 7 to 74 ± 13 (p < 0.05) distal to a severe stenosis, respectively. In contrast, posterior systolic wall thickening [%] (Sonomicrometry) was significantly decreased only by a cumulative dose of 14 μg BRL from 9.7 ± 1.8 (no BRL) to 7.8 ± 2.1 (14 μg BRL) (p < 0.05) in the ischemic and reperfused myocardium. These results demonstrate that BRL 34915 can simultaneously enhance coronary blood flow in the poststenotic myocardium and decrease myocardial function, potentially narrowing the gap between oxygen supply and demand.

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PROBLEMS IN TIMING OF END-SYSTOLE FOR THE CALCULATION OF REGIONAL MYOCARDIAL FUNCTION DURING VENTRICULAR ASYNCHRONY.

R. Bretschneider, R. D. Guth and G. Heusch

The regional myocardial function is often based on a comparison of myocardial wall thickness at end-diastole to that at end-systole (Xs). Consequently, the accurate calculation of regional myocardial function depends on the exact definition of the end of systole. The maximum contractile state at end-systole (Xs) is determined by end-systolic stress, but also by the tension developed during the systolic interval. Thus, the definition of end-systole must be based on an accurate determination of the maximum heart rate during the entire cardiac cycle. The maximum heart rate during the entire cardiac cycle is determined by the difference between the maximum heart rate and the heart rate at the onset of diastole (Xd).

L. H. G. Bretschneider, R. D. Guth, R. J. Oudiz and G. Heusch

The minimal contractile state at end-systole (-dP/dt) is defined as the point at which the maximum contractile state is achieved during the cardiac cycle. The maximum heart rate during the entire cardiac cycle is determined by the difference between the maximum heart rate and the heart rate at the onset of diastole (Xd). The maximum heart rate during the entire cardiac cycle is determined by the difference between the maximum heart rate and the heart rate at the onset of diastole (Xd).

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Macromolecule permeability increases in ischemic myocardium. The dependence of endothelial permeability on the energetic state was investigated in the model of rat coronary microvascular endothelial cells (CMEC) and porcine aortic macrovascular endothelial cells (AMEC). Confluent monolayers of CMEC and AMEC on filter membranes were incubated with 10% fetal calf serum (FCS) in modified Tyrode solution (pH 7.4; 37 °C). Macromolecule permeability was determined with the aid of FITC labelled albumin. Under control conditions, the permeability was 11.3 x 10^-6 cm/sec with CMEC and 5.6 with AMEC. In both endothelial cell preparations, permeability remained unaltered, when incubated for 1 h with 5 mM KCN. When 10 mM deoxyglucose was present together with KCN, macromolecule permeability increased in CMEC by 41%, and in AMEC by 39%. After 1 h with KCN and deoxyglucose, the increased permeability could be completely reversed in both cell preparations by an 1 h-return to culture medium (medium 199 with 10% FCS).

Conclusions: Macromolecule permeability of microvascular and macrovascular endothelial cells is energy dependent. Glycolytic energy production is sufficient to maintain a normal permeability. Permeability changes caused by energy depletion do not indicate irreversible endothelial injury.

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221 CARDIAC ENDOTHelial FUNCTION INVESTIGATED BY EXPERIMENTAL ALTERATION BY GASPERFUSION (GP)
H. Mertens, O. Obst, H. Kammermeier

Isolated rat hearts were perfused under constant pressure with saline solution and to manipulate the endothelial function with carbogenas for 20 min.

The following consequences of the GP could be observed: 1) Purine nucleoside phosphorylase, an endothelial cytosolic marker enzyme, could be detected in the interstitial transudate (IT) (1.3 ± 2.1 µg/mg tissue) but not in the venous effluent (VE) (5.0 ± 2.6 µg/mg with a concentration gradient IT/VE greater than 100:1 (total content: 180 µg/mg heart)). 2) The production rate of the IT was increased up to a factor of 5. 3) The coronary response to 2 min anoxia was inverted from an increase before GP to a decrease. 4) A substantial alteration of the myocytes could be largely excluded because: a) during the first 16 min after GP the LDH release in the IT amounted to less than 1% of the total content (210 U/g heart), b) the VO_2 was unchanged, c) the pressure-rate product was reduced by 20%, d) the coronary flow reached a stable level of about 2/3 of control, e) the myogenic autoregulation was maintained. 5) The rate of uric acid (UA) release was unchanged before and after GP. 6) The transcapillary UA gradient during noradrenaline (NA) administration (10^-7, 10^-6 M) significantly decreased after GP. 7) The transcapillary permeability of NA increased after GP.

The points 1) and 2) are interpreted in terms of an endothelial lesion paralleled by a partial (1) abolition (2),3,6,7) of the barrier function.

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222 CALCIUM-DEPENDENCY OF EDRF/NO SYNTHESIS IN ENDOTHELIAL CYTOSOL IS MODULATED BY CALMODULIN
A. Mülisch and R. Busse

A transmembrane calcium influx is involved in the agonist-induced release of endothelium-derived relaxing factor/nitric oxide (EDRF/NO) from endothelial cells (A. Lüchhoff et al., Br J Pharmacol 95: 189, 1988). Recently we have demonstrated that NADPH-dependent EDRF/NO synthesis from L-arginine in isolated endothelial cytosol is increased by free calcium ions (EC_50 0.3 µM; A. Mülisch et al., Naunyn-Schmiedeberg's Arch Pharmacol, in press). We now studied whether calmodulin, which is known to mediate calcium sensitivity to a variety of target enzymes, could modulate the calcium sensitivity of EDRF/NO synthesis in isolated endothelial cytosol. EDRF/NO was quantified by activation of a purified soluble guanylate cyclase incubated with cytosol from freshly harvested porcine aortic endothelial cells. With 0.1 mg cytosolic protein/ml the calcium-dependent, but not the calcium-independent EDRF/NO synthesis was potently inhibited by the calmodulin antagonists melittin, mastoparan, and calcineurin (IC_50 1 µM, 1 µM and 1 unit/50 µl, respectively), but not by fendiline, trifluoperoxime or calmidazolium (up to 10 µM). The inhibitory potency was increased by preincubation of inhibitors with cytosol (10 min, 30°C). Inhibition was overcome by exogenously added porcine brain calmodulin, or by addition of heat-denatured endothelial cytosol, which contained about 0.3 µM calmodulin, as measured by radioimmunoassay. We conclude that calcium-calmodulin is tightly associated with the enzyme(s) catalyzing EDRF/NO synthesis, thereby providing the calcium sensitivity of endothelial EDRF/NO synthesis.

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The myogenic vascular response to increases in transmural pressure represents a mechanism which potentially reduces the coronary reserve. Since EDRF is released in increased amounts in response to increased shear stress, it was tested whether EDRF interferes with the magnitude of myogenic responses of the coronary vascular bed to increases in perfusion pressure. Isolated rabbit hearts (n=5; Langendorff technique) were perfused under constant pressure conditions with a modified Krebs-Henseleit solution. The resulting flow was measured by means of an electromagnetic flow probe. Flow responses to changes in perfusion pressure were investigated before and after pretreatment of the coronary circulation with the stereospecific inhibitor of EDRF-synthesis N\textsuperscript{\textregistered}-nitro-l-arginine (L-NO\textsubscript{N}A; 30 \textmu M). A sudden increase in the perfusion pressure from 70 to 120 mm Hg induced an initial increase in flow by 108 ±13% (corresponding to a decrease in vascular resistance(VR) by 22 ± 4%). This was followed within 20 seconds by a renewed increase in VR by 12 ± 4%. After L-NO\textsubscript{N}A, this increase in VR was significantly higher (56 ± 15%; p≤0.05). During a stepwise increase from 45 to 120 mm Hg the flow increased from 24 ± 2 to 56 ± 3 ml/min (+136 ± 14; n=7). After L-NO\textsubscript{N}A the flow increased from 14 ± 5 ml/min to only 22 ± 6 ml/min (+47 ± 17%; p≤0.05). In contrast, the D-stereoisomer D-NO\textsubscript{N}A did not significantly affect the vascular responses to changes in perfusion pressure. It is concluded that EDRF significantly attenuates myogenic responses to increases in coronary pressure. This would otherwise tend to reduce the coronary conductivity. This effect may be mediated by an enhanced release of EDRF due to changes in shear stress at the endothelial surface.

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ENHANCEMENT BY L-ARGININE OF ACETYLCHOLINE-INDUCED RELAXATION IN ISOLATED RAT BASILAR ARTERY

U. Pohl, D. Lamontagne, E. Bassenge, and R. Busse

The endothelium can affect vascular tone by releasing vasoactive compounds, one of these termed "endothelium-derived relaxing factor" (EDRF). Recently, NO, probably derived from L-arginine has been shown to account for at least a part of the EDRF-action, which can be elicited by acetylcholine (ACH) or by nitro-l-arginine (NO\textsubscript{N}A; 50 \textmu M) or by Oxy-haemoglobin (Oxy-Hb). Therefore, we studied the effects of L-arginine on precontracted rat basilar artery and on ACH-induced EDRF-dependent relaxation in the presence of NO\textsubscript{N}A or Oxy-Hb.

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THE cAMP-DEPENDENT PHOSPHORYLATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) PREVENTS PROTEOLYSIS BY ENDOPEPTASE-24.11 BUT HAS NO EFFECT ON THE HALF-LIFE OF ANP IN THE BLOOD.

Th. Elber, M. Fleckenstein, D. Hock, M. P. Christmann and W. G. Corssmann

Exogenously administered ANP in the circulation exhibits a short half-life time of 1 to 2 min. In human coronary sinus blood of patients with cardiovascular disease, levels of ANP decreased between Cys-105 and Phe-105 amounts to 20-30% (T. G. Yandle et al. BPC 61:832, 1987) indicating participation of the endopeptase-24.11. This hypothesis was supported by studies showing the cleavage of ANP in the kidney with the highest concentration in the proximal tubulus. In order to determine whether the endopeptase-24.11 contributes to the ANP clearance, endopeptase-24.11 labelled ANP together with unlabelled ANP (27-32k ANP, spec. act. 3Ci) were injected into anesthetized rats (phentobarbital i.p. 60mg/kg) and blood samples were removed. The determination of ANP in the plasma was achieved by measuring the radioactivity following separation by RF-HPLC. (3H-labelled ANP was used for the control's with unphosphorylated ANP.) We found similar clearance rates for phosphorylated and unphosphorylated ANP. Approximately 50% of the peptides were removed from the plasma within 1 to 1.5 min.

The results indicate that the endopeptase-24.11 plays no major role in the clearance of ANP from the blood. However, because of the high endopeptase-24.11 concentration in the kidney the proteases may be involved in the regulation of specific ANP functions in this organ.

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EXCESSIVE MURAL CALCIUM UPTAKE IN DIFFERENT TYPES OF HUMAN ARTERIOSCLEROTIC VESSELS

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Chemical analysis of stenosing coronary plaques which had caused large coronary insufficiency and had revealed tremendous accumulation of calcium (86-fold above normal) whereas free and total cholesterol rose by only 1.8 and 1.7 times, resp. As already shown in previous animal experiments (1-3) calcium overload has to be considered a pathogenic factor in the development of arteriosclerosis. Thus in human coronary walls, the severity of plaque formation (grade I-III according to WHO classification) positively correlates with the degree of Ca accumulation. In contrast, there is no or even an inverse correlation between coronary plaque development and the free or total mural cholesterol contents. Aortic plaques (grade II and III) contain more cholesterol than coronary plaques of grade II or III do. However again, also in these aortic lesions, tremendous Ca overload that positively correlates with the degree of arteriosclerosis is the most spectacular phenomenon. Sclerotic wall tissue of human femoral arteries is also characterized by an 80-90-fold increase in Ca above that of healthy young individuals. Here too, the augmentation of free and total cholesterol is modest. In arteriosclerotic dorsal foot arteries, excessive mural calcium overload takes place without any significant change in the cholesterol metabolism within.

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FLUORESCENT STAINING OF THE TOTAL AND PERFUSED CAPILLARY NETWORK IN THE BRAIN

H. Thalheim, U. Göbel and W. Ruchinsky

Previous studies have described a large fraction (40-50%) of nonperfused capillaries in the rat brain (e.g. H. R. Weiss et al., Proc. Soc. Neurosci. 5:494-504; 1982; H. R. Weiss, Microvasc. Re C. 36:172-180, 1988). These authors used FITC-dextran as an intravascular marker to measure the density of perfused capillaries. The density of morphologically existing capillaries was determined with the alkaline phosphatase (AP-) method. Both methods as used by Weiss et al. can be criticized: air drying of the cryosections may underestimate the intravascular marker; the light microscopic AP-method may not be sensitive enough to stain all morphologically existing capillaries in the brain.

Therefore we have developed a fluorescent double staining technique to quantify the density of perfused and morphologically existing capillaries in rat brain. First the perfused capillaries were visualized by intravascular Evan's blue, which was allowed to circulate for at least 10 seconds. Then the morphologically existing capillaries were stained by using a newly developed histochemical fluorescent method. The capillary wall constituent fibronectin was visualized by a primary antibody directed against fibronectin and a second FITC-coupled antibody (indirect immunofluorescence). The existing capillaries were relocated in the same measuring field of the same brain section. This kind of double staining resulted in identical capillary counts (perfused-existing) in 97%. In contrast, the AP-technique yielded capillary counts that were consistently 20% lower.

The capillary wall constituent fibronectin was visualized by a primary antibody directed against fibronectin and a secondary FITC-coupled antibody. The existing capillaries were relocated in the same measuring field of the same brain section. This kind of double staining resulted in identical capillary counts (perfused-existing) in 97%. In contrast, the AP-technique yielded capillary counts that were consistently 20% lower. These authors used FITC-dextran as an intravascular marker to measure the density of perfused capillaries. The density of morphologically existing capillaries was determined with the alkaline phosphatase (AP-) method. Both methods as used by Weiss et al. can be criticized: air drying of the cryosections may underestimate the intravascular marker; the light microscopic AP-method may not be sensitive enough to stain all morphologically existing capillaries in the brain.

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CEREBRAL AND CARDIAC RESPONSES TO UNILATERAL STIMULATION OF CAROTID SINUS BARORECEPTORS

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The present study was designed to investigate effects of unilateral baroreceptor stimulation on higher brain structures by measuring event-related potentials in response to activation and inactivation of baroreceptors located in either the right or the left carotid sinus.

Ten healthy right-handed men participated in the experiment (mean 33 years). Baroreceptor activation (BA) was evoked by applying a negative pressure for intervals of 6s each, either to the left or to the right sinus region, using two separate small chambers. Baroreceptor inactivation (BI) was achieved by applying a positive pressure to one side at a time. Pressure changes amounted to +28.6 ± 0.4 mmHg during BA and to +19.2 ± 0.2 mmHg for BI. In response to BA heart rate dropped significantly, validating that baroreceptors indeed were activated. Correspondingly heart rate increased when a positive pressure was applied to one of the two carotid sinus regions. Nine of the ten subjects responded with the more pronounced chronotropic effects to pressure manipulations over the right than for those over the left carotid sinus. Compared to BI, the slow negative brain potential was significantly reduced during baroreceptor stimulation, replicating earlier reports (Elbert et al., 1988). Over parietal cortex, the reduction in cortical negativity under BA was more pronounced ipsilateral to the site of stimulation than contralateral. The reverse effect was observed for BI.

Using the same stimulation devices, Tafi-Klawé et al. 1990 demonstrated that the baroreceptors on the left have relatively larger inotropic effects. Results are in line with the morphological and functional asymmetry of cardiac innervation. The carotid sinus nerves project bilaterally to the diencephalic part of the solitary nucleus. Thereby uncrossed pathways seem to be of primary importance. Furthermore, the present study suggests a considerable impact of baroreceptors on higher (cortical) centers.

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PECULIARITIES OF VITAMIN D3-INDUCED EXCESSIVE CALCIUM ACCUMULATION IN DIFFERENT RAT ARTERIES.

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Vitamin D3-intoxication (one single dose of 300,000 I.U./kg I.m.) leads to severe calcific sclerosis in coronary, mesenteric, and renal arteries of rats as well as in the aorta within 4 days (1-2). This type of arteriosclerosis is characterized by necrotization of Ca-overloaded smooth muscle cells and by incrustation of the elastic fibres with Ca salts. Interestingly, not all arteries respond in this way to vitamin D3 overdoses. In fact, the brain arteries (A. basilaris, A. cerebri media) exhibit a totally different behaviour, since (as shown in the Table) they proved to be rather resistant to vitamin D3-induced Ca accumulation. The preferential binding sites for Ca in the vascular smooth muscle cells and the elastic fibres. However, the cerebral vessels, particularly the basilar arteries, are scarcely provided with smooth muscle cells and elastic elements. Thus they are probably lacking a pronounced Ca-binding capacity.

**Table 1:**

| Mural Ca content mmol/kg dry tissue weight | Coronary art. 11.2 ± 0.64 (n=30) 30.7 ± 23.8 (n=94) | Basilar art. 10.3 ± 1.0 (n=21) 12.0 ± 1.4 (n=15) |

(1) Fleckenstein, A.: Calcium Antagonism in Heart and Smooth Muscle - Experimental Facts and Therapeutic Prospects. Monograph, John Wiley & Sons, Inc., Publ. Co., New York: Chichester - Brisbane - Tokyo - Singapore - Toronto.

(2) Fleckenstein, A., Frey, M., Zorn, J., A. Fleckenstein-Grün, G.: Experimental Basis of the long-term therapy of arterial hypertension with calcium antagonists. Am. J. Cardiol. 55, 3N-14H; 1985.

EFFECT OF ENHANCED OXYGEN DELIVERY ON PLASMA ADENOSINE [ADO] IN PATIENTS WITH SEPSIS

H. Bardenheuer, H. Forst, M. Haller, K. Peter

**INTRODUCTION:** IMPAIRED TISSUE OXYGENATION CAN HAVE DELETERIOUS EFFECTS ON THE OUTCOME OF CRITICALLY ILL PATIENTS. SINCE ADO IS A SENSITIVE MARKER OF TISSUE ISCHEMIA IN MAN, THIS STUDY INVESTIGATES THE INFLUENCE OF AN INCREASE IN OXYGEN DELIVERY [DO2] FOLLOWING BLOOD TRANSFUSION [BT] ON PLASMA ADO AND LACTATE IN PATIENTS WITH SEPSIS. THE VELOCITY OF OXYGENATION WAS INCREASED BY OCCLUSION OF THE FEED ARTERY TO ONE OF THESE TWO VASCULAR SECTORS SO THAT DURING OCCLUSION THE EFFECTED SECTOR WAS SUPPLIED BY COLLAGEN FLOW THROUGH THE AA. THE DIAMETER OF THE AA AND RED CELL VELOCITY THROUGH IT WERE MONITORED WITH A DUAL ELIT VIDEO MICROSCOPE SYSTEM /F.C. Johnson and M. Intaglietta, Am J Physiol 232:1666, 1976/.

**METHODS:** IN 18 PATIENTS (AGE 46 +/- 20) WITH HCT < 30% MEAN ARTERIAL BLOOD PRESSURE [MAP], HEART RATE [HR], CARDIAC INDEX [CI], AND PULMONARY CAPILLARY WEDGE PRESSURE [PCWP] WERE DETERMINED BEFORE [C], 10 AND 60 MIN, AND 24 HOURS AFTER BT. ALSO DO2 AND OXYGEN UPTAKE [CALCIMETR.] [VQO2] WERE DETERMINED AND PLASMA ADO, PH, AND LACTATE [LAC] ANALYZED.

**RESULTS:**

| (MEAN VALUES, N=16; * p < .0S) |
|-------------------------------|
| TIME after BT |
| C 18' 68' 24 H |
| MAP (MM HG) 77 90* 93* 81 |
| HR (1/MIN) 92 98 98 88 |
| CI (L/MIN/MB) 5.0 4.9 5.0 5.0 |
| PCWP (MM HG) 14 14* 11 12 |
| BCT (%) 28 32* 29 |
| DO2 (ML/MIN/KG) 13 15* 19* 19* |
| VQO2 (ML/MIN/KG) 3.3 3.5 3.6 3.5 |
| ADD (NMOL/L) 617 169* 310* 210* |
| LAC (NMOL/L) 110 110 103 103 |
| PH 7.42 7.42 7.41 7.48 |

**CONCLUSIONS:** 1.) BT ELEVATES MAP, PCWP, AND DO2. 2.) THE ENHANCED VQO2 [VQO2 = 2.5 + 0.06 X DO2, R = 0.5] WAS DUE TO AN INCREASED VENOUS FLOW TO ISCHEMIC AREAS FLOW-INDUCED CONTROL OF SMOOTH MUSCLE TONE AT MICROVASCULAR LEVEL

V. Cmiško, D.V. Lang and F.C. Johnson

Flow-induced, endothelium-mediated dilation has been demonstrated mainly in large conduit arteries. To determine whether the dilation exists at microvascular level we studied the effect of an increase in blood flow velocity on diameter of arcing arteriole /AA/, connecting two adjacent triangular vascular sectors in the rat mesentery. The velocity was increased by occlusion of the feed artery to one of these two vascular sectors so that during occlusion the affected sector was supplied by collateral flow through the AA. The diameter of the AA and red cell velocity through it were monitored with a dual slit video microscope system /F.C. Johnson and M. Intaglietta, Am J Physiol 232:1666, 1976/.

The occlusion of a feed artery increased red cell velocity in the AA from 9.9 ± 1.1 to 66.2 ± 3.5 mm/s /SEM. After a delay of 7.1 ± 0.6 s the AA dilated by 66.2 ± 3.5 % of the initial diameter /60 ± 2.4 %. The dilation of the AA was equal to the maximum dilation attained with topical application of papaverine, /B/ was sustained for the duration of increased velocity, /C/ was smaller during partial occlusion than during total occlusion, /D/ was absent in adjacent non-arcading arteriole without velocity increase. The dilation enlarged collateral volume flow to ischemic vascular sectors by about 150 % as compared to the increased flow immediately after occlusion. These observations indicate that a potent flow-dependent dilator mechanism is present in arterioles under 100 microns diameter.
Non-invasive assessment of stroke volume (SV) is mainly limited to 2 methods: impedance cardiography (ICG) and calculation from echocardiographic measurements of the systolic and diastolic changes of left ventricular dimensions. Impedance techniques are particularly attractive since they allow for real-time evaluation as well as for measurement of steady state SV. The purpose of the present study was to provide data on the variability of measurements of some key hemodynamic data including SV at several days’ intervals as they are often needed to be done in pharmacological intervention trials. Moreover, data on the reproducibility and the validity of the method as evaluated from the changes when raising from the supine to the seated posture are provided. Variability, 12 healthy male volunteers aged 22 to 29 yrs were studied 3 times at 1-week intervals under rigorously standardised steady state conditions in the supine posture; all parameters were recorded on-line and computed using an OS 9 system. SV was averaged over 20-second intervals. The values used are the means obtained during 10 such intervals; they correspond to a mean value as measured over 200-second periods. Mean values ± SD for SV were 94 ± 27, 109 ± 21 and 98 ± 16 ml without any statistically significant difference. The corresponding values obtained for cardiac output and total systemic vascular resistance were 6.3 ± 1.3, 6.9 ± 1.4, 6.1 ± 0.7 (mean) and 16.2 ± 4.7, 14.4 ± 4.1 and 15.6 ± 2.5 units respectively, again without statistically significant differences. Reproducibility was tested by considering the values obtained at two consecutive 100 msec periods as a duplicate determination. Regarding SV, the mean difference was 5% of the mean value (96 ml, n = 104), the corresponding coefficient of variation 2.7%. Validity: In 5 volunteers the hemodynamic parameters including SV were measured twice at an interval of approximately 5 min in the supine and the seated posture, i.e. in the course of a manoeuvre known to selectively affect SV in a predictable way. Sitting resulted in a mean reduction of 14.6 and 13.4% of the initial (supine) SV without a statistically significant difference. In previous studies it has been shown that the orrheostatic changes of SV measured by ICG compare favorably with those obtained by the Fick method.

It is concluded that ICG can be used for determination of SV changes as a substitute for invasive methods provided adequate standardisation is achieved. This is at least valid under resting conditions.

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RED CELL POTASSIUM CONCENTRATION AND RED CELL VOLUME DURING EXHAUSTIVE EXERCISE WITH A SMALL MUSCLE GROUP

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Red cell volume during exercise is reported to be constant. This is only possible if all factors influencing the volume (mainly pH and tissue osmolality) are counterbalancing each other. Some authors discuss a K⁺ uptake as a volume regulating factor.

Methods: 7 male volunteers performed handgrip exercise until exhaustion. Skin blood flow was reduced by cooling. Blood was drawn from a cubital vein. [Hb], [HCT], [Prot], [PO₂], [K⁺], [Na⁺], and [Cl⁻] in hemolysed blood were measured. [K⁺]ery was calculated and related to the water phase. Results: During exercise the following changes were measured: [PROT] from 7.2 +/− 0.3 to 7.7 +/− 0.4 g/dl; [Hb]: 15.2 +/− 0.6 to 16.2 +/− 0.7 g/dl; MCHC: 34.5 +/− 0.6 to 35.4 +/− 1.0 g/dl; [K⁺]ei: 4.2 +/− 0.15 to 7.2 +/− 0.52 mmol/kg H₂O; [K⁺]eri: 1.36 +/− 9.0 to 138.6 +/− 4.0 mmol/kg H₂O; pH: 7.352 +/− 0.026 to 7.715 +/− 0.046; OSM: 293.8 +/− 4.2 to 319.3 +/− 8.7 mosmol/kg H₂O. All changes were significant except [K⁺]ei.

Discussion: The decrease in pH would cause an increase in red cell volume but that is over compensated by the high tissue osmolality which leads to shrinking of the red cells and thus to an increase of MCHC. [K⁺]eri is influenced by water shifts only but is no volume regulating factor in the red cell under these conditions.

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BLOOD FLOW RESPONSES OF THE INTERNAL MAXILLARY ARTERY AND OF ITS VASCULAR COMPONENTS - AVA AND CAPILLARIES - TO INCREASES IN SYSTEMIC ARTERIAL BLOOD PRESSURE.

J. Mühling, F. Bari and K. Pleischka

To evaluate the role of baroreceptor reflexes in blood flow control of the facial and nasal vasculature, total blood flow and perfusion pressure (PP) of the internal maxillary artery (IMA-FLOW) were recorded bilaterally at rest and during baroreceptor stimulation (ST) in 10 anesthetized, paralyzed and artificially ventilated dogs. ST was achieved by increasing spontaneously established blood pressure (BP) in the aortic arch with an inflatable balloon, positioned in the descending aorta. Mean increases in regional BP were 35.4 (SE 1.94) mmHg (ST1); 53.1 (SE 1.27) mmHg (ST2), and 71.7 (SE 2.53) mmHg (ST3). Distribution of IMA-FLOW to precapillary (CAP-FLOW) and arteriovenous anastomoses (AVA-FLOW) was determined ultrasonographically (Hashimoto et al., Pflügers Arch. 402, 580, 1985) during the three periods of ST and the corresponding resting conditions. Further variables measured or determined were cardiac output (CO) of the right heart, heart rate (HR), and peripheral resistance of the IMA (R-IMA). The results are listed in the table as percentage changes during ST1. (* indicates significance at P < 0.05).

|            | ST1 (%) | ST2 (%) | ST3 (%) |
|------------|---------|---------|---------|
| PP         | +23.9   | +31.5   | +68.0   |
| R-IMA      | -11.0   | -10.5   | -2.5    |
| IMA-FLOW   | -36.2   | +74.2   | +76.0   |
| AVA-FLOW   | +37.8   | +84.7   | +105.1  |
| CAP-FLOW   | +33.2   | +61.1   | +48.1   |
| CO         | -34.0   | -49.3   | -37.5   |
| HR         | -43.4   | -41.9   | -37.5   |

The results demonstrate that ST1 caused significant increases in IMA-FLOW concomitant with significant stimulus strength dependent increases in PP. The increase in IMA-FLOW was mainly due to marked increases in AVA-FLOW rather than to increases in CAP-FLOW. Because of the less pronounced decreases in R-IMA it appears that the IMA-FLOW responses were passively induced. On the other hand the preferential increase of AVA-FLOW during all periods of ST1 could also be reflexive to baroreceptor stimulation. Unimpaired of baroreceptor reflex was indicated by decreases in heart rate in association with decreases in cardiac output during ST1.

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CARDIOVASCULAR EFFECT OF DIPA-DCA COMPARED TO CLONIDIN IN ANAESTHETIZED GUINEA-PIGS

H. D. Schmidt, H. Groß, and W. Loock

In previous studies in cats DIPA-DCA proved to have a strong sympatholytic effect (Schmidt et al., Pflügers Arch. 406, R64, R85, 1986) which is presumably due to activity on the ganglionic level. In the present study DIPA-DCA is compared to the centrally-acting sympatholytic agent clonidin. In 32 anaesthetized guinea-pigs, increasing doses of DIPA/DCA, up to 5mg/kg, progressively decreased aortic pressure (AP -33%), heart rate (HR -10%), cardiac output (CO -12%) and total peripheral resistance (TPR -25%). Similar effects were seen with 1 µg/kg clonidin: AP -39%, HR -10%, CO -9%, and TPR -38%. When clonidin was injected after 5mg/kg DIPA/DCA, AP increased by +23%. This proves that DIPA-DCA had completely blocked the sympathetic system, revealing the effect of clonidin on peripheral α₂-receptors. When DIPA/DCA was given after premedication with clonidin AP no longer decreased, but increased considerably (+88 at 5mg/kg and +16% at 20 mg/kg). TPR increased (+19%) while CO remained unchanged and HR decreased slightly. This effect on peripheral vessels cannot be prevented by prazocin and is also present when the ganglion blocking agent, hexamethonium, is used.

Conclusion: The maximal cardiovascular effect of DIPA-DCA is qualitatively and quantitatively similar to clonidin. It also shows a stimulatory effect on the peripheral vasculature which is not, however, mediated via α₂-receptors. DIPA-DCA seems as suitable as clonidin as a non-β-receptor blocking sympathetic agent.

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A Hall-effect sensor for recording pulse waves and diameter changes in cutaneous veins

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When recording venous pulse waves to determine the pulse wave velocity special problems arise from the low blood pressure in these vessels. Accordingly, most of the numerous pulse transducers designed for recording arterial pulses are not suited for veins. A pulse transducer is presented, which is custom-made for application in the veins. The transducer is based on the Hall-effect. A small magnet is fixed to the skin over a cutaneous vein. The movements of this magnet as transmitted from the vessel wall are detected as changes in the magnetic field in the output voltage of the IC. Thus, the changes in the magnetic field are transformed into a voltage signal, which is fed to the analogue-digital-converter of a personal computer. The output voltage of the IC depends on an exponential manner on the distance between the Hall-sensor and an artificial vectorial veins. The transducer can be used for the calculation of the varying diameter in veins. Using two of the described pulse transducers, which were placed over a vein on the dorsum of the hand and over the cephalic vein respectively, the vascular pattern of artificially induced pulse waves could be measured. First results show this variable to be closely related to changes in venous blood flow velocity and to changes in vein diameter.

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COMPARISON OF THE EFFECTS OF COOLING THE THORACIC SKIN ON CARDIOVASCULAR PARAMETERS IN BROODY AND NON-BROODY HENS

M. Brummermann and R. E. Reinertsen

During the breeding season the ventral skin of many birds undergoes feathering and vasocutaneous changes, limiting the extent of the brood patch.

Stepwise cooling of the brood patch of 14 broody and a corresponding shaved skin area of 13 non-broody Bantam hens with stimulation temperature descriptors (T s) from 35 to 15 °C resulted in a significantly lesser decrease of breast skin temperature in the broody than in the non-broody hens. Deep body temperature was lowered significantly only in broody hens.

Oxygen uptake was inversely correlated to T s in both groups, the effect being up to 2.5 times as high in broody than in non-broody hens.

Cardiac output which was 674±134 in 8 broody and 360±200 ml/min·kg in 8 non-broody hens increased with decreasing T s with a steeper slope in the broody than in the non-broody hens, mainly due to a tachycardia which was much more pronounced in the brooding hens, and partly to a slight increase in stroke volume (SV) in both groups, the broody hens having a 45% higher SV already at resting conditions. Since blood pressure was rather stable and equal in both groups, this accounts for an initially lower total peripheral resistance (TPR) and a greater decrease of TPR during cold stimulations in broody than in non-broody hens.

Thus in incubating hens the heat transfer to cold eggs seems to be achieved by two factors, a permanent enlargement of the vascular bed and an additional vasodilation in reaction to cold stimulations of the brood patch, both increasing the thermal conductance.

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DISOCIATION BETWEEN MYOCARDIAL CAPILLARY AND FIBER DENSITY IN RATS WITH IN VIVO AGED RED CELLS

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In rats, in vivo aging of the erythrocytes increases the cardiac intercapillary distances (H.Rogausch, Int. J. Microcirc.: clin. exp. 1, 1981). In the following experiments we tested whether the increased distances were associated with a greater cross sectional area of individual myocardial fibers. After 5 weeks of erythrocyte aging by the method of Ganzoni (J. clin. Invest. 50: 1373, 1971), the plasma of the animals was stained with FITC-globulin and the hearts were rapidly frozen according to the method of Vetterlein (An. J. Physiol. 242: H133, 1982). In alternating cryostate sections at the greatest circumference of the hearts, the fibers were visualized by v. Gieson's stain and the capillaries by fluorescence microscopy. Mean intercapillary distance increased by 14% (p<0.001), mainly due to a pronounced increase of the larger distances. The mean cross sectional area of myocardial fibers, however, was not significantly altered (364±86 μm² in controls and 269±97 μm² in hearts with aged erythrocytes, p<0.05). Therefore heart hypertrophy was not the cause of the increased intercapillary distances. In HE-stained sections no signs of myocardial cell damage were seen. The results suggest that the decrease in the capillary density is caused by a reduction of microvascular flow without visible myocardial cell damage.

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CHARACTERIZATION OF A LOW AFFINITY COMPONENT FOR OXYGEN IN THE RAT CAROTID BODY IN VITRO

H. Heller and K.-D. Schuster

It has been shown that the rat carotid body superfused in vitro with low PO 2 (20 Torr) exhibited an optical absorbance spectrum which resembles the reported spectrum of the NAD(P)H oxidase in neutrophiles. Diphenyl iodonium (DPI) as a specific inhibitor of the oxidase attenuated the absorbance spectrum in the carotid body (Acker et al., Pflug. Arch. 405: 769-78, 1989). For further characterization microscope fluorescence measurements of FAD (460 nm/523 nm) and NAD(P)H (366 nm/465 nm) have been carried out in the rat carotid body in vitro. Lowering the PO 2 gradually in the superfusion medium from 340 Torr to 20 Torr was followed by a gradual decrease of the FAD fluorescence of about 15% indicating a reduction of FAD (n=6). The gradual PO 2 decrease in the superfusion medium was also followed by a gradual increase of the NAD(P)H fluorescence of about 15% (n=6). The apparent KmPO 2 for FAD as well as for NAD(P)H was 62 Torr indicating a close functional relationship between both components. DPI (10 μM) inhibited the hypoxia-induced NAD(P)H fluorescence of about 40% as well as FAD changes. Concomitantly the hypoxic nervous excitation of the rat carotid body could be depressed by the same dose of DPI. Superfusion of the rat carotid body with low sodium (20 mM) and amiloride (1 mM) attenuated the hypoxia-induced reduction in the carotid body oxygen transport and metabolism, indicating that the Na+ dependent factor forms the major portion of the reduced spectrum of the NADPH oxidase in the carotid body. These results suggest a membrane localization of the NAD(P)H oxidase in the carotid body, which has a low affinity for oxygen and might participate with O2 radical generation as an O2 sensor protein in the chemoreceptive process.

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INVESTIGATION OF OXYGEN TRANSPORT TO TISSUES DURING REST AND ERGOMETER WORK BY USING OXYGEN ISOTOPES

M. Delpiano, E. Dufau, J. Hentschel

Due to the different molecular weights of the isotopic molecules 16O18O and 16O2, a fractionation effect occurs between them when passing through the pathways of oxygen transport and metabolism. Several constituent processes of these pathways such as O2 diffusion and O2 utilization are known to have high fractionations (3 and 1.3 % respectively). In recent experiments performed on healthy humans at rest, the uptake rate of 16O2 was found to be 0.9% above that of the heavier molecule (Schuster and Pflug, Oxygen Transport to Tissue, XI, Adv. Exp. Med. Biol. (1989)). We investigated the question as to whether diffusion becomes a limiting process of oxygen transport during ergometer work, thus causing an increase of overall fractionation effect. Experiments were carried out on 6 healthy humans at rest and during ergometer work for 10 minutes and varying loadings of 50, 100, 150, 200 and 250 W. Samples from inspiratory and expiratory gas were analysed by mass spectrometry. At rest, fractionation effects were below the value of 1.3 % which is in line with the expected value of 0.72 % ± 0.00 % ± 0.00 % higher rate for 16O2 than for 16O18O. During ergometer work this fractionation effect steadily decreased to 0.31% ± 0.04 % at 250 W. These results suggest: (1) At rest the overall fractionation effect of respiration between 16O18O and 16O2 is below the value of 1.3 % which is in line with a limitation of oxygen uptake mainly due to metabolism, (2) the decrease of overall fractionation effect during ergometer work indicates that oxygen transport is not limited by diffusion up to work loads of 250 W. Therefore an increase of fractionation effect should have occurred.
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DISPERSION OF INSPIRED AEROSOL BOLI AND DETERMINATION OF EFFECTIVE AIRWAY DIAMETERS BY A NEW AEROSOL METHOD IN HEALTHY SMOKERS BEFORE AND AFTER BRONCHODILATION. R. Schömöer +, H. Kronenberger +, H. Linn +, Ch.F. Schiller-Scotland +, J. Gebhart +, J. Heyder +, J. Meier-Sydow +, W. Stahlhofen +.

Dispersion and half-width of aerosol bolli can be determined by an aerosol method during in- and expiration. The dispersion of the inhalated aerosol bolli with a half-width of 70 ml is a parameter for convective gases during respiration. Another method recently described by J. HEYDER (J. Aer. Med.;1989; 2:69-77) allows the invasive determination of effective airway diameters (EAD) as a function of volumetric lung depth (LD). This method depends on settling velocity and deposition rate of an inert aerosol in the respiratory tract during a defined time of breath-holding. Methods: Aerosol dispersion and EAD were determined in a group of 18 healthy smokers (age: 25.1 yrs; pack yrs.: 11.6) with normal lung function tests. EAD were assessed in LD between 150 and 600ml. Each subject was measured twice within one week before and after bronchodilatation by a B-agonist (Formoterol) and used as its own standard.

Results: Relative changes (increase) of EAD by Formoterol [% baseline] are shown in table.

| LD [ml] | 150 | 200 | 250 | 300 | 400 | 500 | 600 | 700 | 800 |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| EAD [%] | 37.1 | 28.9 | 23.9 | 18.3 | 12.5 | 10.0 | 7.7 | 6.3 | 66 |

Discussion and Conclusions: As a function of LD bolus half-width increase whereas EAD decrease. Inhalation of the B-agonist causes significant changes of EAD in LD between 150 and 300 ml (<0.05, Wilcoxon). The relative changes of EAD decrease with increasing LD, whereas no significant changes of bolus half-width were found. We suggest that in healthy subjects convective gas transport is not influenced by bronchodilatation.

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A NEW METHOD FOR CONTINUOUS REGISTRATION OF O2-SATURATION AND PO2 OF FLOWING SHEARED BLOOD. E. Nottagby, C. Geisen, W. Richter and J. Beckman

Standard-O2-dissociation curves (OCD) are measured in non-flowing blood under equilibrium conditions, neglecting rheological aspects. The recently introduced rheo-oxymetry in contrast, allowed the evaluation of the oxygen uptake or delivery in flowing blood by continuous PO2 registration. During these measurements, blood is sheared in a Couette-flow-system while being exposed to the oxygen phase across a membrane for defined contact times. An additional simultaneous saturation measurement would allow a complete analysis of the gas exchange properties of sheared blood. In a new approach, this continuous O2-saturation measurement was achieved using a new wavelength scanning oximeter. This device was originally developed for measurements of cutaneous haemoglobin spectra, according to Lübbers et al. We adapted it's photometric sensor to a thin layer cuvette connected to the vent of the Couette rheo-oxymeter. The blood's reflection spectrum is continuously scanned in a wavelength range from 500 to 600 nm. The momentary saturation is calculated on the basis of a non-linear two-component analysis enabling rapid measurements. This, in combination with a simultaneous PO2 measurement now allows the measurement of a 'dynamic' OCD of blood under shear conditions. Haemorheological effects on the oxygen uptake have been proven under several circumstances. Artificial rigidification of RBC's e. g. leads to a slower O2-saturation (shear rate 370 s-1). It has been observed, that impaired RBC-deformability reduces the oxygen uptake even when the standard-OCD is left-shifted.

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PATHOPHYSIOLOGICAL MECHANISMS OF LIPOPOLYSACCHARID INDUCED RESPIRATORY AND CARDIO-VASCULAR CHANGES. W. Marek, and W.T. Ulmer

Lipopolysaccharid (LPS) induced biphasic changes in airway resistance, pulmonary artery pressure and vascular permeability represent an accepted model of adult respiratory distress syndrome (ARDS).

Methods: Besides direct vagal reflex mechanisms, we studied the contribution of mediators like histamine, platelet activating factor (PAF), and leukotrienes by application of their specific antagonist before i.v. infusion of 0.2 ug/kg LPS from Escherichia coli endotoxin in anaesthetized sheep.

Results: LPS infusion results in an increase in dynamic elastance (Edyn) as a representative of airway resistance from 2.0 ± 0.9 to 7.5 ± 3.6 mmHg/100ml VT after 50 min, along with an increase of systolic pulmonary artery pressure from 15 ± 2.3 to 34 ± 10 mmHg with a maximum after 30 min. Both result in a severe arterial hypoxaemia. The late phase of response after 2-5 hours was characterized by a secondary decrease in the number of leucocytes in arterial blood from 4.100 ± 860 to 550 ± 240 cells/ml and the development of lung oedema along with mild respiratory and cardiovascular changes. Despite of allergen induced airway obstruction, bilateral vagotomy only slightly diminished the respiratory and cardio-vascular responses to LPS. While histamine H1-receptor antagonists hardly showed any changes of the responses to LPS infusion, between 30-50 % of the responses were diminished after pretreatment with the PAF-antagonist WEB-2089 or the lipoxygenase inhibitor HWA-214.

Conclusion: Platelet activating factor and lipoygenase products contribute to the LPS-induced respiratory and cardio-vascular changes, while vagal reflex-mechanisms and histamine are of minor importance.

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PERIPHERAL CHEMORECEPTOR RESTING DRIVE IN NORMOTENSIVE AND HYPERTENSIVE SLEEP APNEA PATIENTS. M. Tafsl-Klawe*, A.E.Thiele, F.Raschke, J.H.Peter, G. Hildebrandt

Augmented carotid chemoreceptor resting drive has been suggested to be involved in the pathophysiology of human essential hypertension (1,2,3). Relationship between arterial hypertension and sleep apnea syndrome was frequently reported (4). Do arterial chemoreceptors play a role in the pathophysiology of sleep apnea syndrome (5)?

The ventilatory response to inactivation of carotid chemoreceptors (CCh) was studied in 15 normotensive patients with sleep apnea syndrome (SAS), in 13 hypertensive (H-SAS) and in 30 age-matched controls (C). The inactivation of CCh was due to pure oxygen breathing for 1 min. A significant decrease in ventilation was observed in all groups of subjects: C 16.3 ± 2.5% SAS 15.9 ± 2.1% H-SAS 27.47 ± 3.9% (SEM).

The reduction of ventilation was significantly higher in H-SAS patients as compared to SAS and C subjects p(0.001).

Our results suggest that H-SAS patients have an augmented resting CCh drive whereas the SAS patients did not differ from the C subjects. The increase in tonic CCh activity can probably contribute in causing arterial hypertension in H-SAS patients.

1. Przybylski 1981, Med.Hypoth.; 2. Trzebski et al. 1982, Cardiovasc.Res.; 3. Tafsl-Klawe et al. 1986, Acta Physiol.Pol.; 4. Gislason 1987, Acta Med.Sc.; 5. Przybylski et al. 1986, Med.Hypoth.

Institut für Arbeitsphysiologie und Rehabilitationsforschung, Robert–Koch Str.7A, 3500 Marburg; Zentrum für Innere Medizin, Marburg; *Department of Physiology, Medical Academy, Warsaw.
INFLUENCE OF CHANGES IN LUNG VOLUME ON AIRWAY DIAMETERS IN HEALTHY SUBJECTS: ASSESSMENT WITH A NEW AEROSOL RECOVERY METHOD. H. Kronenberger*, J. Gebhart+, T. Kullmer*, H. Lird+, C.H. Schiller-Scotland+, R. Siekmeier+, J. Heyder+++, J. Meier-Svdow*, W. Stahlhofen++. Our group has developed a new method which enables us to determine non-invasive effective (mean) airway diameters (EAD [mm]) as a function of volumetric lung depth (LD [ml]). The method is based on tidal gas measurements of aerosol recovery of a monodisperse aerosol during a single breath (J. HEYDER: J.Aerosol. Med. 89:2;89-97). It is well known that airway resistance and thus airway calibre is influenced by lung volume. In this study we evaluate the effect of changes in lung volume on EAD. Methods: 13 healthy smokers (age: 20-30 yrs; pack yrs: 10-50) with normal lung function tests were subjected to successive EAD-measurements in LD 100-800 ml. Routine procedure (RP) starts at 1 l below FRC with inhalation of a 2 l of aerosol followed by different respiratory pauses so that the EAD are determined at a lung volume of 1 l above FRC. In the altered procedure (AP) the inhalation starts at FRC, so that the lung volume for the measurement is raised to 2 liters above FRC (1 l above RP-level). Results: EAD for RP (EADRP) and AP (EADAP) are shown in the table (Statistics p<0.05 [WILCOXON]).

**LD 100 150 200 250 300 400 500 600 700 800**

| EADRP | 2.29 | 1.31 | 0.97 | 0.71 | 0.63 | 0.52 | 0.45 | 0.41 | 0.37 | 0.35 |
|-------|------|------|------|------|------|------|------|------|------|------|
| EADAP | 1.98 | 1.19 | 0.91 | 0.75 | 0.68 | 0.58 | 0.49 | 0.44 | 0.40 | 0.37 |

Discussion and Conclusions: As expected the elevated lung volume by 1 l causes a significant increase of EAD in LD between 100 and 500 ml. In more peripheral air spaces no change can be observed. These marked changes of EAD have the same extent as those described in healthy subjects after inhalation of inhaled pharmaceutical drugs (H. KRONENBERGER ET AL: Eur. Resp. J. 89:2;392). Thus, these data confirm the value of the new aerosol method for the evaluation of airway calibres.

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CIRCADIAN VARIATION OF NASAL AND ORAL RESISTANCE A.E. Thiele, F. Raschke and G. Hildebrand

Nasal and oral resistance measurements showed pronounced 24h variations with a maximum of the oral resistance at 6.00h. The maximum of the nasal resistance was preceded for about 4h. Nasal laterality showed a cycle length of 4h on average. HR and rectal temperature showed the well known circadian rhythm with a minimum at 5h.

Discussion: The phase difference between diurnal variations of nasal and oral resistance is indicative for different mechanisms of their regulation. Whereas the neural part of nocturnal increase of oral resistance is known to be mediated by the parasympathetic nervous system, the nocturnal increase of nasal resistance may be induced by a reduction in tone of the adrenergic system. Additionally there is evidence for a reciprocal control of vasomotor activity in the capillaries of the turbinates originating in the lower brain stem (Bamford & Eccles, Pflügers Arch.394:139,1982). Our results have some implications in the pathogenesis of nocturnal breathing disorders.

Respiratory Effects of Aminophylline in Adult Rabbits G. Kostakou-Burghof, R. Kiwul-Shonke and P. Kiwul

Since aminophylline* (A), the complex of theophylline and the solvent ethylenediamine (EDA), is suitable for the treatment of respiratory failure after birth, it often has been studied in newborn rabbits. For adult rabbits, only few data are available about respiratory drug and/or solvent effects at central and effector level. Therefore, anaesthetized adult rabbits, spontaneously breathing (urinary air), were studied before and during intravenous infusion either of A (loading dose: 80mg/kg for 10 min, maintenance dose: 10mg/kg/h for about 90 min) or only of EDA in equivalent molar amounts. Tidal volume and inspiratory/expiratory durations (VT, T1, T2), transpulmonary pressure (Ptp), phrenic nerve activity (PNA) and end-tidal PCO2, were continuously determined, as well as from blood samples, the arterial PCO2, pH, standard HCO3 and theophylline concentration.

After the initial loading infusion of either A or EDA, VT, Ptp and PNA invariably decreased by 10-20%. During the maintenance infusion, VT returned to control, and Ptp rose beyond these cases. Although PNA rose only in ca. 20% of A. Upon the onset of infusion, A led to a pronounced (70%) and EDA to a smaller (20%) rise in respiratory rate. Therefore, ventilation (V) was enhanced immediately and then maintained at a high level by A, but EDA caused even an initial depression, followed by a delayed small and transient rise. Both response-types of V were accompanied by the same decrease in arterial PCO2 by 0.5-1.0 kPa.

We conclude that EDA in aminophylline partly inhibits the respiratory drive elicited by theophylline at central level, but at the same time enhances the effectiveness of respiratory control for the gas-exchange at effector level.

* Ephyllin®

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FADE AT THE NEUROMUSCULAR JUNCTION IS CAUSED BY
POSTSYNAPTIC EFFECTS
Ronald Bradley, Reinhold Storz and Klaus Pfeiffer

Fade or rundown at the mammalian neuromuscular junction (nmj) can be observed in the presence of 1 ~M d-tubocurarine (d-TC) or in myasthenic patients: in a nerve train stimulation the 4th pulse elicits an amplitude in compound action potential (CAP) of, e.g., only 25% of that of the first pulse. It was assumed that positive feedback via postulated presynaptic AChR is necessary to counteract a natural fade in ACh-release. If the presynaptic AChRs are blocked by d-TC, a decreasing amount of ACh is released in each stimulation. We tried here to bypass the presynaptic part of nmj by applying constant doses of 4000 pCoulomb ACh ionophoretically very close to a voltage-clamped endplate. Under this condition, the fade ratio 4th/1st (endplate current, epc) was 0.93 (baseline shift was corrected). If, however, 1 M d-TC was added, the fade ratio became 0.60 (i.e., fade occurs). With much smaller ACh-pulses of 66 pCoulomb the ratio remained at 0.98 (i.e., no fade). It was further observed that small doses of α-toxin indeed cause severe fade, although it is generally assumed that this toxin does not bind to presynaptic structures. We therefore propose here that the phenomenon of fade can be explained, partly or completely, by postsynaptic effects. Since fade was observed only at high saturating concentrations of ACh (as is the case for ACh release by the nerve), we propose that open-channel blocking or some other allosteric effect of d-TC on the post-synaptic AChR is the real cause of fade.

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VOLTAGE DEPENDENT CONTRACTILE ACTIVATION AND INTRANERABRANIE CHARGE MOVEMENTS IN MYOTOME CELLS OF THE LANCELET (BRANCHIOSTOMA LANCEOLATUM).
By R. Benterbusch and W. Melzer

According to a recent hypothesis Ca release from the sarcoplasmic reticulum becomes physically linked during evolution of vertebrates (the scheme is a modified (dPAK-pyrophosphate) receptor) resulting in a more efficient control of this process by the membrane potential. By studying the protocordate Branchiomisstias we expect gaining information about the voltage-controlled Ca release in an early evolutionary state. We performed whole-membrane voltage clamp on resealed fractions of myotome cells and studied contractile activation as a function of voltage. Contractions persisted after eliminating the Ca currents indicating a current-independent but voltage-controlled mechanism. The voltage dependence of fractional isometric shortening S/Nmax could be fitted by a Boltzmann distribution of 66 at 100 pC per pF linear capacitance, a half maximal charge displacement at V50 = -6mV and k = 8mV. Searching for a voltage sensor signal we eliminated virtually all transmembrane currents and short transient current with the characteristics of an intramembrane charge movement persisted. Fitting charge-voltage relation by a Boltzmann distribution we obtained a maximal charge density of 1pC per pF linear capacitance, a half maximal charge displacement at V50 = -10mV and a value for k of 10mV corresponding to the movement of 2.5 electronic charges per voltage sensor unit across the membrane. With its voltage dependence and kinetics this charge movement signal could be involved in the control of intracellular Ca release as well as in the gating of the Ca current present in Branchiomista myotome cells.

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CONTRACTILE PROPERTIES AND FIBRE TYPE COMPOSITION OF SLOW AND FAST TWITCH MUSCLES OF THE WALTZING MOUSE

S. Aamussen, I. Kunze and K.-G. Pieper

Due to a genetically determined degeneration of the neuroepithelium of the vestibular apparatus was observed in slow muscle fibres. The disease, characterized by stereotypically performed rotatory movements. The influence of such a hypermobility on the contractile, histochemical and some biochemical properties of the slow-twitch soleus (SOL) and the fast-twitch extensor digitorum longus (EDL) muscles of adult (3 month old) WM was investigated. SOL and EDL of age-matched mice with normal motor activity (CM) served as controls. In comparison to controls the time parameters of the single twitch and of the tetanic tension development of the SOL as well as the EDL of WM are prolonged. The SOL of WM develops a higher maximum tetanic force per unit cross-sectional area. The EDL of WM shows a smaller post-tetanic potentiation (40%) than that of CM (44%). Also, the cooling potentiation of the EDL is smaller in WM (50%) than in CM (65%). The SOL of WM contains more type I fibres (84%) than that of CM (65%) and the activities of the lactate dehydrogenase, malate dehydrogenase and phosphoglycerate kinase are significantly lower in the SOL of WM. There are no striking differences in fibre type composition and enzyme activities measured between EDLs of WM and CM. However, the results are discussed in comparison with other models of muscular hyperactivity.

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THE SITE OF CAFFEINE ACTION IN SLOW MUSCLE FIBRES OF THE FROG (RANA ESCULENITA)

J. Steinmetz and H. Schmidt

It is known that caffeine elicits contractures in frog twitch and slow muscle fibres. Axelsson & Theeff (1958); Scornik & Oetliker, J. Physiol. Lond. 194:51, 1968; Nasledov et al., Experientia 28:1305, 1972). In the present experiments caffeine was applied extra- and intracellularly in frog slow fibres. When added to the bath solution the threshold caffeine concentration was near 2 mmol/l; maximum tension was obtained with 12-20 mmol/l. The dose-response (DR) curve was shifted towards lower concentrations after reducing the Ca-superscript concentration of the medium. Long-lasting Ca-superscript-deprivation did not affect the contracture elicited with high caffeine concentrations, but reduced the effect of submaximal caffeine concentrations. Increasing the Ca-superscript concentration had opposite effects. In contrast to K-contracts there was only a very small shift of the caffeine DR-curve when Ni replaced Ca sup +.

Caffeine was also applied locally by pressure ejection from a micropipette positioned near the outer surface of a slow fibre. Localized contractures were obtained upon application of 47-138 pl caffeine solution (40 mmol/l; four fibres). After impalement of the fibres similar caffeine doses were never observed to elicit a tetanic response. It is concluded from these results that caffeine releases Ca sup + from an intracellular store by acting on an external membrane site different from that which is involved in Ca-superscript-release following depolarization of the membrane with a K-rich solution.

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DIVALENT CATIONS AND ACTIVATION OF CONTRACTILE FORCE IN SLOW MUSCLE FIBRES OF THE FROG

P. Krippelt-Drews and H. Schmidt

Isometric contracture tension was recorded from single slow fibres of Rana temporaria and esculenta. Omission of Ca from the medium had time dependent effects on the amplitude of contractures elicited by application of K-rich solutions. After short exposure to Ca-free (0 Ca sup +) Ringer the threshold K-concentration was reduced, but the dose-response (DR) curve was flattened, and maximum force was reduced by about 5-10%. Replacement of Ca by 1.8 mmol/l Ni or Co sup + shifted the DR-curve clearly towards higher K-concentrations. Substitution of Mg for Ca slightly increased the K-threshold, but the DR-curve was much less steep than those for Ca, Ni sup + and Co sup +. Long-term exposure (40-60 min) to 0 Ca sup +-Ringer completely abolished the response to high K-Ringer. Contractile force could not only be restored by readmission of Ca, but also by application of 1.8 mmol/l Ni or Co sup +. Mn and Mg were less effective. In contrast to K-contracts the response to high caffeine concentrations (12-20 mmol/l) was very little affected, even after several hours of exposure to 0 Ca-superscript-Ringer. The following conclusions can be drawn from these results: 1. Excitation-contract coupling in frog slow fibres is supported by divalent cations bound to an extracellular site; their order of efficiency is Ca sup + > Ni sup + > Co sup +. 2. Block of excitation-contraction coupling by a Ca-superscript-free solution is not due to exhaustion of intracellular Ca sup +.

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SIZE AND HISTOCHEMICAL PROFILE OF RAT MUSCLE FIBERS AFTER DEAFFERENTATION AND IN COMBINATION WITH PERIPHERAL NERVE CRUSH

U. Hübschen and A.C. Nacimiento

Whereas much information has accumulated about changes in motor units after peripheral nerve lesion much less is known about the role of afferents during posttraumatic regeneration. To approach this question we first performed a unilateral rhizotomy of dorsal roots L2-L5 in rats and tested the influence of deafferentation. Then we added an ipsilateral peripheral crush of the common peroneal nerve 1,2,3,4 and 6 weeks postoperatively. Muscle fibres were identified enzym histochemically with the help of single muscle fibres (slow twitch oxidative, SO; fast twitch glycolytic, FOG; fast twitch glycolytic, FG) in the tert anterior and extensor digitorum longus muscle. Deafferentation produces an ipsilateral decrease of SO- and FOG-fibers'size at 2 weeks, a temporary recovery at 4 weeks and a decrease again at 6 weeks. FOG-fibers were larger than normal after 6 weeks. Contralateral fibers of all types behaved like ipsilateral SO and FOG-types. The relative amounts of FOG-fibers increased ipsilateral in the first 4 weeks and decreased later; the contralateral SO-fibers'amount increased steadily in the whole observation time. The combined lesion produced ipsilateral a reduced FOG-fiber size up to 6 weeks. FOG-fibers' size was first reduced but recovered at 4 weeks. The relative amount of FOG-fibers was higher than normal in the first 3 weeks. FOG-fibers' amount was subnormal in the 2nd week. Possible mechanisms causing these changes will be discussed.

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GABA and inhibitory nerve stimulation produce different effects on intracellular pH in crustacean muscle fibers

D. Gunzel, S. Gallner and W. Rathmayer

Most arthropod skeletal muscle fibers receive, in addition to their excitatory innervation, input from inhibitory motoneurons. The transmitter of these inhibitory neurons was shown to be γ-aminobutyric acid (GABA), which opens Cl- channels. Inhibitory activity is able to release catch-like contraction, which is a prominent feature of many arthropod muscles. K. Kallal and J. Volpio (Nature 330:163, 1987) showed for the opener muscle in crayfish that GABA, in addition to increasing Cl- permeability, also increased HCO3- conductance, which resulted in intracellular acidification of the muscle fibers.

To determine whether activity of inhibitory neurons produces a similar change in intracellular pH (pHi) of crustacean muscle fibers, pHi was continuously monitored in fibers of walking leg muscles of the crab Dripa spinifrons and the crayfish Pacifastacus leniusculus during repetitive stimulation of specific or common inhibitory motoneurons with 40/s for 2 to 5 min. Muscles were bathed in salines containing CO2 (1%) / HCO3- (19mM). pH i was measured with microelectrodes filled with a sensor based on the neutral ionophore ETH 1907 (time constant smaller than 0.6 s). In no preparation a change in pHi was observed upon stimulation of the inhibitors (8 preparations, 25 fibers). On the other hand, in all muscle fibers investigated (including fibers which lack inhibitory innervation), 10^-5 M GABA produced a fall in pHi by 0.17 ± 0.07 (S.D.) pH units (n=12). We conclude that HCO3- conductance can not be activated through synaptic GABA-receptors. Therefore, release of catch-like contraction is evidently not caused by intracellular acidification of muscle fibers.

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INFLUENCE OF FIBRE TYPE COMPOSITION OF SKELETAL MUSCLES ON TEMPERATURE DEPENDENCE AND ACTIVATION ENERGIES OF CONTRACTION PARAMETERS

U. Gaunitz and G. Asmussen

The temperature dependence of contractile parameters of slow twitch soleus (SOL) and fast twitch extensor digitorum longus (EDL) muscles were measured from leg muscles of guinea pigs in a range from 15° to 37°C. The logarithm of time parameters recorded were plotted versus the reciprocal temperature (Arrhenius plot). The slope of the relation is a formal measure of the energy of activation of the basic processes. Straight lines (constant activation energies) are not achieved in all cases. The best mathematical approximation is obtained by a polynomial of second order (3 coefficients) that means linear changing activation energies. The activation energies of the contraction process of EDL and SOL are comparable. Considerable differences were found in the temperature dependence of the relaxation process of SOL of different species. In the sequence mouse-ext-guinea pig the activation energies increased. In the same sequence the proportion of slow twitch fibers in SOL increased. The SOL of guinea pig is composed by slow twitch fibers only but the SOL of mice and rats contains a remarkable amount of fast twitch fibers (15%). It is suggested that the strong temperature dependence of the twitch relaxation is caused by the amount of slow twitch fibers in the muscles. The more complicate kinetic mechanism cannot be explained by a single-step-reaction. The interpretations are supported by previous data of single fibers from a mouse foot muscle (J. Nürnberg and H. Westerblad, J. Physiol. 390: 285, 1987).

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THE PENTAPEPTIDE PROCTOLIN INDUCES ELECTRICAL MYOTONIA IN MAMMALIAN MUSCLE

E.Wiehnemeier, D. Könlen & H. Jochum

In mammalian muscle, mutations as well as a number of agents that interfere with Cl-conductance lead to a hyperexcitability of the plasma membrane and thereby to the symptoms of myotonia i.e. unscheduled runs of action potentials and aftercontractions. Proctolin (R-Y-L-P-T) has recently been reported to induce aftercontractions in insect muscle (Herrmann, C. E. & Schmitz, J. in: N. Elmer & W. Singer (eds.): Dynamics and Plasticity in Neuronal Systems, Thieme, Stuttgart, New York, p 129, 1989). Therefore we tested the effect of proctolin on mammalian muscle, both by intracellular recording and by contraction measurements. Whereas 10 μM proctolin after 30 minutes caused mouse sternocostalis muscle to produce 'myotonic' runs and visible aftercontractions of the stimulated fibers, no aftercontractions were measured in whole EDL muscles.

The same phenomenon had been observed after treatment of muscles with 4-phospholiposteres and other activators of protein kinase C (E. Wiehnemeier & H. Jochum, J. Biochem. Biophys. Res. Commun. 169, 1383-1389, 1987). The discrepancy between electrical and mechanical myotonia has not yet been resolved. As activators of protein kinase C reduce Cl-conductance, proctolin may act likewise on ion channels via a second messenger mechanism, but it remains to be resolved which channels are affected (Walther, C. & Zitrin, K. E., J. of Physiol. 410, 32P, 1989).

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ADULT MOUSE INTEROSSEOUS MUSCLE HAS ION CHANNELS THAT ARE ACTIVE AT THE RESTING POTENTIAL

H. Brinkmeier, E. Zachar and R. Rödel

We investigated the single channel activity in patches of sarcolemmal vesicles obtained from intact fibers by treatment with 140 mM KCl, and also in membrane patches of collagenase-treated fibers voltage-clamped in the cell-attached and inside-out configurations. The 3 methods yielded corresponding results. Under physiological ion conditions, we regularly observed several Ca2+-dependent K channels and several delayed rectifier channels. In a patch the Ca2+-dependent K channels (145 pS) opened only at voltages positive to +40 mV, the delayed rectifier channels (17 pS) were activated by voltage steps from -60 to -20 or 0 mV. The inactivation of the delayed rectifier was never complete as the channels kept opening and closing at constant voltages between -50 and +20 mV. In about 1 out of 5 patches, we recorded channel activity (see Fig. 1) near the resting potential (from -110 to -70 mV) with a linear current-voltage relationship yielding a conductance of about 10 pS. We have not yet established whether these channels conduct Cl or Na ions.

Fig. 1. Channel activity observed in an inside-out patch with 140 mM NaCl. 1 mM CaCl2, 1 mM MgCl2 in the pipette and 140 mM KCl, 1 mM EGTA, 1 mM MgCl2 in the bath. Both solutions were buffered with 1 mM Tris·Cl to pH 7.4.

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EFFECTS OF MYOSIN LIGHT CHAIN PHOSPHORYLATION ON ISOMETRIC FORCE AND CROSS-BRIDGE TURNOVER KINETICS

B. Brenner, J. Morano

Isometric tension and the rate constant of force redevelopment after a short period of shortening and restretch to the initial sarcomere length \( k_{\text{m,app}} \) increase upon Ca\(^{2+}\) (Brenner, PNAS 85, 1988) and myosin P-light chain phosphorylation (MPLC-p) (METZGER et al. J.Gen.Physiol. 93, 1989). Here we studied the effect of incubation of Ca\(^{2+}\)-activated skinned psoas fibers with myosin light chain kinase (MLCK) on the time-course of k\(_{\text{m,app}} \) and MPLC-p. Fibers were incubated at 15°C with contraction solution containing 5mM calmodulin and 100mM MLCK. Single fibers were analyzed by a Micro-2D-PAGE-technique after TCA-denaturation. Values are means±SEM.

Incubation with MLCK at pCa 5.6 (15% activation) increased tension from 15.1±3mN to 30.5±13mN, k\(_{\text{m,app}} \) from 1.8±0.2s\(^{-1}\) to 2.8±0.1s\(^{-1}\) (n=5) while MPLC-p was not detectable. The half-time for the increase in k\(_{\text{m,app}} \) and MPLC phosphorylation ranged between 30s and 60s. Effects of MPLC-p on k\(_{\text{m,app}} \) was lower at higher Ca\(^{2+}\) concentrations: around 89% of the increase in k\(_{\text{m,app}} \) was observed at 25mM Ca\(^{2+}\) while K\(^{+}\) effect was marked at 5mM Ca\(^{2+}\). The decrease in force was preceded by a decrease in k\(_{\text{m,app}} \) and MPLC phosphorylation.

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References

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ACETYLCHOLINE-INDUCED CURRENTS IN DENERVATED MOUSE SOLEUS:
EFFECTS OF ANTAGONISTS
H. Lorkovic

ACh-induced currents were measured in partially depolarized mouse soleus muscles denervated for 3-6 days by using a point voltage clamp. When 0.25 mM d-tubocurarine (d-Tc) were used, the weak currents provoked by 0.1 nM ACh at a holding potential -20 mV were barely affected, while the large currents provoked by 2-5 nM ACh were decreased by more than 50%. By contrast, weak and strong ACh-induced currents were proportionally diminished when, under similar conditions, 20-100 ωM ipratropium was used. Currents were proportionally diminished by d-Tc when the holding potential was set at +15 mV, a level corresponding to the reversal potential of the current provoked by low ACh concentrations. In non-denervated flexor digitorum brevis muscles, d-Tc had the same relative effect at low and at high ACh concentrations independent of the holding potential. The reversal potential for the ACh-induced currents was about +14 mV for [ACh] and decreased to about +3 mV with 4 nM ACh in denervated soleus muscles. It was concluded that denervated soleus muscles, in contrast to endplate regions of non-denervated mouse muscles, contain a low proportion of highly ACh-sensitive, weakly d-Tc sensitive, predominantly Na+-permeable ACh receptors. These receptors are presumably responsible for the non-fading ACh-induced currents described before for the denervated mouse soleus muscle.

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DIHYDROPYRIDINE-SENSITIVITY OF THE TRANSIENT, CALCIUM INDEPENDENT POTASSIUM OUTWARD CURRENT IN SINGLE SMOOTH MUSCLE CELLS OF GUINEA PIG Portal VEIN

Th. Nonn, P. Deitmer, K. Golenhofen, E. Lammel

Under voltage clamp, single cells from guinea pig portal vein responded to positive going step voltage commands from a holding potential of -70 mV with an initial inward current (I\text{To}) which inactivated in approximately 20 ms. It was followed by an outward current consisting of three components: a transient outward current (I\text{To}) inactivating within 5-10 s, a longer lasting 'steady state current' (I\text{ss}) and a brief current peaks called 'spontaneous transient outward currents' (STOCs). I\text{To} was calcium independent, it was not altered by e.c. calcium removal and addition of 1 mM EGTA. I\text{ss} was also unaffected by increasing i.e. EGTA from 1 mM/l to 10 mM/l. The reversal potential of I\text{To} was at the potassium equilibrium potential, and classical potassium channel blockers like TEA (10 mM/l) and 4-aminopyridine (5 mM/l) had a suppressing effect on this current. Caffeine (1 mM/l e.c.) selectively blocked I\text{To}, Nifedipine (NIF) at a concentration of 10^{-6} mol/l (sufficient to block the greater part of the calcium inward current) had at negligible effect on I\text{To} and I\text{ss}. However, at a concentration of 3 x 10^{-6} mol/l, in calcium-containing as well as in calcium-free solution with EGTA (1 mM/l), NIF completely blocked I\text{ss} without affecting I\text{ss}. This effect was reversible. In normal calcium-containing solution, the calcium agonistic dihydropyridine, the (-)-enantiomer of BAY K 8644 (BAY K), increased I\text{ss} and STOCs, and at high concentrations (3 x 10^{-6} mol/l) selectively inhibited I\text{ss}, similarly to NIF. The inhibitory effect of the dihydropyridines NIF and BAY K at high concentrations is therefore independent of the typical effect of these substances on calcium channels. Moreover, it is not an unspecific effect on membrane channels, since I\text{To} persisted and STOCs even increased in frequency and amplitude during BAY K-treatment.

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WHOLE-CELL RECORDING OF HORMONALLY REGULATED
CALCIUM CURRENT IN SMOOTH MUSCLE
Andrea Welling1, Jochen Felbel2, Franz Hofmann* and Klaus Peter1

Calcium currents were examined in single, freshly isolated bovine tracheal smooth muscle cells by the whole-cell patch-clamp technique. After blocking potassium currents by cesium and tetraethylammonium-chloride (TEA-Cl) small inward currents were detected and identified as L-type calcium currents by the I-V relationship and organic and inorganic calcium channel blockers.

The calcium peak current ($I_{\text{Ca}}$) was increased 2.7 ± 0.2 (n = 54) fold by isoproterenol ($EC_{50}$ = 3 nM). The isoproterenol effect was mediated by the $\beta_2$- but not by the $\alpha$-adrenergic receptor. Carbachol decreased the isoproterenol enhanced $I_{\text{Ca}}$. The inhibition disappeared with atropine.

Internal dialysis of the cell with cAMP (100 $\mu$M) or cAMP-kinase had no effect on basal or isoproterenol stimulated $I_{\text{Ca}}$.

Conclusion: L-type calcium current can be regulated by the $\beta$-adrenergic receptor in freshly isolated bovine tracheal smooth muscle. Apparently, this regulation does not involve cAMP or cAMP-kinase.

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SPONTANEOUS TONIC CONTRACTIONS OF PORCINE DUODENUM AND INHIBITORY INNERVATION WITH VIP AS A PUTATIVE TRANSMITTER
K. Mandrek and K. Milenov

Mechanical activity was recorded in isolated preparations from the circular and longitudinal layers of different sections of porcine duodenum. Strong spontaneous tonic contractions were observed in the circular preparations. These tonic duodenal contractions (TDC) were weak or absent in the first 1 or 2 cm postpyloric (pp), reached a maximum in the region 3 to 10 cm pp, and disappeared further distally. In the longitudinal preparations no spontaneous tonic contractions could be observed. Strips with strong TDC also possess a strong inhibitory innervation. Electrical field stimulation (EFS) with a single pulse of 10 V and 3 ms duration produced a short lasting inhibition to near zero tone. Phasic rhythmic contractions of both circular and longitudinal preparations could be suppressed by application of the calcium channel blocker nifedipine. The TDC, however, were resistant to nifedipine and could be suppressed by sodium nitroprusside, similar to tonic contractions of fundic preparations from the stomach.

Vasoactive intestinal peptide (VIP) inhibited both the spontaneous tonic and rhythmic activity (half suppression at 2 × 10⁻⁵ mol/l).

Neither the inhibitory effect of VIP nor that of EFS were influenced by guanethidine, atropine, propranolol, phentolamine, ampin and methysergide. This suggests that the transmitter system is neither cholinergic, nor adrenergic, nor serotoninergic, nor purinergic (ATP had no effect).

Conclusion: Strong tonic contractions exist in circular preparations of porcine duodenum, which are similar in nature to fundic tone of the stomach and different in their mechanism from phasic rhythmic contractions. Duodenal tone is probably part of a control process for the regulation of duodenal transit which has not yet been described in the literature. A strong inhibitory innervation is observed in the tonic regions of the duodenum with VIP as a putative transmitter.

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Ureteric motility of rodents can be modulated by a release of substance P (SP), neuromedin A (NKA) and calcitonin gene-related peptide (CGRP) from capsaicin sensitive afferents. Since birds are highly resistant to different actions of capsaicin, we have compared the effect of sensory peptides and capsaicin on the ureteric motility of guinea-pigs and chicken. For this purpose, guinea-pigs were cannulated and superfused with oxygenated artificial interstitial fluid at 37 °C. Changes in intraluminal perfusion pressure indicated ureteric motility. The distribution of SP and CGRP in the ureter were examined using immunohistochemistry.

In the guinea-pig ureter NKA (0.005-2.5 $\mu$M) and SP (0.1-2.5 $\mu$M) evoked dose dependant rhythmic contractions, whereas CGRP (0.1 $\mu$M) inhibited spontaneous or provoked rhythmic peristalsis. Contractions were also produced by capsaicin 1 $\mu$M. This effect was completely desensitized upon repeated applications of capsaicin. After desensitization, however, capsaicin in concentrations >10 $\mu$M inhibited contractions induced by NKA, indicating an unspecific direct effect of the drug on smooth muscle. In the chicken ureter neither SP or NKA (up to 5 $\mu$M) nor capsaicin (0.1-100 $\mu$M) evoked contractions. Rhythmic peristalsis, however, was produced by KCl (10-30 mM) or Noradrenalin (1-10 $\mu$M) and was suppressed by CGRP (>0.3 $\mu$M) or capsaicin (>100 $\mu$M). The demonstrated species differences appear to be not due to the absence of peptidergic afferents, since SP and CGRP containing nerve fibres were found in the ureter of both species.

These results suggest the lack of a specific action of capsaicin on afferent neurones (release of tachykinins) in the chicken.

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MECHANICAL RESPONSES OF ISOLATED PORCINE CORONARY ARTERIES TO INTRACELLULAR ALKALINE OR ACID LOADING
H. Wiederhold, A. Lepolle-Wienhues, C. Korbmacher, H. Haller, M. Wiederhold

As previously reported (Nguyen-Duong, Pflügers Arch., 411, R193, 1988 and Nguyen-Duong, Bamsos and Spurway, Proc. of the
Int. Union of Physiol. Sciences, Helsinki, p.398,1989), the pattern of contractile responses of isolated porcine coronary arteries (PCA) to application and removal of weak acids or bases at constant external pH coincides exactly with expected pH changes, pH decrease leading however to contraction and pH increase to relaxation. We have investigated whether this may be explained by changes in intracellular calcium concentration ([Ca++]i). As previously shown (Nguyen-Duong, 1988), the stimulation of the ciliary muscle cell line (HTCM) and the isolated myocytes with NO-releasing agonists induced a biphasic increase of intracellular free calcium. The initial calcium peak was not abolished either by omission of extracellular Na+ or by applying inhibitors of the Na-H exchange. The [Ca++]i-induced effects were completely abolished after 2 h incubation in Ca-free EGTA-containing PSS. All aforementioned effects could also be observed with several weak acids (Na acetate, Na butyrate) and bases (Methylamine, Trimethylamine) in the presence of Ca++, suggesting that the HTCM cells are equipped with a general pH regulatory mechanism involving the activation of a Cl-HCO3 exchanger for recovery from alkaline loads and may be interpreted on the basis of a model that involves Ca++- and H+ ions for common intracellular binding sites.

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ACETYLCHOLINE INCREASES INTRACELLULAR CALCIUM IN SINGLE CELLS OF CULTURED HUMAN CILIARY MUSCLE CELLS
P. Popple, L. Wiederhold, A. Lepolle-Wienhues, C. Korbmacher, H. Haller, M. Wiederhold

The ciliary muscle is known to play an important role in accommodation. Aqueous humor outflow. In order to get more insight into cellular mechanisms involved in ciliary muscle contraction we performed measurements of free intracellular calcium ([Ca++]i) in single cells of confluent monolayers of an established human ciliary muscle cell line (HTCM). [Ca++]i was measured using the fluorescence properties of the calcium indicator FURA-2.

Application of acetylcholine lead to a typical biphasic increase of intracellular free calcium. First, a transient peak elevation of [Ca++]i occurred, followed by a recovery to a sustained elevated [Ca++]i level. In most of the experiments oscillations of [Ca++]i occurred during the recovery phase and the sustained elevation of [Ca++]i. The acetylcholine induced increase of [Ca++]i was dose dependent (10^-8 to 10^-7) and blocked in the presence of atropine.

The initial calcium peak was not abolished either in the presence of verapamil (10^-4 M) or in the absence of extracellular calcium (1 mM EGTA). In contrast, the sustained elevation of [Ca++]i could be completely blocked by withdrawal of extracellular calcium and was partly blocked by verapamil. We conclude that the initial [Ca++]i peak is due to calcium release from intracellular stores and the sustained [Ca++]i elevation is most probably mediated by opening of Ca-channels of the muscle cell membrane.

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CA-INFLUX THROUGH ATP-OPERATED NON-SELECTIVE CATION CHANNELS IS LARGE ENOUGH TO INACTIVATE VOLTAGE-OPERATED CALCIUM CURRENTS
P. D. Schmieder and K. A. Wiederhold

In myocytes isolated from the urinary bladder of the guinea-pig, we studied the effect of ATP on membrane currents with the voltage clamp method. K-currents were reduced with CsCl-electrodes. At 22°C, the isolated myocytes were continuously superfused by solutions containing 2.6 mM Ca++ and 5 mM Mg++. At -60 mV, bath-application of ATP induced an inward current IATP that rapidly activated. IATP decayed in the constant presence of ATP. The amplitude and reversal potential of IATP were unaffected by incubation in Ca-free, EGTA containing PSS. The results suggest that the inactivation of IATP is due to influx of Ca++, not to Ca-release from the SR. Hence, Ca++-influx through ATP-operated channels is large enough for increase in [Ca++]i and contractile activation.

[1] Benham, C.D. and Tsien, R.W., Nature 328, 275-278 (1987)

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ENDOTHELIN-EVOKED CONTRACTIONS IN ISOLATED BOVINE CILIARY MUSCLE STRIPS
A. Lepolle-Wienhues, F. Stahl, W. Gebauer, U. Willner, C. Korbmacher and M. Wiederhold

Endothelin was found to mediate contractions in many different types of vascular, uterine, intestinal, and bronchial smooth muscle tissue. Recently, we described changes in the membrane potential of a human ciliary muscle cell line evoked by endothelin.

Small ciliary muscle strips (5 x 0.5 mm) were prepared from freshly enucleated bovine eyes. Force measurement was performed using a "position clamp" device similar to that described by Brutsaert et al. (Circ. Res. 1988;62:358). A coil was suspended in a magnetic field, and shortening of the muscle strips resulted in a small rotation of the coil. This rotation was instantly converted by an optoelectronic feed-back mechanism. Coi current was calibrated in Micronewton (μN). Maximal contractions by 10^-7 M acetylcholine were adjusted near 100 μN.

Endothelin evoked concentration-dependent contractions in bovine ciliary muscle. 10^-9 M had no effect. Related to the maximal acetylcholine response, the maximal contraction amounted 274 ± 2.8 % (n=8), and was reached at 5·10^-6 M/1. 1·10^-7 M1/1 evoked no further contraction. In contrast to cholinergic agonists the effect of endothelin was slow in onset, the maximum was reached after 10 min. In presence of the drug tension decreased to the basal level within 30 min. Repeated stimulation diminished the answer to endothelin without apparent reduction of the acetylcholine response. Recovery of the endothelin response could not be seen below 60 min.

Endothelin seems to be a potent contracting agent in ciliary muscle. The observed action of endothelin in this tissue is comparable to that in other smooth muscle tissues. Endothelin may play a role in accommodation and regulation of the intraocular pressure.

DFG WI 328/11

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The composition and organization of the cytoskeleton of cultured vascular smooth muscle cells and the influence of culture conditions

Peter C. Dartsch

The cytoskeleton components of smooth muscle cells can be divided into three categories: 1. Microfilaments, i.e. actin and actin-binding proteins; 2. Intermediate-sized filaments; and 3. Microtubules. The pattern of distribution of these components in stationary cultured smooth muscle cells is presented in detail. Alterations of this pattern were observed when cells were subjected to a mechanical stimulus (cyclic and directional stretching) or to the wash-out of Ca+2. Inhibition of MLC-phosphatase by okadaic acid reverses the latch state, i.e. acts as a low shortening velocity (v). In intact smooth muscle strips from chicken gizzard, slow tonic contractions may be elicited by decreasing the K+-conductance with 3,4-diaminopyridine (Kirsch and Narahashi, Biophys J 22:507, 1978). How-ever, at least in the chicken gizzard, certain potassium channels appear to be involved in the control of the expression of the latch state, i.e. tonic contractions.

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REGULATION OF MYOSIN ISOENZYME EXPRESSION IN THE RAT HEART AND UTERUS DURING PREGNANCY

I. Morano, C. Bialojan, M. Lengsfeld and V. Kyrtatas

We studied myosin isoenzymes (MI) of rat ventricle and uterus during pregnancy and compared these MI with those from rat aorta and portal vein using the native polyacrylamide gel electrophoresis and western blot analysis with antibodies directed against smooth muscle myosin heavy chains and filamin. All MI of smooth muscle revealed a higher mobility in the gel than the three ventricular MI (VI, V2 and V3). In the uterus of non-pregnant and early pregnant rats we observed two bands designated with increasing mobility as F and SMu. In late pregnant rats (19-21 d gravida) SMu disappeared and a second myosin band (SMu2) appeared with an even higher electrophoretic mobility than all the other smooth muscle MI. F but not SMu and SMu2 reacted with the filamin antibody, while SMu and SMu2 but not F reacted with the antibody against smooth muscle myosin.

SMu2 revealed the same electrophoretic mobility as the main MI band of aorta and portal vein. In the late stage of pregnancy ventricular MI shifted to the V3-Form when the V1/V3-ratio being 57/15 in the non-pregnant and early pregnant rats (up to 12 d gravida) and 31/35 after 20 d of pregnancy. We conclude that especially during late stages of pregnancy regulation of MI expression in uterus and ventricle is distinct from non- or early pregnant stages.

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LATCH-LIKE CONTRACTIONS IN INTACT AND SKINNED SMOOTH MUSCLE STRIPS OF CHICKEN GIZZARD

G. Pfitzer and W. Fischer

In skinned chicken gizzard, latch-like contractions can be induced at submaximal Ca2+ (1.7 µM) and low CaM (CaM, 0.12 µM), which are characterized by high force (95% of the maximum elicited at 15 µM Ca2+, 1 µM CaM), low levels of myosin light chain phosphorylation (MLCP: 0.1 mol Pi/mol MLC) and low shortening velocity (v = 0.22 Li/sec). Inhibition of MLC-phosphatase by okadaic acid reverses the latch state, i.e. increases 2-fold (0.64 Li/sec) in concert with MLCP (0.85 mol Pi/mol MLC) but force is barely affected (117% of max.). In intact smooth muscle strips from chicken gizzard, physiological stimuli (carbachol, norpinephrine, membrane depolarization-both electrically or with high K+) elicit only plastic contractions preceded by a rapid phosphorylation transient; e.g. in electrically stimulated preparations, the tension transient lasts only 15 sec (time to peak tension 6 sec) and the MLCP transient lasts 10 sec (time to peak MLCP 3 sec). However, slow tonic contractions may be elicited by decreasing the K+-conductance with 3,4-diaminopyridine (Kirsch and Narahashi, Biophys J 22:507, 1978) which are not associated with an increased MLCP. The active state in these contractions, as estimated from quick-release experiments, is lowered exponentially. In the skinned gizzard preparations are in line with the hypothesis of a dominant role of MLC-phosphatase in the development of a latch state (Hasi and Murphy, Am J Physiol 254:C99, 1988). However, at least in the chicken gizzard, certain K+-channels appear to be involved in the control of the expression of the latch state, i.e. tonic contractions.

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MINOR ROLE OF THYROXINE AS AN INHIBITOR OF CALMODULIN/MLCK INTERACTION IN SMOOTH MUSCLES

T. Lenz, P.J. Boels, U. Theil, I. Morano, and V.A.W. Kreye

Thyroxine has been shown to act as a competitive antagonist on calmodulin-dependent myosin light chain kinase (MLCK) activation in human platelets (Mamiya et al., J.Biol.Chem. 264:8575, 1989). This leads to inhibition of collagen-induced aggregation response and release reactions as well as to marked reduction of phosphorylation rate of the 20-kDa myosin light chain. Since calmodulin and MLCK are also involved as essential biochemical mediators in the contraction processes of vascular and nonvascular smooth muscle, it is conceivable that thyroxine exerts comparable inhibitory effects in these tissues. Therefore we studied the influence of thyroxine (10^5 - 10^6 M) on contraction responses induced either by noradrenaline or by KCl in intact smooth muscle preparations obtained from uterus of the guinea-pig and from the thoracic aorta of the rabbit. Chemically skinned fibers from resistance vessels of the guinea-pig (diameter of 0.1mm), contracted with 10^3 M Ca^2+, either in the absence or presence of calmodulin (0.1 and 0.3mM), were used to study more directly the effect of thyroxine on calmodulin/MLCK interaction. Force development induced with noradrenaline (10^5 - 10^3 M) and KCl (5 - 80mM) in guinea-pig uterus and rabbit aorta were not appreciably altered by thyroxine (n=4). There was also no significant difference in the Ca^2+-induced contraction response of skinned fibers with thyroxine if calmodulin was added. If, however, no calmodulin was added (nominally calmodulin-free) thyroxine (10^5 M) was capable of reducing force production by about 50%, indicating a minor inhibitory effectiveness of thyroxine.

Conclusion: In all smooth muscle preparations tested we were unable to demonstrate a significant physiological role for thyroxine as a competitive antagonist of calmodulin/MLCK interaction, such as has been described for human platelets. Whether this discrepancy is due to a cell- or species-specific variant of MLCK isoenzyme remains to be elucidated.

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CHANGES IN FATTY ACID COMPOSITION IN THE RAT AORTA AFTER PHYSICAL EXERCISE

T. Ohkubo, H. Popp and R. Jacob

Physical exercise is known to reduce the high blood pressure in SHR. Although the underlying mechanisms are not known, prostataglandins (PG) are likely to be involved. Because arachidonic acid (C20:4) is the essential precursor of PG, the effect of swimming (max. 2x90 min/day, 35°C, 6 wk) on C20:4 was studied. Fatty acids were determined in the aorta by gas chromatography (Carlo Erba GC 6000; CP-Sil.88 capillary column; I00-227 m, methyl esters/BF3/MeOH method). Essentially, the swimming rats exhibited an increased percentage of C20:4, whereas linoleic acid (C18:2) was markedly reduced; gamma-linoleic acid (C18:3) was not affected. Noteworthy is that eicosapentaenoic acid (C20:5) is increased and alpha-linoleic acid (C18:3) was reduced:

FA                      male sedentary               male swimming
C18:2 (m6)             20.2±2.6                            10.8±1.3*  
C20:4 (m6)             11.3±1.3                                           14.3±1.8*
C18:3 (m3, alpha)      1.6±0.2                                              1.2±0.4*  
C18:4 (m3)              0.5±0.1                                              0.4±0.1  
C20:5 (m3)              0.5±0.2                                              0.7±0.1* 
C22:6 (m3)              1.9±0.5                                              2.0±0.6

The changes in FA composition resemble those seen after nor-epinephrine injections, suggesting that this type of exercise is associated with a marked adrenergic drive. In accordance, the norepinephrine or epinephrine stores of heart and adrenal glands were increased which is indicative of an enhanced biosynthetic capacity. The reduced C18:2 cannot be attributed to a lower intake of C18:2 because in female swimming rats where the food intake was not reduced, comparable changes were seen. The reduced C18:2 level suggests that the biosynthetic pathways leading to C20:4 and PG are greatly stimulated. The data show for the first time that changes in a subcellular level can occur not only after dietary interventions or administration of catecholamines, but also after physical exercise.

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MEASUREMENTS OF INTRACELLULAR PH IN SMOOTH MUSCLE CELLS OF THE RABBIT EAR ARTERY

M. Vonderlage and V. Fricke

The pH-sensitive dye 2',7'-bis-(2-carboxyethyl)-5(6')-carboxyfluorescein (BCECF) was used to measure intracellular pH (pHi) in everted segments of the rabbit ear artery (REA). To measure fluorescence, segments were investigated in a manner as described previously (Vonderlage and Schreiner, Am.J.of Physiol.257,H649-657,1989). The wavelength for excitation was 506 nm, bandwidth 0.2 and for emission 530 nm, bandwidth 8.0. There was a loss of dye with time, which however was very slow (decrease in fluorescence < 3%/5 min).

By using the KCl - ionophore nigericin, fluorescence signals were calibrated. Resting level of pHi was found at 7.2 in a bicarbonate-free and at 6.8 in a bicarbonate-containing buffer solution. After a latency of about 5 sec, norepinephrine (1 µM) induced a decrease in pHi down to 7.0 probably due to hydrolysis of ATP. After reaching this minimum, pHi increased and returned more or less to its initial value. Using a sodium-free solution pHi decreased continuously. Due to these procedures only slight changes in pHi could be observed when a bicarbonate-containing solution was used. It is suggested that in cells of the REA there is a Na+/H+ - exchange which is directly and/or indirectly activated by norepinephrine.

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ENHANCED RUNNING ACTIVITY CAUSES INCREASE IN TRANSMITTER RELEASE IN MOUSE EDL MUSCLE

M. Dorlöchter, M. Brinkers, A. Iritachev and A. Werdig

Activity possibly influences structural and functional parameters in neuromuscular junctions. We studied effects of long-term (2-8 months) running in wheels on transmitter release in leg muscles of mice. In the trained group each animal was provided with a running wheel which was used ad libitum to an average amount of 3-15 km per day. Intracellular recordings in Mg-blocked preparations (2.75 mM Mg, 0.4 mM (Ca) at 10 Hz stimulation revealed that quantal content of endplate potentials (EPPs) was higher in trained than in control EDL (g = 1.75 ±0.19 versus 1.35 ±0.35, n=7 each, p<0.05). In curare-block the amplitudes of the first, maximum and plateau EPPs in a train of 100 Hz for 400 ms or 1 s were increased in trained EDL by 28% each (p<0.05). Resting membrane potentials and muscle fiber diameters measured from frozen cross-sections did not differ significantly in trained and control EDL. Training effects were particularly evident in two pairs of monosynaptic twitches in which a drastic increase in quantal content and EPP amplitudes the time course of facilitation and depression in a 100 Hz train were changed: in trained EDL, the maximum EPP was reached earlier (in the average at 2.0 impulses versus 2.6 in controls, p<0.01) and was followed by a somewhat earlier decline below the value of the first EPP (EPP amplitude versus 5.0 in controls, 1.4 in trained, p<0.05). In isometric tension measurements block resistance in Mg-block was higher in trained than control EDL for single twitches, depression in a train of four single twitches at 2 Hz and curare-block (10 µM) was lower in trained EDL (p<0.05). The results suggest that prolonged elevated activity causes a marked increase in transmitter release and safety margin of transmission in EDL muscles. Histochemical studies showed mainly an increase in Type IIb (FG) fibers and a decrease in Type IIa (FG) fibers (p<0.05) which can be considered as a rise in oxidative capacity caused by endurance exercise.

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REPEATED OBSERVATION IN VIVO OF IDENTIFIED FROG NMJ JUNCTIONS
A. Wernig and B. Langenfeld

Recently dyes have been used for repeated staining of endplate membranes in the living animal. In the present experiments we compared vitally stained endplates in adult frogs at intervals of 4-5 weeks. Before the second in vivo staining, transverse sections of the c.p. nerve were performed (3-5 h per day for 6-13 days, 10 Hz, 0.5 ms pulses in anesthetized). Twelve (14) cutaneous pectoral muscles of six stimulated and one unstimulated frog (c.p. nerve 7 cm) were investigated. In deep anesthesia (MS 222) the outer muscle surface was stained with 10 µM 4-4'-di-2-ASP (Molecular Probes) and viewed with epifluorescence (40/0.75 w, HBO 50, BP 450-490 nm filter) through a coverslip. Images were videotaped and later digitally drawn on lucite sheets. From a total of 92 synapses 47% showed growth, 27% growth and retraction, 12% retraction only and 14% no change. Individual length changes were as large as 20% of the total synapse length (median=35). The occurrence of both, sprouting and retraction even within a single junction and in the unstimulated frog suggests the existence of junctional remodeling. Stimulations had no obvious effects on synaptic length changes. In three stimulated and three control animals (without stimulation and in vivo stainings) signs of sprouting (spotted arrangement of AChRs) and retraction were quantified after staining the muscles with rh-AcChR and cholineesterase in vitro. Sprouts were present in both, but less frequent in untreated controls (p<0.01, t-test). This indicates that the experimental procedures might enhance but do not initiate the structural changes. Sprouts were more and abandoned gutters less frequent in the unstimulated control than in stimulated muscles (p<0.05). This indicates that stimulation reduced sprouting and enhanced retraction without significantly affecting junctional size. This is consistent with previous findings that stimulation caused reduction in transmitter release (Hinz and Wernig, 1988) and a slight increase in abandoned gutters relative to contact length. This work is supported by DFG Wa 859

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DECLINE OF QUANTAL AND VESICULAR STORES OF TRANSMITTER AT NERVE-MUSCLE SYNAPSES OF CRAYFISH IN THE PRESENCE OF VERATRIDINE
W. Finger, H. Wollburg and A. Beer**

At the crayfish neuromuscular junction on application of veratridine the quantal release rate increases instantaneously to several thousand quanta per second and afterwards declines exponentially with a time constant of about 50 s. This effect can be induced only once in a single nerve-muscle preparation suggesting that veratridine largely depletes the presynaptic store of transmitter quanta (Finger and Martin 1989, Pfliigers Arch. 414:437-442). In the present study we employed morphological techniques to find out whether a correlation exists between the veratridine-induced decline in the rate of quantal release and the number of vesicles stored in the nerve terminals. About 5 min after veratridine-evoked quantal release had declined to a low level preparations were fixed in glutaraldehyde. For control, preparations not treated with veratridine were also fixed. Electron microscopy was performed on ultrathin sections obtained from control muscles and muscles treated with veratridine. The results show that compared to nerve terminals in control muscles nerve terminals in preparations treated with veratridine were largely depleted of their synaptic vesicles. This suggests that in the presence of veratridine the decline of quantal secretion results from the loss of vesicles. In addition, our results suggest that during or after excessive quantal release evoked by veratridine synaptic vesicles not only have the ability to fuse with the presynaptic membrane but also may fuse with each other with other intraterminal organelles.

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PROLONGATION OF ACTION POTENTIAL INCREASES THE DELAY OF QUANTAL RELEASE

Ch. Schweida, J. Dudel

It was shown in Dudel (1986, Pfliegers Arch 407: 134-144) that prolonged depolarization of nerve terminals by applied current increased the delay of first releases and of the median delay of release. Therefore we investigated the effect of prolonged depolarization on the correlation between lengthened action potentials and the time course of release in crayfish muscle. The K+ channel blockers 4-aminopyridine, 3,4-diaminopyridine (3,4 DAP) and tetraethylammonium (TEA) were applied in order to prolongate action potentials. The motor axon was excited via a suction electrode, and quantal currents were recorded by a macro-patch-clamp electrode which was perfused with the drug-containing solution (Dudel 1989, Pfliegers Arch 415: 289-298). This limited the action of the drug to about 20 um diameter of the terminal. Recordings of quantal release were performed using 16000 stimuli to construct distributions of the number and of the median delay of release. Therefore we investigated the delay of first releases and of the median delay of release. Therefore we investigated the effect of veratridine on the correlation between lengthened action potentials and the time course of release. The K+ channel blockers 4-aminopyridine, 3,4-diaminopyridine and tetraethylammonium were applied in order to prolongate action potentials. The delay of first releases and of the median of the distribution of releases were increased by up to 1.6 ms and on average by 1.3 ms ±0.35 ms (7 experiments). Most efficient was a combination of 0.1 mM 3,4-DAP und 1 mM tetraethylammonium. If release after membrane depolarization were only controlled by the increased intracellular Ca2+ concentration, the initial delay of the transmitter release should be independent of the duration of the action potential. The present results indicate that although Ca inflow starts during the upstroke of the action potential and Ca concentration rises within the terminal simultaneously, prolongation of the action potential can repress release for more than the usual minimal delay. It seems that depolarization has a repressing effect on the onset of release (Dudel 1986).
Spreading depression (SD) may be a correlate of migraine. A possible role of the excitatory amino acids (EAA) aspartate (asp) and glutamate (glu) in the generation of SD has been demonstrated in hippocampal slice preparations. The present study was undertaken to determine in vivo whether the extracellular concentrations of these EAA's vary during such attacks. Therefore, a microdialysis (MD) probe was implanted into the cerebral cortex of anaesthetized rats. Dialysate fractions were collected in one min periods and the glu and asp content determined by HPLC. A microelectrode for recording of DC or extracellular ion changes was placed in a distance of 100 to 200 μm from the tip of the MD probe. Single SD's were induced by a 1 - 2 min application of a perfusate containing a high K-CSF (128 mmol/l K+). SD's were characterised at the remote electrode by a negative shift of the field potential (fp) of about 1 min duration and 19.6 ± 2.31 mV amplitude (n = 8) and by large shifts in [K+]o, [Ca2+]o or by alkaline/acidic shifts of the pH. Parallel to the negative fp shift the EAA content in the perfusate increased by a factor of 18.4 for asp and by a factor of about 9.9 (n = 8) for glu. Local application of the NMDA antagonists APV (0.1 mmol/l in perfusate) or ketamine (1 mmol/l in perfusate) blocked the K+-induced SD. The K+-induced rise in EAA's was somewhat reduced. A triggering role of the K+-induced glu and asp release for the generation of SD was also suggested by the fact that local application of NMDA (1 mmol/l in perfusate) caused SD's which were sensitive to APV or ketamine. However, ketamine had to be applied in higher concentrations (20 mmol/l in perfusate). These results demonstrate in vivo, that K+-induced glu and asp release precede SD similarly to NMDA in vitro. We conclude, that asp and/or glu release can be a trigger for the generation of SD in vivo.

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HYPOLYCAEMIA-INDUCED HYPERPOLARIZATION PRECEDES RISE IN CYTOSOLIC FREE CALCIUM IN HIPPOCAMPAL PYRAMIDAL CELLS

Thomas Knöpfel, Andreas Spuler, Peter Grafe and Beat Gähwiler

The mammalian brain relies on a persistent supply of glucose. Lack of this energy delivering substrate results in a loss of consciousness and an isoelectric electroencephalogram. These symptoms are reversible depending on the duration of the hypoglycemia. In hippocampal pyramidal cells glucose deprivation as well as anoxia induces a membrane-hyperpolarization by inducing a potassium conductance (gK). It has been proposed that this hyperpolarization results from a rise in cytosolic free calcium ([Ca2+]i) which appears to be one of the causes of cell death following anoxia. The gK might, therefore, be activated by a rise in [Ca2+]i and would then simply reflect the onset of cell deterioration.

We used intracellular recordings combined with microfluorometric measurements of [Ca2+]i to investigate the relation between [Ca2+]i and the gK induced by glucose deprivation in slice cultured CA3 pyramidal cells. We found that this gK does not result from a rise in [Ca2+]i, but, on the contrary, might serve as an emergency measure to lower further energy consumption otherwise leading to calcium accumulation and cell death.

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MODULATION OF LIGAND-GATED POTASSIUM CONDUCTANCE IN HIPPOCAMPAL CA3 NEURONS

M. Böjk, U. Misgeld and W. Müller

In the hippocampus acetylcholine (ACh) and norepinephrine (NE) are considered to produce an excitatory and inhibitory effect, respectively. Yet the depolarization evoked by ACh and hyperpolarization induced by NE are small unless they are applied in high concentrations. Under single electrode voltage and current clamp carbachol (Cch, 0.05-0.5 μM) significantly reduces (by 40-80%) hyperpolarization and outward current induced by adenosine (5-10 μM), serotonin (5-10 μM) and bacoferen (0.05-0.5 μM) in CA3 cells of guinea pig hippocampal slices. The effect of Cch is antagonised by pirenzepine (0.1 μM), suggesting an involvement of the M1 muscarinic receptor subtype. In contrast to Cch, NE (5-10 μM) and α-adrenergic agonists phenylphrine and clonidine (0.5-5 μM) potentely augment (up to 300%) the ligand activated K-conductance. The effects of Cch and NE are observed after blockade of Na spikes with TTX, suggesting a direct postsynaptic interaction. Cch and α-adrenergic agonists modulate ligand-gated K-conductance without having any effect of their own on membrane potential or holding current. The potency of both modulatory effects on the baclofen-induced K-conductance increase depends on concentrations of Cch and noradrenergic-agonists as well as on the magnitude of the baclofen response. In conclusion, Cch and α-adrenergic agonists can exert strong excitatory and inhibitory effects via suppression and enhancement, respectively, of ligand-gated potassium conductance increase in the hippocampus.

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EFFECTS OF PENTYLENETETRAZOL ON SYNAPTIC ACTIVITY AND [Ca2+]i IN RAT HIPPOCAMPAL SLICES

G. Rauscha, E. M. Leweke and U. Heinemann

Effects of pentyleneetetrazol (PTZ) on synaptic activity of dentate granule cells and CA1 pyramidal cells were investigated with intra- and extracellular recording techniques. PTZ bath-applied with doses of 0.5 to 20.0 mM/l induced spontaneous epileptiform field potential transients in area CA1 (concentration optimum of 2 mM/l) but not in dentate gyrus. Dihethylamino effects of PTZ on paired pulse stimulus induced responses were already seen with concentrations of 0.5 mM/l in both areas. PTZ augmented paired pulse potentiation in area CA1 and reversed frequency habituation into potential in dentate gyrus at all stimulus intervals investigated (25, 50, 200 ms). This effect was strongest at 50 ms interval with 5 mM/l PTZ as quantified with a coastline index. With larger concentrations and intense stimulations, however, the second response could become depressed in both areas when evoked at stimulus intervals of more than 100 ms. Antidromically evoked field potentials were unaffected in dentate gyrus but enhanced in area CA1. Repetitive stimulation (20/s, 100/s) induced decreases in extracellular calcium concentration were enhanced by PTZ. This effect was optimal with 5 mM/l PTZ in granule cell layer as well as in stratum moleculare of dentate gyrus. In area CA1 the effect was optimal in pyramidal cell layer with a PTZ concentration of 5 mM/l while in stratum radiatum optimal effect was seen with 7 mM/l PTZ. Intracellularly four effects of PTZ could be seen: 1. block of the early part of the synaptically induced IPSP (optimal effect with 2 mM/l PTZ) while the late part was unaltered, 2. dose dependent increases in input resistance, 3. an average increase between 1 and 2 mM/l PTZ and as a result of this 3. facilitated bursting during depolarizing current injection with a reduced frequency accommodation and afterhyperpolarization, 4. prolongation of action potentials. All effects were fully reversible and could be seen in both CA1-pyramidal cells and dentate gyrus granule cells.

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CAFFEINE INDUCED PAROXYSMAL DEPOLARIZATIONS IN HIPPOCAMPAL NEURONS IN VITRO (GUINEA PIG)

D. Bingmann, J. Morselli and E.-J. Speckmann

It is well known that caffeine intoxication of patients may lead to epilepsy. The present experiments aimed to study this epileptogenic effect in hippocampal slices (400 μm thick).

When the slices were superfused by a 32°C warm saline, a spontaneous aperiodic bioelectrical activity predominated in granule cells and in CA1-CA3 neurons. After adding caffeine to the superfusate (final concentrations: 0.2-5 mmol/l) the following reactions were observed: (1) At caffeine concentrations exceeding 0.3 mmol/l all CA3 neurons (n=28), but only 2 out of 9 CA1 neurons started to generate paroxysmal depolarizations (PD) which periodically occurred 60 to 3 times per min. The latency until the onset of PD typically ranged between 2 and 5 min. In granule cells PD were absent. During washing with control saline, PD of CA3 neurons disappeared within two hours. (2) To study, whether transmembrane calcium currents contribute to the generation of PD, effects of the organic calcium antagonists verapamil (40-80 μmol/l bath concentration; n=14) and flunarizine (40 μmol/l added to the bath; n=4) on caffeine induced PD were tested. Both calcium antagonists reversibly reduced PD in amplitude, duration and frequency of occurrence until failure. During a first application of these drugs, PD disappeared after 20-60 minutes. A second application reduced this latency markedly. When tetrodotoxin (TTX 0.2 μmol/l; n=5) was added to the caffeine containing saline, PD stopped as soon as the amplitude of action potentials was reduced. However, even after abolition of sodium spikes, intracellular current injections evoked PD, which again were reduced reversibly in amplitude and duration by verapamil.

As a whole, the findings indicate that caffeine induced PD are generated endogenously and that verapamil sensitive calcium currents contribute to these endogenous mechanisms.

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SUPPRESSION OF BICUCULLINE-INDUCED PAROXYSMAL ACTIVITY IN HIPPOCAMPAL AND NEOCORTICAL NEURONS (IN VITRO) AFTER APPLICATION OF THE ORGANIC CALCIUM ANTAGONIST VERAPAMIL

H. Straub, D. Bingmann, E.-J. Speckmann, J. Walden, R.E. Baker

The organic calcium antagonist verapamil has been shown to suppress epileptic activities in architected and neocortical neurons elicited by pentylentetrazol (PTZ; cf. Bingmann and Speckmann, Exp. Brain Res. 64: 99-104, 1986, Bingmann et al., Exp. Brain Res. 72: 439-442, 1988). The present investigations tested whether verapamil is also able to block epileptic discharges elicited by the GABA-A antagonist bicuculline. The investigations were performed on hippocampal slices (CA3, guinea pig) and on organotypical explants (neonatal rat). The membrane potential was recorded by microelectrodes filled with 2 mol/l KCl, 30 μmol/l HEPES (pH 7.4) and 20 μmol/l EPPH (pH 7.4) at a temperature of 30°C. Two-nozzle voltage-clamp technique was used. PTZ (1 to 100 μmol/l) was applied for 30 to 120s using a concentration-clamp technique (exchange of solutions in less than 30s).

With administration of PTZ an inward current with an amplitude of up to 30nA was elicited at holding potentials of -50mV. The current remained constant or slightly increased during application (up to 100nA). The time to peak of rise and decay was ca. 40 and ca. 40ms, respectively. Sucrose solutions of comparable concentration failed to elicit currents.

With PTZ application the conductance was reduced for up to 30% and reincreased with ongoing application nearly to initial values. The equilibrium potential of the inward current ranged between -65 and -95mV. With elevating extracellular potassium concentrations to 40mmol/l the equilibrium potential was shifted for 20 to 30mV in positive direction. With administration of the anion channel blocking substrate 4-Acetamido-4'-Isothiocyanato stilbene-2',2'-disulfonäure (SITS) 10μmol/l the amplitude of the inward current was reduced.

From these observations the conclusion may be drawn that PTZ reduces the conductance for potassium ions and increases the conductance for chloride ions.

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A versatile highly sensitive CCD camera system for quantitative fluorescence microscopy

M. Hans, D. Swandulla and H. D. Lux

Fluorescent probes have been proven to be a useful tool in the investigation of cellular functions (Tsen, R.Y., In: Methods in Cell Biology, D. L. Taylor and Y.-L. Wang (eds), Vol. 30, 1989, Academic Press, San Diego, CA). We have developed a system for simultaneous measurements of low-level fluorescence emission signals and membrane currents derived from biological samples. The image acquisition and processing system consists of an epifluoresced Zeiss fluorescence microscope, a solid-state detector unit (Photometrica) and an image processing unit (TEX151).

The nitrogen-cooled detector nitrogen-cooled CCD detector with a 384x576 pixel array which has a very low intrinsic noise (dark current < 5e-/pixel/sec) and shows linear response over the full dynamic range with a quantum efficiency of 30% (700-700 nm). An analog processor digitizes the signals with high resolution (14 bit/50 kHz). The main feature of this system is that fluorescence signals as low as five photons can be reliably detected. This allows detection of emission signals from single fluorescent labelled ligands such as monoconal antibodies. The small pixel size of the detector results in a spatial resolution of 0.2 μm²/pixel (40x objective), a value which enables resolution of the spatial distribution of free ion concentrations (Ca²⁺, H⁺, etc.) or membrane potential in single cells using appropriate fluorescent probes.

In order to minimize photodecomposition processes, the fluorescence excitation light path is controlled by an electronic shutter. For dual wavelength measurements, excitation filters are changed using a stepper motor. All camera functions and components of the imaging system are operated by a microcomputer (Intel 60386), which allows selection of subarrays of the detector. For a 100x100 pixel area frames are obtained at a frequency of 10 Hz. The maximal temporal resolution of the system is less than 5 ms/frame (100x100 pixel area with 25x25 pixel binning).

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The excitatory glutamate subreceptor agonist N-methyl-D-aspartate (NMDA) is suggested to be involved in the generation and spread of epileptic discharges. Since the neurotransmitter noradrenaline (NA) has been described to reduce epileptic activity (D.I. Barry et al., Proc. Natl. Acad. Sci. 84: 9712-8715, 1987; R.S. Neumann, Epilepsia 27, 359-366, 1986), the influence of NA on NMDA induced negative cortical field potential changes (CFP) was tested.

The experiments were carried out in anesthetized and artificially ventilated rats. Transmitters were ejected by pressure pulses with a 3-barrelled micropipette which was glued in parallel to the recording microelectrode. CFP were led from different laminae of the rat motorcortex.

NMDA induced negative CFP were reduced in amplitude when NA was applied together with or 10 to 30 s before the excitatory amino acid. This effect was mimicked by the alpha-1 agonist phenylephrine, but not by the beta agonist isoproterenol. The alpha-2 agonist clonidine de- or increased the NMDA response.

In summary, the results suggest that the reduction of glutaminergic effects is involved in the antiepileptic action of NA.

H. Pawelzik, H.U. Doett and W. Ziegglänsberger.

A prerequisite for the understanding of high complex structures like the neocortex is the evaluation of single cell-to-cell interactions. The in vitro brain slice technique allows stable intracellular recording and pharmacological manipulation of synaptic transmission. However conventional electrical stimulation of an in vitro preparation simultaneously activates heterogeneous synaptic inputs.

To study potentials elicited by excitation of single cells we employed the method of spike-triggered averaging: a neocortical neuron (layer II/III) was recorded intracellularly; the discharge activity of an adjacent neuron which was recorded extracellularly was increased by microiontophoretic application of glutamate or NMDA. The spikes of the extracellularly recorded unit (presumptive presynaptic) were used to trigger averages of the intracellularly recorded unit. Two pure inhibitory connections were found. In 17 cases spikes in the presynaptic cell resulted in excitation of the follower cell with amplitudes of 162 ± 111 μV (mean ± S.D.). Only two purely inhibitory connections were found. The excitatory postsynaptic potentials were followed in 9 of 17 neurons by hyperpolarizing potentials with time to peak of about 60 ms and amplitudes of 27 ± 16.3 μV. Time to peak of this potential lies between the time to peak of fast (20 - 25 ms) and slow (150 - 250 ms) inhibitory post synaptic potentials in neocortical pyramidal cells. Thus this potential may represent a novel synaptic component which can only be revealed by spike-triggered averaging. One possible origin of this potential may be the co-release of a second neurotransactive substance, e.g. a neuropeptide together with the classical neurotransmitter glutamate.

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Experimental allergic neuritis (EAN) is an acute polyneuritis caused by the peripheral nervous system. The morphological changes of the nerve fibres are characterized by segmental demyelination and are similar to those found in the Guillain-Barré-Strohl-syndrome in humans. EAN can be induced in Lewis rats by immunization with bovine myelin plus Freund's complete adjuvants. Within 12-16 days motor deficits occur ranging from limp tail to tetraparesis. In diseased rats conduction velocity is decreased and latency of the H-reflex is increased (Wiethöter, Springer 1989).

Strobe-syndrome in humans. EAN can be induced in Lewis rats by immunization with bovine myelin plus Freund's complete adjuvants. Within 12-16 days motor deficits occur ranging from limp tail to tetraparesis. In diseased rats conduction velocity is decreased and latency of the H-reflex is increased (Wiethöter, Springer 1989).

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DENDROTOXIN BLOCKS ONE TYPE OF PARANODAL FAST K+ CHANNEL IN RAT MYELINATED NERVE
B.J. Corrette, F. Dreyer1, H. Repp1 and J.R. Schwarz

As in the frog, slow and fast K+ channels have been shown to occur in the rat node of Ranvier, but the distribution of these channels is different. Slow K+ currents predominate in the nodal membrane, whereas large fast K+ currents can only be recorded after paranodal demyelination (Röper & Schwarz, J Physiol 1989). We have investigated the effect of dendrototoxin (DTX), a specific blocker of one type of fast K+ channel in the frog (Benoit & Dubois, Brain Res 1986), on the K+ currents before and after demyelination.

Single myelinated nerve fibres were mechanically isolated from rat sciatic nerve and voltage clamped. K+ currents were measured in isocitric KCl solution. The relative contributions of fast and slow K+ channels were derived from kinetic analysis of tail currents. In intact nodes, the ratio of slow to fast K+ channels was found to be as high as 9:1. This ratio could be inverted by paranodal demyelination with promine. 140 nM DTX blocked 85-100% of fast K+ currents in the intact node, whereas demyelination of the same concentration was only able to block 73-86%. 14 nM DTX blocked about 50% of the toxin-sensitive K+ channels. No consistent effect of DTX on slow K+ currents was observed. Binding studies with fibre membrane indicated high affinity binding sites for DTX. Furthermore, iodinated DTX could be completely displaced from its sites by DTX. 1, MCD-peptide and charybdotoxin. Immunocytochemical methods showed that binding sites for DTX are only present following paranodal demyelination. Our results show that the fast K+ channels present in the rat paranode can be further subdivided into DTX-sensitive and DTX-insensitive channels.

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INTRACELLULAR CALCIUM TRIGGERS LONG-TERM DEPRESSION IN THE CEREBELLUM
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Synaptic transmission between parallel fibers and cerebellar Purkinje cells (PC) is depressed by asynchronous activation of the climbing fibers. This form of synaptic depression can last for hours or longer and has been termed long-term depression (LTD). We have performed two tests of the hypothesis that LTD is initiated by increases in free calcium concentrations ([Ca2+]i) in PC. First, we asked whether activation of climbing fibers raises [Ca2+]i in PC. Whole-cell patch clamp methods were used to record [Ca2+]i from PC in thin cerebellar slices of 12-18 d old rats. Synaptic currents were elicited in these neurones by extracellular stimulation of parallel and/or climbing fibers. The recording pipette contained fura-2 (100-400 μM) and the fluorescence of this dye was measured with a SIT video camera or a photomultiplier to determine [Ca2+]i. We found that climbing fiber stimulation of parallel and/or climbing fibers produced a large rise in [Ca2+]i in PC. The rise was transient, usually lasting less than 5 s, and was much more prominent in dendrites than in somata. The rise in [Ca2+]i appeared to be due to the climbing fibers activating voltage-gated Ca channels, because the [Ca2+]i changes were small when Ca spikes were absent. The second test of the hypothesis was to examine whether raising [Ca2+]i depressed transmission at the parallel fiber-PC synapse. Elevating [Ca2+]i, by depolarizing the membrane potential of the PC to open voltage-gated Ca channels, often produced a dramatic reduction in the magnitude of the synaptic current evoked by parallel fiber activation. This depression was long-lasting (> 30 min), greatly exceeding the duration of the [Ca2+]i rise that triggered it. In conclusion, both tests support the hypothesis that climbing fiber synapses initiate LTD by raising [Ca2+]i in PC.

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DECLINE OF EXTRACELLULAR FREE CALCIUM CONCENTRATION DURING CAFFEINE-INDUCED PAROXYSMAL DISCHARGES OF CA3 NEURONS IN HIPPOCAMPAL SLICES (GUINEA PIG)
I. Moradis, A. Lehnenkühler, H. Straub and D. Blüthmann

Recently, Kostyk et al. (J. Membrane Biol. 110, 11-18, 1989) have described caffeine-induced periodic increases of the free intracellular calcium concentration ([Ca2+]i) in small neurones. The present experiments were carried out to analyze whether calcium entry from the extracellular space contributes to increases of [Ca2+]i. Therefore, changes of extracellular free calcium concentration ([Ca2+]o) and field potentials (FP) were recorded with double-barreled liquid membrane micro-electrodes in hippocampal slices (n=8) during exposure to a saline containing caffeine (0.5 mmol/l; T=32°C). The experiments revealed: (1) In the stratum pyramidale of the CA3 region FP periodically occurred at a rate of 5 to 20 per min. They consisted of an initial large positive deflection (amplitude: 0.5-1 mV; duration: 0.2 s) followed by a smaller negative one (amplitude: 0.1 mV; duration: 0.6 s). FP reversed in polarity in the stratum radiatum. (2) The bioelectrical events were accompanied by decreases in [Ca2+]o of 0.3 to 0.5 μmol/l. Minimum calcium values were observed after 0.3 s. [Ca2+]o recovered within 6 s. The findings indicate that caffeine-elicited epileptic bioelectric phenomena are associated with transmembrane calcium currents which may contribute to oscillations of [Ca2+]i.

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ELECTROPHYSIOLOGICAL STUDIES ON CO-CULTURES OF THE HIPPOCAMPUS AND THE HYPOTHALAMUS
D. Büsselberg, B. Heinrich1 and H. L. Haas

Histaminergic neurons in the tuberomammillary nucleus (TM) project to different brain regions, including the hippocampus. We used organotypic co-cultures of the hypothalamus and the hippocampus in vitro to study their neuronal connections. 400 μm thick slices of the TM region in the posterior hypothalamus and the hippocampus were prepared from 3-5 day old rat pups. The two slices were placed on coverslips at a distance of about 1 mm. The surface of the hypothalamus was orientated to the stratum oriens of the hippocampus. The slices were imbedded in a clot of chicken plasma and were cultured for 16-35 days with the roller tube technique. The histaminergic neurons were identified by histidine-decarboxylase immunoreactivity. CA1 neurons were impaled with glass microelectrodes (filled with 3 M KC1; 40-70 MG) while the TM region was electrically stimulated at 0.02-0.1 Hz. Signals were recorded, digitized and analyzed using "pclamp" software. Both structures displayed spontaneous action potential firing. The response was a complex depolarizing synaptic potential which appeared about 3-7 msec after the stimulus artefact and declined over twenty to several hundred msec, sometimes triggering action potentials. Spontaneous postsynaptic potentials (PSPs) were regularly observed. Adding P2+ (10 μM; a blocker of synaptic transmission) to the bath abolished all potentials. Bicuculline (10 μM), a GABA A antagonist, only partially reduced the spontaneous and evoked PSPs. Histamine H3-antagonists did not affect the potentials but H1-antagonists enhanced their latency and number significantly. Thus excitatory and probably inhibitory (gabaergic) as well as modulatory (histaminergic) projections were established between hypothalamus and hippocampus in vitro.

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LONG-TERM POTENTIATION (LTP) IS NOT SUPPRESSED BY ANTIDEPRESSANTS AND ANTIEPILEPTICS

S. Birnstiel and H.L. Haas

Recent investigations indicate an inhibitory role of the tricyclic antidepressants and antiepileptics on the N-methyl-D-aspartate (NMDA) receptor complex (Neuron 2:121; Brit.J.Pharmacol. 95:19, Naunyn Schmiedebergs Arch. 339:613). Therefore we studied the impact of the antidepressant agents imipramine, (+)-oxaprotiline and (-)-oxaprotiline as well as midazolam and phenytoin on tetanus-induced LTP in the CA1 region of hippocampus.

Recordings were obtained from Sprague-Dawley rats weighing 150-200 g. Stimulation was at 0.1 Hz, tetanic stimulation consisted of 4 trains at 100 Hz and the same stimulation strength. Drugs were added 11 min before tetanus and were present until the end of the experiment (22 min after tetanus). At 67% of maximum stimulation, neither Phenytin (10 μM, n=7) nor Midazolam (0.1 μM, n=9) at their ED 50 to inhibit NMDA-mediated discharges in Mg-free medium influenced LTP. Midazolam was equally ineffective at 1 μM (n=8). Similar results were obtained with the antidepressants at 10 μM and 67% maximum stimulation. When tested at halfmaximal stimulation however, all antidepressants significantly enhanced LTP (p < 0.05).

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SEROTONIN ACTION ON TUBEROMAMILLARY NEURONES

B. Schönrock, D. Büsselfberg and H.L. Haas

The tuberomammillary nucleus (TM) contains large neurones, the only histaminergic neurones in the brain, which project to most regions of the central nervous system and receive inputs from many sources including serotonergic neurones in the raphe nucleus. Both nuclei exhibit congruent marked changes in firing rate across behavioural states.

We report here the responses of TM histamine cells and other mammillary neurones of the rat in vitro to serotonin. Histaminic cells fire spontaneously and display two characteristic A-currents and an inward rectifier current (J.Physiol. 399:633-646) while neighbouring mammillary neurones often have a low threshold Ca-spike. Both types of neurones could be depolarized (n=16) or hyperpolarized (n=8) by serotonin 1-10 μM. Biphasic actions consisting of an initial hyperpolarization followed by a longer lasting depolarization were also observed. These actions were accompanied by an increase or decrease of membrane resistance respectively and occurred in the presence of tetrodotoxin too. In some neurones, 5-HT induced a long lasting depolarization or a membrane potential oscillation (ca 1 second) after a hyperpolarizing pulse, the low threshold Ca-spike was markedly prolonged and enhanced.

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SHIFT OF THE STEADY-STATE INACTIVATION CURVE OF THE A-CURRENT BY VALPROATE-SODIUM

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Valproate-sodium (VPA) has been found to decrease the slope of depolarization of paroxysmal discharges and to suppress paroxysmal depolarization shifts of single neurones. It was studied whether the A-current (IA) is responsible for these effects.

In identified neuronal individuals in the buccal ganglia of Helix pomatia semihexameric currents were measured using conventional voltage clamp techniques. VPA was administered extracellularly (dissolved in control medium; 10 mmol/l) or was applied intracellularly (dissolved in KCl solution (150 mmol/l); 1.7 mmol/l; pH 7.4) by pressure pulses. The final intracellular concentration of VPA was estimated to be ca. 1 mmol/l.

Extra- or intracellular applications of VPA had no effect on the current-voltage relation and the time to peak of IA. The steady state inactivation function (hIA) was shifted to more positive potentials after VPA application (Fig. 1). Thus the value of half inactivation (IhIA/IhIAmax= 0.5) changed from -76.5 ± 3.8 mV to 69.3 ± 2.8 mV (n=5).

In summary, the findings may explain the flattening of paroxysmal depolarization shifts after VPA application.

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DIFFERENTIAL EXPRESSION OF FUNCTIONAL SEROTONIN, GLUTAMATE, GABA, AND GLYCINE RECEPTORS IN XENOPUS OCYTES INJECTED WITH RAT BRAIN RNA

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Oocytes from Xenopus laevis are able to express functional receptors for a variety of neurotransmitters after injection of RNA from nerve tissues. The rate of co-expression was examined after injection of rat brain RNA. RNA was prepared by a guanidin/LiCl method (Cathala et al., DNA 2: 329, 1983). Poly (A)+ mRNA was isolated by affinity chromatography on oligo (dT)-cellulose. Oocytes (stage V or VI) were pressure-injected with 75μg total RNA or mRNA and kept for up to 14 days in a modified Barth medium. Membrane currents were measured by conventional voltage-clamp technique. Transmitters were applied by the concentration-clamp technique: serotonin (5-HT) 10 μmol/l, glutamate 200 μmol/l, GABA 200 μmol/l, glycine 1 mmol/l. Oocytes injected with total RNA or mRNA developed differential sensitivity to the transmitters. 75% of oocytes responded to 5-HT (60 of 80 tested). Only oocytes sensitive to 5-HT responded also to glutamate (14 of 60 tested; 23%), GABA (14 of 60 tested; 23%) and glycine (11 of 45 tested; 24%). Co-expression of three and four receptors appeared in 15% and 7%, respectively. Control oocytes (non-injected or water-injected; n=30) showed no responses.

One possible reason for the different rate of expression might be due to the different complexity of receptors.

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The kappa opioid-receptor agonists, U 50488H and U 66593, reduce postsynaptic potentials, but increase direct excitability in hippocampal CA3 neurons in vitro.

C. Alzheimer and G. ten Bruggencate

Within the hippocampal formation, CA3 neurons receive a strong peptidergic innervation by mossy fibers of the dentate granule cells which is mediated by proenkephalin-derived peptides. Dynorphin has been shown to act as an endogenous ligand at the kappa opioid-receptor subtype, which is also present in the CA3 subfield. In order to examine whether the activation of kappa opioid-receptors might affect neuronal excitability in this area, we tested the electrophysiological actions of two kappa opioid-receptor agonists, U 50488H and U 66593, on guinea pig CA3 neurons in vitro using intracellular recording techniques.

Within 30-40 min following bath-application of U 50488H (30-100 μM), the neurons' direct excitability was substantially enhanced as indicated by the following observations: (1) U 50488H dose-dependently increased the neurons' input resistance by 10-38% (50 μM: 16.7 ± 2.5% [± S.D.] n = 6, 100 μM: 32.7 ± 4.7% n = 3), whereas the resting membrane potential was not significantly altered. (2) Measurements of the neurons' I/V relations revealed a reduction of inward rectification in the hyperpolarizing direction in the presence of U 50488H. (3) Spike repolarization was found to be impaired by U 50488H causing a broadening of the spike and the appearance of a depolarizing afterpotential. In contrast to the excitatory actions on direct excitability, U 50488H, within the same concentration range, reduced mossy fiber-evoked postsynaptic potentials. Similar effects on both intrinsic properties and postsynaptic potentials of CA3 neurons were observed in the presence of U 66593, although the concentrations required were moderately higher (100-200 μM). The electrophysiological actions of the kappa opioid-receptor agonists were at least partially reversible following prolonged superfusion (60-90 min) with normal bathing solution. None of the effects were blocked by naloxone (2.5 μM, n = 4). However, since naloxone is only a weak antagonist at the kappa opioid-receptor and apparently exerts agonistic effects at higher concentrations, it remains to be determined whether the actions produced by the two kappa opioid-receptor agonists are mediated by the corresponding opioid receptors, or whether they are due to mechanisms unrelated to opioid receptors.

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CENTRAL ALPHA ADRENOCEPTORS AND REM SLEEP REGULATION
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It has generally been accepted that brainstem cholinergic mechanisms play a major role in REM sleep but a monoamine involvement in REM sleep regulation was also proposed. Narcolepsy is a disorder of REM sleep, and canine narcolepsy is an animal model of the human disease with essential homologies, that allow the investigation of brain neurotransmitter abnormalities potentially involved in this disorder. The present study aimed at investigating a possible involvement of central alpha-2 receptors in the narcoleptic symptomatology.

Alpha-2 receptors in the canine brain were pharmacologically characterized with [3H]Ptycholine binding. Tissue samples from five normal and five narcolectic Doberman brains were used. The receptor density was compared between normal and narcolectic dogs by Scatchard analysis in frontal cortex, hippocampus, locus coeruleus, nucleus caudatus and thalamus. The main result was a significant difference in the number of alpha-2 receptors in the locus coeruleus: narcoleptic dogs had a higher number of receptors in this region (p<0.01) while the other investigated brain areas revealed no significant difference.

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three different types of interictal activity were observed. Spikes occurred in irregular, composed or regular patterns. In cases of irregular patterns, the mean interval length between successive spikes ranged from 1700 to 4800 ms. The lengths of successive intervals varied to a great extent. The composed patterns consisted of two classes of intervals with lengths of about 250 to 500 ms and 1 to 3 short ones or by a further long interval. The slow component of this composed pattern resembled the irregular rhythm. The third pattern was characterized by very regular intervals ranging from 650 to 1200 ms in different experiments. During such sequences of regular discharges spike frequency gradually decreased. Within the experiments the foci switched between different patterns; a certain pattern could persist for several seconds, minutes or hours. The investigations show that pentylentetrazole induced interictal spikes can occur in different neuronal networks and can be modulated by various factors such as the time course of intrinsic and synaptic inhibitory processes following paroxysmal depolarization shifts in neurons (With, Uhlig, Valle, Neurosci. Lett. 1989, 101, 51-56). It is suggested that interictal spike rhythms and refractoriness of epileptic foci are determined by cellular inhibitory processes.
EEG-MAPPING IN NEONATES - EXPERIMENTAL AND CLINICAL STUDIES

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EEG mapping is commonly used today in cerebral function analysis of adults. Local disturbances like seizures, cerebral infarction, cortical bleedings and disturbed regional cerebral blood flow demand better topographical description of cortical electrical activity in neonates, too. Multichannel (8 or 16) EEG were recorded simultaneously at different anatomical structures (dura, os, skin) in neonatal piglets. It could be shown, that EEG maps recorded from surface position represent fairly good the cortical EEG-topography.

In the clinical study 8 healthy term newborns were examined by 16 channel unipolar recordings related to the international 10/20 system. Different EEG-topography were found for different typical EEG-patterns occurring in the term newborn. Low intra- and interindividual variability for the maximum of electrical activity was found, if the typical patterns were considered. Thus, we had introduced a new methods of pattern recognition. This maximum were located in the centroparietal region corresponding to the region of the maximum of the regional cerebral blood flow.

Topographic changes of epileptogenic discharges were studied by experimental seizures (penicillin-induced) in newborn piglets and rabbits by dynamic EEG-mapping. The results were compared to the dynamic EEG-topography of benign epileptogenic patterns of the human neonate (frontal sharp transients) and discharges during neonatal status epilepticus.

EFFECT OF NEONATAL SEIZURES ON VEGETATIVE BRAIN STEM FUNCTION

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Epileptogenic discharges may play an important role in autonomic dysfunction and for sudden unexplained death in adults with epilepsy. We tried to explain whether neonatal seizures may influence vegetative brain stem function. Seizures were defined by polygraphic recordings of human neonates including at least EEG, ECG and respiratory movements. Cardioeireography was inspected visually to determine gross changes of mean heart rate and respiration related to seizure activity, e.g. bradycardia, tachycardia, apnoea and tachypnoea. Furthermore, spectral analysis of cardioeireography were performed during periods with and without seizures to detect subtle changes. Experimental seizures were induced by local cortical penicillin application in neonatal rabbits. Spectral analysis was performed during control and periods with penicillin induces seizures.

32 out of about 300 newborns were found to show neonatal seizures. 8 out of these 32 showed gross changes of vegetative parameters. Subtle changes of heart rate fluctuations were found by spectral analysis, whereas both an increase and decrease of such parameters as Respiratory Sinus Arrhythmia may occur. Only an increase of the mean heart rate was found during experimental seizures indicating an increase of the sympathicotonus. No direct sign of brain stem involvement could be shown in that study.

In summary, we concluded that neonatal seizures may affect vegetative brain stem functions and may be partly responsible for their poor prognosis.

THE INFLUENCE OF DIFFERENT RATIOS OF RARE TO FREQUENT VISUAL STIMULI ON VISUAL P-300 COMPLEX

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Visual P-300 Complex was elicited in 21 male subjects 38 - 50 years old by flashing two different kinds of L/s randomly presented checkerboard patterns of 100 ms on a viewing screen 1 m in front of the subjects' eyes. Both, frequent A-stimulus (16x16 caskets) and rare B-stimuli (64x64 caskets), were always kept constant in size. Every subject was investigated with 5 different B/A ratios in a random sequence (I: 10:90, II: 20:80, III: 30:70, IV: 40:60, V: 50:50). In three instances during an extra session, the 5 ratios were presented vice versa (A-stimuli being rare). The potentials were derived from Oz to Fz, Cz was ground. The subjects counted silently only the rare stimuli.

Results: (1) The N-250 latencies from I through V of frequent A-stimuli as well as of the rare B-stimuli are not significantly different (Friedmann-Test: = 0.05). (2) The N-250-P-300 amplitudes are highest at the ratio I and show a steep decrease to II, also from II to III, but little difference in III:IV and in IV:V. The differences I-II, I-III, I-IV, I-V are significant (Wilcoxen-Vilcox.Test: = 0.05). (3) With A-stimuli being the rare ones the same results were obtained as above in respect to P-300 Complex.

Conclusions: The finding, that N-250 latency is independent of the ratio of frequencies of the two stimuli to be differentiared, is additional evidence of the hypothesis of N-250 latency being a discrimination-potential (Teghaye and Kugler 1988, Intern. J. Neuroscience, 17-28). The impact of the reversal of the ratio of A/B-stimuli on N-250-P-300 amplitudes as well as systematic changes of ratios of visual stimuli support the hypothesis of N-250-P-300 being an endogenous task processing potential.

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DIFFERENCES IN THE CONTRIBUTION OF NMDA RECEPTORS TO SYNAPTIC TRANSMISSION BY LATERAL AND MEDIAL PERFORANT PATH AND COMMISSURAL FIBERS IN RAT DENTATE GYRUS

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The degree of NMDA receptor participation in synaptic transmission in dentate gyrus has become controversial. We therefore studied the relative distribution of NMDA receptors versus quisqualate receptors in the area dentata of rat hippocampal slices by measuring the Qu- and NMDA-induced decreases in extracellular Na⁺ concentration ([Na⁺]o). Previous studies have shown that part of these dose dependent decreases persist in the presence of TTX. They can therefore be attributed to Na⁺ fluxes through the respective receptor related ionophores. Quis- and NMDA- induced [Na⁺]o decreases were small in the hilus and stratum granulare. Quis induced large [Na⁺]o decreases in all of stratum moleculare (SM), whereas amplitudes of NMDA-evoked [Na⁺]o decreases declined from the inner to the outer SM. Since the lateral perforant path (LPP) innervates the outer SM, the medial perforant path (MPP) the medial SM and the commissural fibers (CF) predominantly the inner SM, it appeared likely that the NMDA receptors would contribute to synaptic transmission in the different pathways to a different degree. This was tested by lowering extracellular Mg²⁺ concentration ([Mg²⁺]o) and then applying the NMDA receptor antagonist ketamine during selective stimulation of the three pathways. In low [Mg²⁺]o, field potentials evoked by CF stimulation were enhanced to the greatest degree, while those evoked by MPP stimulation were less, and LPP-evoked potentials were least enhanced. All enhancements were sensitive to ketamine.

These findings show that during synaptic transmission NMDA receptors can be activated to a different amount by the three dentate gyrus input pathways with the greatest amount of NMDA receptor participation in CF, a lesser amount in MPP and the least in LPP. Controversial findings indicating NMDA receptor participation in synaptic transmission in dentate gyrus can be explained by various stimulation sites within these three pathways.

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ELECTROPHYSIOLOGICAL AND BEHAVIOURAL CHANGES PRODUCED BY 2 VESSEL OCCLUSION (2VO) IN THE RAT

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In contrast to experimental models of ischemia 2VO does not lead to consistent neuronal damage in vulnerable structures of the brain (Niehl et al., Eur J Neurosci Suppl 1, 1988, 58). The metabolism, measured by local PG and lactate, was markedly influenced during occlusion and early reperfusion (Block et al., Eur J Neurosci Suppl 2, 1989, 151). To gain more information about this model electrophysiological measurements and behavioural observations were made. Induction of 2VO was done in pentobarbital anesthesia (60mg/kg i.p.) by transient (40 min) occlusion of both common carotid arteries. The electrophysiological experiments were carried out during the occlusion and the first hour of reperfusion. By electrical stimulation of a forepaw somatosensory evoked potentials (SEP) could be recorded from the corresponding somatosensory cortex. Visual evoked potentials (VEP) were elicited by photic stimulation using a strobe and responses were recorded from the primary visual cortex. The behaviour was monitored during the night (12 h) at intervals of 20 min using an automatic system. One group of animals was observed 2 days after 2VO, the other one 1 year afterwards. The SEP’s were not altered by 2VO. VEP’s revealed a significant reduction in amplitude and increase in latency during the occlusion compared to sham-operated controls. These changes were reversible since during reperfusion no differences between the groups were seen. The acute effect in behaviour, measured 2 days after 2VO, resulted in an increase of stereotypic movements and rearing at the occluded animals. One year afterwards 2VO animals had significantly less resting periods (defined by distance travelled = 0, ambulation = 0, rearing = 0) compared to controls. These results suggest that besides reversible changes during occlusion 2VO leads to long-lasting alterations. They might be interpreted as early aging.

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EFFECTS OF TEMPERATURE ON SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES

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We are interested in comparing the effects of temperature on neuronal functions in hibernators, which survive body temperatures down to 2°C, and in non-hibernators. In the present study we have investigated the effects of cooling on synaptic transmission in area CA1 of rat hippocampal slices. Evoked field potentials were recorded extracellularly in stratum radiatum (SR) and stratum pyramidale (SP). Stimulation electrodes were positioned in SR for orthodromic stimulation and in the alveus for antidromic stimulation of the pyramidal cells. The temperature was varied between 37°C and 8°C. In normal artificial CSF and with constant stimulus intensities the latency and duration of orthodromically induced population spikes of the pyramidal cells increased continuously with cooling. In contrast, the amplitude of these spikes first increased between 37°C and approximately 28°C and then decreased with further cooling (see figure). Below about 30°C the ability of the tissue to follow high frequency stimulation was impaired. At temperatures below 20°C the stimulus threshold increased strongly and in the range of 17-13°C the synaptic transmission from Schaffer collaterals and commissural fibers to the pyramidal cells was completely blocked. This was demonstrated by the loss of orthodromically evoked population spikes, even with very high stimulus intensities; with direct antidromic stimulation pyramidal cells were excitable down to temperatures below 10°C. The effects were largely reversible. The temperature at which synaptic transmission was blocked was strongly dependent on extracellular calcium concentrations. For example, it increased from approximately 15°C in the presence of 5 mM K+ to about 25°C in 10 mM K+. Differences between rats (non-hibernators) and hamsters (hibernators) are currently investigated.

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FASTER FOVEOPETAL THAN FOVEOFUGAL MOTION
EXTRAPOLATION REVEALED IN A VISIBLE-MOTOR TASK

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Six subjects indicated, by pressing a button, the moment at which a moving target (direction of motion: left to right; velocity 6 deg/s) "passed" a stationary reference line, marked by two bright dots 1 deg above and below the horizontal motion path (12 deg long). The reference line was randomly presented at seven positions. For two of the positions the alignment between the moving target and the reference was visible while in the other five positions an imaginary alignment had to be based on motion extrapolation. Two conditions of binocular fixation were tested: (A) at the point of target disappearance; (B) at the actual position of the reference. Extrapolation of motion was thus away from fovea (foveofugal) in condition A and towards fovea (foveopetal) in condition B.

A linear relationship between response time and physical duration of extrapolated motion (both measured from the moment of target disappearance) was obtained. Whereas no systematic differences between conditions A and B were found for the indicated moments of visual alignment, response times, indicating imaginary alignment, were consistently longer in A. The differences increased with extrapolation distance. The results suggest that motion is faster (22% on the average) for foveopetal as compared to foveofugal extrapolation. The data are discussed in relation to recent electrophysiological findings of foveopetal-foveofugal asymmetries in the responses of visual neurons in primate retinal cortex.

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CORTICOSTEROIDE DECREASES INHIBITORY POST-
SYNAPTIC POTENTIALS IN RAT NEOCORtical AND
HIPPOCAMPAL CELLS IN VITRO

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Steroid hormones are known to act through the genome inducing mRNA synthesis. Recent evidence suggests that steroids may also affect membrane responses of neurons directly. We investigated the action of corticosterone (CT) on membrane properties and synaptic potentials in neurons of the rat hippocampus and neocortex. Conventional intracellular recordings were performed in slices of the frontal neocortex and the hippocampus (CA1). CT (10^-7 - 10^-5 M) was dissolved in ethanol and added to the perfusion fluid. The excitability of the neurons decreased due to a diminution of the inward going rectification. CT (10^-5 M) did not change resting membrane potentials (RMPs) or thresholds for eliciting postsynaptic potentials. Early and late IPSPs were both reduced under CT. At low stimulation intensities (EPSPs not contaminated by IPSPs) EPSPs were not affected by the steroid. In about half of the hippocampal neurons, CT increased the afterhyperpolarization (AHP) following repetitive spiking. When CT was applied locally close to hippocampal neurons, AHPs were reduced almost immediately. This implies that in central neurons CT acts not only through a genomic mechanism mediated via cytosolic receptors but also exerts effects also at membrane receptors. The present data are in line with an action at GABA receptors.

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DC RECORDINGS AT THE SURFACE OF THE SKULL DURING CORtical SPREADING DEPRESSION AND GENERALIZED SEIZURE ACTIVITY

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Characteristic negative DC potential shifts can be led from the surface of the brain cortex during spreading depression (SD) and generalized seizure activity (cf. Caspers, H, Speckmann E.-J., Lehmenkühler A., in: Physiol. Biochem. Pharmacol. 106: 127-178, 1987). The present experiments on anesthetized and artificially ventilated rats tested whether and to what extent these cortical DC shifts appear at the intact surface of the skull. SD waves were produced by continuous local application of KCl solution (0.5 to 1 mol/l) to the surface of the brain skull lying over the motorcortex. This was done via a recording outflow electrode with a porous ceramic membrane. The first SD started ca. 1 hour after positioning of the electrode and was associated with a negative transient DC shift (amplitude: 4 to 6 mV; duration: 5 to 7 min). Generalized tonic-clonic seizures were produced by systemic administration of pentylentetrazol. Generalized seizures were associated with negative DC shifts (amplitude: up to 3 mV). This type of DC shift had a steep ascending slope while the return to baseline was rather slow and nearly monoeponential. The results demonstrate that SD and generalized seizures are associated with negative DC transients not only at the surface of the brain cortex but also of the skull.

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341 SYNAPTIC CONTACTS BETWEEN LUNG STRETCH RECEPTOR AFFERENTS AND BETA-NEURONES IN CAT
K. Anders, W. Ohndorf*, R. Demetzlof* and D.W. Richter

Light- (LM) and electronmicroscopical (EM) analysis revealed terminal arborization of slowly adapting lung stretch receptor afferents (SARs) within various subnuclei of the solitary tract (TS). These regions are the localization of somata and dendrites of inspiratory beta-neurones (R8) which receive monosynaptic inputs from SARs as shown electrophysiologically. In the present experiments, we have double-labelled SARs and R8-neurones to verify the synapses and further describe their morphological characteristics.

Experiments were performed in pentobarbitone (40 mg/kg) anaesthetized and artificially ventilated cats. SARs were identified within the TS by lung inflation and excitation following vagal nerve stimulation. R8-neurones were identified by their depolarization during sustained lung inflation and central inspiration, as indicated by phrenic nerve activity. One R8-neurone and 1-7 SARs per experiment were labelled. With intracellular injection of fluorescent dye, the R8-neurone was backfilled with horseradish peroxidase (HRP).

In LM, we found 40 boutons of SARs terminating on proximal dendrites of R8-neurones; only one SAR ended on the soma. Boutons were of the "en passant" or "terminal" type. 12 presumed contacts were analyzed in EM. Single axodendritic synapses were verified for 2 SARs terminating on R8-neurones. Multiple synaptic contacts became evident in 2 terminal branches of a single SAR synapsing on proximal dendrites of the same R8-neurone. The boutons had a diameter ranging between 1.5-3 μm, contained round, clear vesicles of a diameter of 40-50 nm and formed asymmetrical synapses. There was only one active zone per synapse.

The findings verify monosynaptic connections between SARs and R8-neurones, partly forming multiple synapses.

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342 PROTO-ONCOGENE C-FOS INDUCTION IN THE SPINAL CORD: DIFFERENTIAL MODULATION BY MORPHINE, ANTI-EPILEPTIC DRUGS AND NMDA ANTAGONISTS
T.R. Töll, J.M. Castro-Lopes and W. Ziegglänsberger

The proto-oncogene c-fos is expressed in dorsal horn neurones of the rat spinal cord following various noxious stimulation (NS). Excitatory amino acids, such as L-glutamate, serve as transmitters of primary afferent fibers and involve actions mediated by one or more combinations of receptors (quisqualic, kainate, NMDA).

Seven rats anaesthetized with halothane were injected i.v. with morphine (5, 7.5, and 10 mg/kg) or the NMDA antagonists ketamine (4 and 10 mg/kg) or MK-801 (1 mg/kg). In another set of experiments the anti-epileptic drugs carbamazepine (50 mg/kg) or valproate (200 mg/kg) were injected i.p.; these agents have an analgesic effect in some forms of chronic pain, such as trigeminal neuralgia. The spinal cord lumbar segments L4-L5 were immunohistochemically processed for the detection of the nuclear c-fos protein 2 hrs after stimulation. Following NS c-fos positive neurones were found in the medial half of the ipsilateral laminae I-II and to a smaller extent also in deeper laminae (III-VI). Morphine and to a smaller extent carbamazepine and valproate reduced the number of stained neurones. Of the drugs tested morphine (10 mg) caused the most extensive depression; 70 % in superficial and 85 % in deep neurones. Morphine dose-dependently (antagonized by naloxone) depressed c-fos expression when injected 10 min before NS, but not 10 min after finishing the NS. Naloxone per se (5 and 10 mg/kg) slightly increased the number of stained neurones. The NMDA antagonists ketamine and MK-801 produced no differences in the number or distribution of labelled neurones, although both drugs showed analgesic potency verified in behavioural tests at this dose-level.

NMDA receptor mediated processes are involved in somato-sensory processing at the first stage in the dorsal horn. However, the present study shows that NMDA receptor blockade does not prevent c-fos induction. Since an increase in intracellular Ca²⁺ induces c-fos expression in vitro, it remains to be shown whether an activation of the inositol phosphate metabolism by L-glutamate or co-released substance P leading to an increase in intracellular Ca²⁺ is the adequate trigger for c-fos induction in the spinal cord.

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Priming of Proto-Oncogene Expression in Neurones of the Spinal Dorsal Horn by Contralateral Noxious Skin Stimulation

J. Sandkühler, J.D. Leeh and T. Herdegen

Noceptive inflow to the spinal cord may be followed by facilitation of spinal nociception at the contralateral site. Here we report that the induction of the proto-oncogene c-fos in neurones of the spinal dorsal horn is 'primed' by contralateral noxious stimulation.

Rats which were either awake or lightly or deeply anaesthetized with subanesthetic or a-chloralose were used. A 20 min noxious mechanical (pinch), or radiant heat (56°C for 10 s at 2 min intervals) stimulus, or a chemical (formalin 2%, or cepacain 10⁻⁷ M, 50 nl injected s.c.) stimulus was applied to the central pad at one hindpaw. Sixty minutes later this stimulus was followed by an identical one applied to the corresponding skin area at the contralateral hindpaw. The rats were killed one hour after the second stimulus, perfused transcardially with PBS and paraformaldehyde and the lumbar spinal cord was removed. Transverse sections were processed with conventional immunocytochemical techniques to detect the nuclear c-fos like protein (primary antibody at 1:10,000; avidin-biotin method).

The number of labelled neurones in the superficial and in the deeper dorsal horn was 60-200% higher at the site of the second stimulus, irrespective of the level of anaesthesia, the anaesthetic used or the type of noxious stimulation. The only exception was capsaicin induced c-fos expression which was equally rare on both sides of the cord even though these animals were anaesthetized. The time dependent decline of the c-fos protein accumulation could, in itself, not account for these large differences in labelling.

This priming of the expression of the c-fos gene in neurones of the spinal dorsal horn may be involved in longterm changes in the central nervous system following peripheral trauma.

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Noxious Transsynaptic Stimulation of Spinal Neurons is Followed by Complex Nuclear Events: Induction of Immediate Early Genes in the Rat

T. Herdegen, J.D. Leae and M. Zimmermann

Jun, JunB, Krox-24, FosB and Fos proteins belong to the group of immediate early genes (IEGs) which are supposed to play a pivotal role in fundamental processes like cell division, differentiation or tumorgenesis. All proteins exert their function in the control of transcription of genes. Here we have investigated the expression of these proteins as induced by transsynaptic activation of nerve cells in spinal cord. The sciatic nerve of pentobarbital anaesthetised adult rats was stimulated electrically and the immunoreactivity (IR) of the proteins was investigated in lumbar spinal cord. Stimulation of Aδ-fibers (0.7 V, 20 Hz, 20 min) did not induce for c-fos expression. Stimulation of Aδ- and C-fibers (20 V, 5 Hz, 10 min) induced the expression of all 6 proteins in neurones of the dorsal and ventral horn, except motoneurons. A minimum stimulation period of 2 min was required for expression of all proteins. The increase of intensity of stimulation was followed by an increased number and intensity of IR. All proteins appeared ipsilaterally between 30-30 min following the onset of stimulation. The maximum of IR was observed between 1-2 h, then the number of labeled cells decreased within 15 h. Contralaterally there was an increase of labeled cells after 6h. All proteins showed a similar pattern of appearance resp. disappearance. Repetition of stimulus (Aδ- and C-fiber stimulation 2 h to 24 h following the first stimulation) provoked changes in the temporal and spatial pattern of IR: compared to the first stimulus the number of labeled cells was increased and the presence of the proteins was prolonged. It is conceivable that the alteration of induction and of stability of proteins following repetition of noxious stimulation reflects genetic mechanisms which might underly lasting changes in the nervous system such as memory formation.

II. Institute of Physiology, TNP 326, 6900 Heidelberg, Germany. Supported by the Deutsche Forschungsgemeinschaft.

Reduction of Pain Perception to Noxious Stimuli by Activation of Carotid Sinus Baroreceptors is Naloxone Reversible

M.K. Herber1, M.K.C. Marquet1, K.-D. Kniffki2

Aim of investigation: As shown on previous experiments stimulation of carotid sinus baroreceptors (CSBs) reduces experimentally evoked pain in healthy volunteers as well as pain perception in chronic pain patients. The aim of the present study was to explore whether this pain reducing effect in healthy humans is naloxone sensitive and may be mediated by endogenous opioids.

Methods: In 6 male and 5 female untrained healthy volunteers experimental pain was induced by applying constant pressure on a 2.6 mm² area to the third and fourth finger for 20 s. One of two different stimuli randomly presented in intervals of 4 min to each of the 4 spots on the 2 fingers was of noxious intensity. During pressure stimulation subjects rated their pain sensation continuously on a 50 point category scale. CSBS were activated by negative pressure applied bilaterally to the neck using a suction chamber beginning 15 s before and ending with the painful stimulus. Trials of 3-5 stimuli of either intensity were performed under (a) control conditions, (b) activation of CSBS and (c) activation of CSBS following i.v. application of 1 mg naloxone or saline solution.

Results: In 7 of 11 subjects tested CSBS activation with an individually tolerated strength of -55 mmHg to -95 mmHg lowered the magnitude of pain ratings significantly as compared to control trials. The pain reducing effect of CSBS stimulation was naloxone sensitive and fully reversible in 4 of these 7 subjects. Saline solution had no effect at all. Pain ratings under CSBS activation were not influenced in one and enhanced in 3 of 11 subjects. Efficiency of neck suction in activating CSBS was confirmed in all cases by the reduction of blood pressure and heart rate monitored during CSBS stimulation.

Conclusion: Neck suction induced activation of CSBS is capable of reducing pain ratings to experimental mechanical noxious stimuli in 7 of 11 subjects tested. This effect was naloxone reversible in 4 of these 7 and therefore might be mediated by endogenous opioids released during CSBS activation.

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DISTRIBUTION OF CALCITONIN-GENE-RELATED PEPTIDE-LIKE IMMUNOREACTIVITY IN THE CAT'S THALAMUS

R. Linker, C. Yahi-Hyn and K.-D. Knobl

Calcitonin-gene-related peptide (CGRP) is a widely distributed neuromediator which is thought to be involved in processing viscerovisceral, cardiovascular, olfactory, gustatory, and noxious stimuli. We studied the distribution of CGRP at dienecephalic sites involved in these functions.

Adult cats not pretreated with colchicine were perfused transcardially under isoelectric conditions. All four spinal nerves on the side of the injection were isolated. Serial sections of thalamus were cut and adjacent sections were immunostained for CGRP (Peninsula) using the peroxidase-antiperoxidase (PAP) method. Immunostaining was visualized by goat-anti-rabbit, Dakopatts; 3rd antibody: PAP complex, Dakopatts.

A dense terminal field of CGRP-immunoreactive (CGRP+) fibers was observed in the ventral part of the principal ventro medial nucleus adjacent to the dorsal hypothalamic area. This network of fibers of intermediate density was seen in the subparafascicular nucleus. A second dense terminal field was located in the anterior hypothalamic area medial to the fornix. Here small and large boutons outlined the somata and primary dendrites of unstained cells. Less dense fiber populations occurred in the dorsal part of the rostral submedial nucleus, in the mediodorsal nucleus, in the lateral part of the lateral habenula, in the dorsal and lateral hypothalamic areas, and in the ventral tegmentum. A few fibers were found in the medioventral part of the ventral thalamic complex. However, these fibers displayed distinct terminal boutons in some cases situated on somata. CGRP+ cells were also found but their number might be underestimated due to the lack of colchicine pretreatment. Scattered immunopositive cells were located at the caudal border of the basal forebrain nucleus in a row running in a caudal and lateral direction to the peripenduncular nucleus. Some cells were also found at the medial border of the medial geniculate body. A third location was in the anterior hypothalamic area surrounding the dense terminal field of CGRP+ fibers.

The distribution of CGRP+ fibers and cell in the cat's brain appears to be similar to that in the rat (Kruger et al., J. Comp. Neurol. 273:149, 1988). The results suggest that at the thalamic level CGRP is more likely a neuromediator of ascending gustatory and visceral pathways and to a lesser degree involved in processing noxious information.

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DIVERGING EFFECTS OF BOMBESIN ON THE TEMPERATURE SENSITIVITY OF NEURONES IN DIFFERENT HYPOTHALAMIC AREAS

J. Schenda, K. Matsumura and Fr.-K. Pierau

Previous investigations have demonstrated that bombesin increases the temperature coefficient (TC) of neurones in the hypothalamic anterior hypothalamic area (AHA). Since bombesin affects temperature regulation only after injection into the PO/AH area but has no effect by injection into the posterior hypothalamus (PH), we have studied the effect of bombesin injection on the TC of neurones in different nuclei of the AH and PH. Extracellular recordings were accomplished from rat hypothalamic slices in a perfusion chamber (artificial cerebrospinal fluid, 95% O2/5% CO2 atmosphere with periodic temperature changes between 35 and 41°C. Bombesin was injected as a bolus (0.1 mg in 0.1 ml).

The proportion of warm sensitive units in different areas of the AH varied between 30 and 50% (threshold TC: 0.6 imp/s). Bombesin increased the number of warm sensitive neurones in 3 of the 4 investigated AH areas (medial preoptic area, mPPO, medial preoptic nucleus, mPPO, and ant. periventricular nucleus, aPPO), indicating a conversion of temperature insensitive neurones into warm sensitive ones. By contrast, bombesin decreased the TC of some of the neurones in the suprachiasmatic nucleus (SCN), resulting in a 5% reduction of warm sensitive neurones. Similarly, the number of warm sensitive neurones was reduced by about 8% in the arcuate nucleus (AN) of the PH, 11% in the dorsomedial nucleus of the PH, and only 20% of the neurones were warm sensitive. The neurones of the other PH area investigated - the pPVN - resembled the AH neurones, in that the proportion of warm sensitive neurones was increased to 80% by bombesin. The results suggest that functional role of hypothalamic neurones in temperature regulation is not simply defined by their affiliation to the AH or PH.

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AXOTOMY AND BLOCK OF AXONAL TRANSPORT INDUCE EXPRESSION OF PROTEINS INDUCED BY THE JUN PROTO-ONCOGENE FAMILY: MOLECULAR GENETIC CHANGES DEPENDENT ON THE SUCCESS OF NERVE REGENERATION

T. Herdegen, J.D. Leah and M. Zimmermann

Proteins of jun and fos proto-oncogene families (JUN and FOS), control transcription of genes and belong to the immediate early gene whose expression plays a central role in development, differentiation or tumorgenesis. We have studied the expression of JUN and FOS proteins in nerve cells following axotomy and block of axonal transport (axt). In pentobarbitonal anesthetized adult rats, the sciatic nerve was transected. Both JUN- and FOS-immunoreactive neurones in different nuclei of the PH and AH were examined. Injection of the sciatic nerve as a bolus appeared 30 min after the sciatic axotomy and block of axonal transport (axt). In pentobarbitonal anesthetized adult rats, the sciatic nerve was transected. Both JUN- and FOS-immunoreactive neurones in different nuclei of the PH and AH were examined.

The proportion of warm sensitive neurones in the suprachiasmatic nucleus (SCN) and in the ventromedial nucleus (VMN) was reduced by about 8% in the arcuate nucleus (AN) of the PH, 11% in the dorsomedial nucleus of the PH, and only 20% of the neurones were warm sensitive. The neurones of the other PH area investigated - the pPVN - resembled the AH neurones, in that the proportion of warm sensitive neurones was increased to 80% by bombesin. The results suggest that functional role of hypothalamic neurones in temperature regulation is not simply defined by their affiliation to the AH or PH.

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348 DIVERGING EFFECTS OF BOMBESIN ON THE TEMPERATURE SENSITIVITY OF NEURONES IN DIFFERENT HYPOTHALAMIC AREAS

J. Schenda, K. Matsumura and Fr.-K. Pierau

Previous investigations have demonstrated that bombesin increases the temperature coefficient (TC) of neurones in the diencephalic anterior hypothalamic area (AHA). Since bombesin affects temperature regulation only after injection into the PO/AH area but has no effect by injection into the posterior hypothalamus (PH), we have studied the effect of bombesin injection on the TC of neurones in different nuclei of the AH and PH. Extracellular recordings were accomplished from rat hypothalamic slices in a perfusion chamber (artificial cerebrospinal fluid, 95% O2/5% CO2 atmosphere with periodic temperature changes between 35 and 41°C. Bombesin was injected as a bolus (0.1 mg in 0.1 ml).

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350 HOW REPRESENTATIVE ARE THE RESPONSES OF SINGLE NOCICEPTIVE DORSAL HORN NEURONS?

J. Sandkühler, D.-G. Fu and C. Forster

It is not known as to whether recordings from single nociceptive spinal dorsal horn neurones provide representative informations. We have addressed this question by recording action potentials of single individual neurones in the spinal cord and of axotomy-induced expression of proteins encoded by the JUN proto-oncogene family: molecular genetic changes dependent on the success of nerve regeneration. The results suggest that at the thalamic level CGRP is more like-ly a neuromediator of ascending gustatory and visceral pathways and to a lesser degree involved in processing noxious information.
LACK OF EFFECT OF BACLOFEN ON RELEASE OF SUBSTANCE P FROM THE CAT SUBSTANTIJA GELATINOSEA IN VIVO
W. D. Hutchison* and C. R. Morton

Baclofen reduces the presynaptic release of excitatory transmitter from large diameter primary afferent fibres terminating in the spinal cord. Analgesia results upon intrathecal administration in animals and humans, suggesting a similar reduction in release of neurotransmitters from small diameter nociceptive fibres. The antibody microprobe technique (Duggan et al. J. Neurosci. Methods 23: 241, 1988) was used to measure the intraspinal release of immunoreactive substance P (irSp) in barbiturate anaesthetized cats before and after the administration of (+/-) baclofen. Glass microelectrodes bearing antibodies to the C-terminal end of Sp are inserted into the lower lumbar spinal cord during noxious thermal or mechanical cutaneous stimulation of the ipsilateral hind paw or electrical stimulation of the tibial nerve at an intensity sufficient to excite C-fibres. Microprobe tips were then incubated in radiolabeled Sp and zones of release identified as deficits in tracer binding seen on autoradiographic images. A video camera and computer were used to scan the images and calculate average scans. In addition, extracellular recordings of dorsal horn neurones allowed the gated C-fibre response to tibial nerve stimulation to be measured before and after (+/-)baclofen administration.

Noxious cutaneous stimulation of the hindpaw or electrical stimulation of the tibial nerve evoked irSp release at a rate associated with the spontaneous activity of the substantia gelatinosa. There was no effect on the height or shape of the zone of release of irSp induced by tibial nerve stimulation following administration of 4 mg/kg of baclofen. A similar result was found for release of irSp induced by noxious cutaneous stimulation where alteration was observed only in a slightly elevated zone of release at the spinal cord surface in both stimulation groups after baclofen administration. This dose of baclofen, however, strongly depressed the gated C-fibre responses of the dorsal horn neurones to tibial nerve stimulation. These findings suggest either that the subpopulation of Sp-containing C-fibres plays a minor role in nociceptive transmission or that baclofen does not induce analgesia by a presynaptic release of irSp from these C fibres.

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CONTROL OF LOCOMOTOR ACTIVITY DURING ISOLATION J. Anchors

Human subjects who live in isolation without temporal cues usually develop circadian rhythms which "free-run" with periods close to 25 h. However, in about 30% of the subjects the sleep-wake cycle is suddenly lengthened to more than 28 h, or shortened to less than 20 h. If this happens, the rhythms of other functions such as body temperature continue to free-run with a period of about 25 h. During such states of internal desynchronization, subjects can be awake for up to 30 h, or for only 10 h. In spite of these drastic changes in the duration of wakefulness, the subjects adhere to their usual number of meals and stretch or compress the intervals between meals proportionally to . The caloric intake per meal remains constant, and body weight neither decreases on long "days", nor increases on short "days". This is possible only if the energy expenditure is adjusted to the duration of .

Since muscular work contributes substantially to the total energy expenditure, it could be assumed that activity is negatively correlated with the duration of . In search of such a relationship, locomotor activity was measured by means of contact plates installed below the carpet within the isolation unit. Electric impulses elicited by steps on the plates were recorded continuously and averaged in 1-h bins. Protocols from 14 subjects revealed a high correlation between the hourly means of activity and . On the average, the mean activity decreased by 4.9% per h increase in . As a consequence, the total amount of "daily" activity remained almost constant within a range of values from 12 to 22 h.

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MEDULLARY RESPIRATORY NEURONES IN RAT Z. Wilhelm*, S.W. Schwarzacher**, K. Anders and D.W. Richter

Many studies of respiratory control and respiratory substrates have been performed in the rat. The information about the organization of the respiratory network in the rat brainstem is, however, sparse. Our present knowledge derives from extracellular recordings that revealed the existence of a dominant ventral group (VRG) and a small dorsal group of respiratory neurones (RNs) similar to the cat (Ezure et al., Brain Res. 455: 262-270, 1988; Saether et al., Brain Res. 419: 87-96, 1987).

To obtain more detailed information about the organization of the respiratory network, we recorded from 70 RNs within VRG and analyzed the respiratory activity in 41 RNs in urethane-(2.5 g/kg) or pentobarbitone (60 mg/kg) anaesthetized Wistar rats. Phrenic and recurrent laryngeal nerve discharges were used to determine the central respiratory rhythm (CRR) and to functionally identify RNs. Some RNs were identified as bulboispinal by their antidromic response to spinal cord stimulation at C4. The RNs examined were not antidromically excited by peripheral stimulation. Six classes of RNs were identified: early-inspiratory (e-I), throughout-inspiratory (r-I), late-inspiratory (l-I), post-inspiratory (p-I), late-expiratory (E-2) and phase-spanning E-I neurones. Intracellular analysis of postsynaptic activities, IPSP reversal following chloride injection and changes in input resistance revealed e-I and p-I inhibition in E-2 neurones, e-I and E-2 inhibition in p-I neurones, and E-2 inhibition in r-I neurones. E-2 inhibition was weak but p-I inhibition strong in E-1 neurones. Tonic excitatory inputs not shunted by weak E-2 inhibition, therefore, might explain the excitatory effects of baclofen administration. The results reveal that in the rat the CRR is organized in three (I, p-I and E-2) phases and that synaptic interaction between RNs occurs similarly as described for the cat (Richter et al., NIPS 100: 109-112, 1986).

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The findings reveal that synaptic interactions between medullary neurons originate in the pons~

The findings reveal that synaptic interactions between medullary neurones when the lungs were ventilated. Non-inflation that caused augmentation and lengthening of inspiratory activity under control, resulted in apneas after MK 801 was administered. During apneusis, MP of E-2 and p-I neurones remained slightly hyperpolarized by continuation of chloride-mediated synaptic inhibition. Superior laryngeal nerve stimulation stopped apneusis and MP hyperpolarization of E-2 neurones. The findings reveal that synaptic interactions between medullary respiratory neurones are not normally mediated through NMDA-receptor activating pathways. The latter are also not used by pulmonary and laryngeal afferents. NMDA-receptor controlled pathways seem to be activated only during non-inflation and to trigger inspiratory bursts (Foutz et al., Neurosci. Lett. 87: 221, 1988).

To study NMDA-receptor activating mechanisms, we analyzed the postsynaptic activity of medullary late–expiratory (E-2) and post-inspiratory (p-I) neurones before and after MK 801 application.

Experiments were performed in pentobarbitone (40 mg/kg) anaesthetized cats which were ventilated with a cycle-triggered pump. Membrane potential (MP) trajectories of 46 E-2 and 8 p-I neurones were analyzed before and after MK 801 (0.3-0.7 mg/kg) was administered intravenously. The neurones were tested for their response to inflation and non-inflation.

MK 801 had no effect on mean values and periodic fluctuations of MPs of E-2 and p-I neurones when the lungs were ventilated. Non-inflation pressures we used the retrograde axonal transport of exogenous fluorescent tracers to demonstrate the intraspinal localisation of motor nuclei projecting to the cat's shoulder muscles. The findings indicate that synaptic interactions between medullary neurones are not normally mediated through NMDA-receptor activating pathways. The latter are also not used by pulmonary and laryngeal afferents. NMDA-receptor controlled pathways seem to be activated only during non-inflation and to trigger inspiratory bursts.

**TOPOGRAPHY OF SHOULDER MOTOR NUCLEI AFTER RETROGRADE LABELING WITH FLUORESCENT DYES IN THE CAT SPINO-MOTOR CORD**

M. Hörner, H. Kümmlle

Detailed studies on the neuronal organisation of forelimb motor control have so far focused on the proximal and distal muscle groups, excluding the shoulder girdle. Behavioural experiments, however, indicate that the shoulder muscles are specifically activated during different movements of the forelimb such as locomotion or target reaching. To reveal the structural basis for further studies on the functional interaction between forelimb muscles we used the retrograde axonal transport of exogenous fluorescent tracers to demonstrate the intraspinal localisation of motor nuclei projecting to the cat's shoulder muscles. The findings indicate that synaptic interactions between medullary neurones are not normally mediated through NMDA-receptor activating pathways. The latter are also not used by pulmonary and laryngeal afferents. NMDA-receptor controlled pathways seem to be activated only during non-inflation and to trigger inspiratory bursts.

**SYNAPTIC INTERACTION BETWEEN RESPIRATORY NEURONES AFTER BLOCKADE OF NMDA-RECEPTORS IN CAT**

U. Windhorst, J.L. Feldman*, K. Anders and D.W. Richter

Termination of inspiration seems to be mediated by two mechanisms: "reversible" inhibition during late-inspiration and "irreversible" inhibition during postinspiration. Small lesions in the rostral pons disturb this inspiratory termination when lung afferents are present, whereas anaesthetized animals, cause spontaneous "irreversible" non-inflation. Similar disturbances to the rhythm were observed after systemic application of the NMDA-receptor antagonist MK 801 (Foutz et al., Neurosci. Lett. 87: 221, 1988). To study NMDA-receptor activating mechanisms, we analyzed the postsynaptic activity of medullary late-expiratory (E-2) and post-inspiratory (p-I) neurones before and after MK 801 application.

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Properties of the "silent period" of human α-motoneurons evoked by magnetic brain stimulation

F. Ansüze, H. Feintheil and H.-J. Heinze

Magnetic brain stimulation elicits a (presumably) monosynaptic excitation of an α-motoneuron which is followed by a time interval during which the motoneuron has a low probability to fire an action potential (Day et al., J. Physiol., 412:449, 1989). The following experiments on healthy volunteers were performed to characterize some properties of the period following monosynaptic excitation evoked by magnetic brain stimulation.

Spike trains of 31 single motor units were recorded from the first dorsal interosseous muscle using a conventional concentric needle electrode. The units were made active by a slight voluntary contraction. Each unit was exposed to 50-120 magnetic brain stimuli (Dantec Stimulator) with the stimulating coil positioned over the vertex.

It was found that one inter spike interval of the investigated motor units exhibited a rapid decrease at monosynaptic latency, i.e. 24-34 ms after the stimulus. During the following time interval the probability to fire an action potential was so low that it could be regarded as a "silent period". The duration of this period varied from unit to unit (range 28-64 ms) in a characteristic way as it was found to be linearly correlated to the amount of inter spike interval shortening at monosynaptic latency. This result is consistent with the notion that the "silent period" appears merely as a consequence of a stimulus-induced motoneuron excitation and does not require motoneuronal inhibition as proposed by Boniface and Mills (J. Physiol., 412:8P, 1989).

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DOES THE LACK OF RECURRENT INHIBITION IN FORELIMB MOTONEURONES TO THE EXTRINSIC DIOIT EXTENSORS CORRELATE WITH ABSENCE OF RECURRENT AXON COLLATERALS?

M. Höhner, M. Ilbert, H. Kümmel

When Hahne et al. (1988) investigated the distribution of recurrent inhibition (RCI) in motor nuclei to cat forelimb muscles with electrophysiological techniques, they found pronounced RIPSPs in elbow motor nuclei, smaller effects in wrist motor nuclei and no effects in the motor nuclei to the extrinsic digit extensor. To verify the latter results and to correlate the electrophysiological data with their morphological substrates, we labelled identified motoneurones (MNs) with HRP. The experiments were performed in anaesthetized, immobilized cats whose dorsal roots were cut. For antidromic identification of spinal MNs the ventral roots of these segments were cut. The dorsal surface of the cervical spinal segments C2 - C5 was exposed by dorsal laminectomy. The dorsal roots of these segments were cut. The involvement of excitatory amino acid neurotransmitters in the excitation of motoneurons of the phrenic nerve (PNA) was studied by pneumatic injection of 50 - 100 nl of solutions of NMDA- or non-NMDA-receptor antagonists into the ventral horn of the cervical spinal segments C3 - C5. The blocking action of D-2-amino-5-phosphonovaleric acid (APV), ketamine, F-D-glutamylaminomethylsulphonic acid (GAMS) or 6,7-dinitro-quinazoline-2,3-dione (DNQX) was studied. After injection of the competitive NMDA-receptor antagonists APV or of the non-competitive NMDA-receptor antagonist dizocilpine the amplitude of IPNA, i.e. the neuronal equivalent of the tidal volume, was reduced. The results indicated that NMDA- as well as non-NMDA-receptors are involved in synaptic excitation of phrenic motoneurons. Whether the synaptic excitation of phrenic motoneurons by descending inputs from bulbo-spinal inspiratory neurons is induced by the release of excitatory amino acids cannot be answered on the basis of the present results.

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363 DIFFERENTIAL RELATION OF SINUS-ARRHYTHMIA TO RESPIRATORY PARAMETERS

H.C. Abildstrom and H.P. Koepchen

Heart rate fluctuations in the frequency range of respiration originate from an interaction of central cardiovascular-respiratory coupling, respiratory phase-dependent baroreceptor processing and respiration-modulated afferent inputs. We analysed different components of heart rate fluctuations in relation to different components of respiration at rest. In 17 sitting, young volunteers, ECG and respiratory movements were recorded during 45'. Means of RR-interval (RR), heart beat differences (D-variability), and respiratory phase at 0.1 and 0.25 Hz, as well as respiratory cycle time (TT), tidal volume (VT) and mean inspiratory flow were computed from successive 2' periods. After relative transformation of the 2 min means, mean RR was classified in three ranges. For each RR range, D-variability and both rhythmicities were sorted according to respiratory parameters which were also classified in three ranges. For each RR range, each triplet of D-variability or rhythmicity was tested (Kruskal-Wallis, α=0.01). Comparison of D-variability and both rhythmicities with all respiratory parameters resulted in only two significant relations. At middle RR, D-variability is related to VT and rhythmicity at 0.25 Hz is related to TT. In conclusion, during stable, low level of activity, heart rate fluctuations in a restricted RR range have no significant dependency on spontaneous changes in the respiratory pattern. This indicates an absence or weakness of coupling of the respective parameters. When there is common activation of the cardiorespiratory system, a decrease in heart rate fluctuations is likely to be caused primarily by common central nervous excitation. Corresponding changes in heart rate pattern do not occur obligatorily with each change in respiratory parameters.

365 CHEMOSENSITIVITY OF SYMPATHOEXCITATORY NEURONES IN THE ROSTROVENTRAL LATERAL MEDULLA OF THE CAT

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Cromosensitivitv of sympaIIheoxeitatory neurones in the rostrventral lateral medulla (RVLm) is highly vascularized with a high local blood flow and glucose metabolism (Gobel et al., Pflügers Arch 1987, 297:112-129). These features and the location of this group of neurones in the intermediate chemosensitive zone described by Schläfke and Loeschcke (Schläfke, Loeschcke, Pflügers Arch 1967, 297:201-220) have led to the suggestion that the activity of these neurones might be directly affected by pCO2 and/or pH of the arterial blood. This hypothesis was tested in chloralose anesthetized cats by artificial perfusion of the RVLm with the brainstem afferent afferent neurones were denervated by bilaterally dissecting the carotid sinus and vagus nerves. Either WH-T3 or renal n. were recorded as indication of sympathetic activity (SA). Perfusion with saline or Ringer solution bubbled with CO2 produced a marked increase in SA and BP. This effect was significantly smaller with solutions of the same pH, achieved with HCl, but without CO2. Unbuffered CO2-bubbled solutions were more effective in stimulating SA than buffered CO2-bubbled solutions. A linear relationship between pCO2 of the perfused solution and SA was found. During prolonged perfusion (90 s) SA returned to control level after 40 s and was reduced well below control levels after the end of perfusion. The SA response to perfusion with solutions bubbled with CO2 was unchanged after blockade of synaptic input by local microinjection of COX into the RVLm, whereas sympathoexcitatory neurones the supra- nesous SA and the supraspinal somato-sympathetic reflex from IC-T4 to WR-T3 were abolished. From these results it is concluded that sympathoexcitatory, bulbohypothalamic neurones within the RVLm are directly exccitatory to changes of arterial pCO2 and pH. (This work was supported by the DFG within the SFB 320.)

366 POSTROSTRAURAL GRADIENT OF NEURONAL SUBTYPES IN GUINEA PIG PREVENTRAL SYMPATHETIC GANGLIA.

R.L. Meckler and E.M. McLachlan

Sympathetic neurones have distinctive discharge characteristics which depend on populations of ganglion cells and their networks. Three types of neurones have been identified: phasic (P), tonic (T), and LAH long afterhyperpolarizing neurones. The present experiments assessed the distribution of these cell types in the rostral, intermediolateral (IC) and intermediolateral (IM) ganglia. Tonic neurones were found. During prolonged perfusion (90 s) SA returned to control level after 40 s and was reduced well below control levels after the end of perfusion. The SA response to perfusion with solutions bubbled with CO2 was unchanged after blockade of synaptic input by local microinjection of COX into the RVLm, whereas sympathoexcitatory neurones the supra- nesous SA and the supraspinal somato-sympathetic reflex from IC-T4 to WR-T3 were abolished. From these results it is concluded that sympathoexcitatory, bulbohypothalamic neurones within the RVLm are directly exccitatory to changes of arterial pCO2 and pH. (This work was supported by the DFG within the SFB 320.)

Intracellular recordings were made from ganglia in vitro. Neurones were classified and passive properties were determined. Synaptic and antidiastic responses were evoked by stimulation of the coeliac and/or colonic nerves. The proportion of T neurones increased progressively from CG to IM, whereas LAH neurones decreased in number towards the IMG and numbers of P neurones were relatively consistent. P neurones had higher input resistances (Rin) relative to T neurones, whereas in the caudal direction the input time constant of T neurones increased as a result of their greater Rin.

Multiple excitatory synaptic potentials (e.s.ps) were evoked in CG T neurones but not in P neurones. In contrast, e.s.ps were produced in IMG and IMG P as well as T neurones. LAH neurones normally received no CO2 input. Equivalent proportions of all three neurone types could be activated antidromically.

We conclude that the distributions and characteristics of the thymus of sympathetic neurones vary in relation to their rostrocaudal location. This may reflect the functions regulated by these neurones (phasic,vasoconstriction; tonic,matility; LAHsecretion), although this remains to be proven.

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364 pH-SENSITIVITY OF NEURONS IN SLICES OF THE RAT VENTRAL MEDULLA OBLONGATA

W. Jarolimek, U. Misgeld and H.D. Lux

Hypercapnia enhances ventilation even when peripheral chemoreceptors are denervated, however, cells in the medullary chemosensitive area are poorly characterized (E.N. Bruce J appl Physiol 62:389 1987). Therefore, the effects of extracellular pH ([H+]o) changes on neurons were investigated in slices of rat ventral medulla oblongata. Changes in [H+]o in the slice compared to changes in the actual [H+]i in the tissue as measured by H+-selective microelectrodes. pH was altered by varying the bicarbonate concentration ([HCO3-]) in the superfusion solution. In 138 pH-sensitive neurons, the discharge rate increased with increasing [H+]o and decreased with decreasing [H+]o while in 180 neurones, the discharge rate was depressed or unaffected by increasing [H+]i. Changes of only 0.01 to 0.04 pH units in either direction affected pH-sensitive neurones, but the response was always transient, lasting 1.5-10 min. The pH-sensitivity persisted in the presence of 0.5μM atropine, 20μM bicuculline and after replacing Ca2+ by Mg2+ in the superfusion solution to reduce synaptic transmission. The H+-induced response was significantly stronger with increased pCO2 than with reduced [HCO3-], and decreased during hypoxia. In conclusion, central pH-sensitive neurones respond to small [H+]o changes in both directions, the response is transient and independent from peripheral or local network synaptic inputs. The reactivity of pH-sensitive neurones, however, depends on influences of local pCO2 and pO2.
WHAT IS THE INFLUENCE OF THE SACRAL AFFERENT SUPPLY ON VESICO-SYMPATHETIC REFLEXES IN THE CAT?

A. Boczek-Puncke, H.-J. Hübner, W. Jänig, M. Michaelis

The urinary bladder receives a dual afferent innervation projecting through the pelvic nerves to the sacral spinal cord and through the hypogastric nerves to the lumbar spinal cord. Both preganglionic and postganglionic parasympathetic nerves have been shown to be critical for the bladder's function. The present study aimed to investigate the role of the sacral afferent supply in modulating vesico-sympathetic reflexes.

Experiments were performed on chloralose-anesthetized, immobilized and artificially ventilated cats. Multunit activity was recorded from strands isolated from both the deep and superficial peroneal nerves (innervating muscle and skin). Vesico-sympathetic reflexes were elicited by isometric distensions of the urinary bladder in a graded manner. In four of the experiments a sacral laminectomy was performed and during the experiments the cauda equina was cut to eliminate the sacral afferent supply leaving the lumbar vesical afferents intact.

The reactions of MVC and CVC neurones induced by distension of the urinary bladder were dramatically diminished or abolished after elimination of the sacral afferent supply. Blood pressure responses were also attenuated, although they are still significant at intravesical pressure steps above 60 mmHg. This blood pressure rise may be due to vasoconstriction in other parts of the body. We conclude that the vesico-sympathetic reflexes examined in the present experiments are almost exclusively mediated by excitation of sacral vesical afferents.

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RESPONSES OF THE ECG T-WAVE TO ELECTRICAL STIMULATION OF THE SUPERIOR LARYNGEAL NERVE

H. Dehner, O. Funcke, H.-J. Hasler, W. Jänig, M. Michaelis

Activity in sympathetic preganglionic neurones innervating the kidney, heart (Bainon et al., J Auton Nerv Syst 12, 77, 1986), and resistance vessels (Boczek-Puncke et al., J Physiol 407, 41P, 1988) is modulated within the respiratory cycle: the neurones are activated during inspiration, followed and sometimes preceded by a depression of activity during postinspiration (PI) and early inspiration (EI), respectively. Both inhibitions are probably related to excitation in respiratory interneurones in the brain stem that are active during PI and EI. Sympathetic inhibition arising from these neurones is thought to be important in the generation of the respiratory rhythm. Electrical stimulation of the afferents in the superior laryngeal nerve (SLN) activates the PI interneurones and leads to a respiratory arrest in PI (Fuenn et al., Pflügers Arch 407, 190, 1986). If the PI interneurones mediate the PI depression, the activity of these neurones might be hypothesized to be blocked by electrical stimulation of the SLN.

The study investigated the responses of preganglionic neurones projecting in the sympathetic trunk and vasoconstrictor neurones to electrical stimulation of the SLN anesthetized, immobilized and artificially ventilated cats. The stimulus strength was adjusted to abolish the phrenic nerve discharges. The activity in the sympathetic neurones was not depressed but slightly enhanced, though the respiratory modulation was abolished. This finding is in variance with the above hypothesis: the inhibition in PI is either not mediated by the PI interneurones or the afferents in the SLN have additional excitatory effects on the sympathetic preganglionic neurones via other interneurones in the medulla oblongata.

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RESPONSES OF SYMPATHETIC PREGANGLIONIC NEURONES TO ELECTRICAL STIMULATION OF THE SUPERIOR LARYNGEAL NERVE

H. Dehner, O. Funcke, H.-J. Hasler, W. Jänig, M. Michaelis

The amplitude of the T-wave (TWA) of the electrocardiogram (ECG) varies with psychological stimulation and provides important information for psychophysiological work. The present paper presents additional evidence that phase changes in TWA depend on task conditions and beta-adrenergic drugs. Three experiments were designed to test the sensitivity of TWA to manipulations in sympathetic arousal. In the first experiment TWA was recorded during an active behavioral task in which 32 subjects believed they could control the duration of an aversive white noise and during a passive behavioral task in which another 30 subjects knew they had no control. TWA decreased to a significantly greater extent in the active behavior group than in the passive group (t(60) = 3.87; p < 0.001). In the second experiment 9 subjects receiving beta-adrenergic blockers and 10 subjects receiving placebo completed the active task. TWA decreases were significantly blocked in the beta blocker group (t(17) = 2.91; p = 0.01). In the third experiment 12 subjects received placebos and 24 received one of two different beta-blockers. All subjects performed a mental arithmetic task. Subjects receiving placebos responded with a significant reduction in TWA. Beta-blockers blocked this reduction significantly (F(2,27) = 5.35; p < 0.05). Results show that TWA responds distinctly to psychological as well as to pharmacological manipulations. Since in all cases TWA changes were blocked through beta-blockade it follows that beta-adrenergic activity is related to phasic TWA responses.

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ALTERATION OF FEVER BY MANIPULATION OF NORADRENERGIC INPUT INTO THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS IN THE GUINEA-PIG

H. Ungar, G. Merker, J. Roth and E. Zeisberger

Evidence for the release of arginine-vasopressin (AVP) in the brain septum during endogenous anti-pyresis was recently reviewed (N. W. Kasting, Brain Res. Rev. 14: 143, 1989). Based on our immunocytochemical studies in guinea pigs (Merker et al., Exp. Brain Res. 45: 722, 1989) vasopressinergic terminal areas in the hypothalamic paraventricular nucleus (PVN). The PVN and other thermoregulatory structures in the hypothalamus may be influenced by aminergic afferents from the lower brain stem (Brück & Zeisberger, Pharmac Thdr 35: 163, 1987). Therefore we investigated in guinea pigs whether the febrile response to bacterial endotoxin could be altered by manipulation of the noradrenergic input into the PVN. Electrical stimulation of the PVN neurons by implanted microelectrodes reduced the febrile response to 45% of the control values (n = 11). This confirms the antipyretic role of these neurons. Chronic destruction of noradrenergic afferents to the PVN by microinjected 6-hydroxydopamine (6-OHDA) also resulted in a significant reduction of febrile responses to 36% of the control values (n = 103), whereas a microinfusion of noradrenaline into the PVN enhanced febrile responses by 39% in comparison to animals microinfused with the solvent (0.9% NaCl; n = 6). Since the febrile response of guinea-pigs was increased after microinfused noradrenaline and decreased by 6-OHDA we assume an inhibitory influence of noradrenergic brain stem afferents on the antipyretic vasopressinergic system of PVN.

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THE EFFECT OF ELECTRICAL STIMULATION OF THE ACUPUNCTURE POINT K3 ON BLOOD PRESSURE AND THE ACTIVITY OF THE RENAL SYMPATHETIC NERVE IN ANAESTHETIZED CATS

H. Gao, M. Hunold, F. Kirchner and K. Takano

Stimulation of the skin or peripheral nerves has effects on visceral organs, and this might explain the effects of acupuncture (for review see SATO and SCHMIDT, Jap. J. Physiol. 37,1-17,1987). In an attempt to combine the experiences of acupuncture and neurophysiology we studied the effect of electrical stimulation of the acupuncture point, which is used in China for controlling the blood pressure e.g. during acupuncture anesthesia. Anaesthetized cats (60 mg/kg chloralose) repetitive stimulation (1 Hz, with an intensity so that a small movement of the foot is observed) of the acupuncture point produced a decrease in blood pressure as well as in the activity of the renal sympathetic nerve. The decrease of the activity of the renal sympathetic nerve was produced by an inhibition which began 50 ms after the single acupuncture stimulus. With higher stimulus intensity a supraspinal reflex discharge was also observed which began about 90 ms after the stimulus. The effect of acupuncture on blood pressure as well as on sympathetic activity disappeared after transection of the sciatic nerve. In some experiments a minor decrease of sympathetic activity and blood pressure was observed during insertion of the acupuncture needle into the point. Stimulation on points on the leg and on the ventral skin, which are not used in acupuncture, did not produce any effect except the small movement of the neighboring muscles which was used for adjusting the stimulation intensity.

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DIFFERENT HYPOTHALAMO-SEPTAL NEUROPEPTIDE SYSTEMS PARTICIPATING IN ENDOGENOUS ANTIPYRESIS

E. Zeisberger

The febrile elevation in body temperature seems to be limited, and sometimes even prevented, by the action of endogenous antipyretic substances liberated within the brain septum during fever (cf. N. W. Kasting, Brain Res. Rev. 14:143-153, 1989). Different neuropeptides were proposed for this function by several authors working with diverse species and inducing fever mostly by i.m. injections or central release into hypothalamic sites. To assess the proposed antipyretic function of the respective neuropeptides the following experiments were made. In several series in conscious freely moving guinea pigs the febrile responses to i.m. injections of bacterial endotoxin (E. coli, 20 µg/kg) have been compared (several times in each animal at 1-week intervals) during a continuous microinfusion (0.1 µl/min for 6 h) of solutions of either arginine vasopressin, α-melanocyte stimulating hormone, angiotensin II and adrenocorticotropic hormone at nearly physiological doses (8 pmol in 6 h) or of a solvent (saline) into a site in the ventral lateral septum. Here all these substances have been found effective in suppressing the febrile responses to i.m. injections of bacterial endotoxin, except saline. Since the immunohistochemical studies revealed that all these peptides are present in the nerve fibers projecting from different hypothalamic cell nuclei into the septum, it is concluded that several neuropeptide systems participate in the control of maximum febrile temperature at about 41°C to a complete suppression of temperature increase.

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 transection of the spinal cord, disrupting the descending vasomotor drive, offers an opportunity of studying the effect of missing supraspinal influences on heart rate (HR) control. In this study we investigated the possibility that the stimulatory HR responses to hypoxia and hypercapnia would be less in the cord injured. We determined the HR responses to acute progressive isocapnic hypoxia and hyperoxic hypercapnia from the linear slopes of HR on oxygen saturation (SaO2) or on alveolar PCO2 in 16 conscious patients with chronic, complete, traumatic spinal cord transection at C4-C7, who consented to study procedure. We found that the HR responses were unattenuated: \( \Delta \text{HR}/\Delta \text{SaO2} = 0.83 \pm 0.14 \) (SE) beats/min per 1% decrement in SaO2 and \( \Delta \text{HR}/\Delta \text{PCO2} = 0.30 \pm 0.13 \) beats/min per 1 Torr increase in PaCO2. Arterial blood pressure was increased by \( \sim 10 \) Torr during both hypoxia and hypercapnia. Since pulmonary reflexes mediated by pulmonary stretch receptors are stimulatory for HR, we compared the contribution of tidal volume (VT) to HR increase in each gas condition. The \( \Delta \text{HR}/\Delta \text{VT} \) slope was 12.6 \pm 10.1 beats/min per 1 l of hypoxic VT and 6.8 \pm 2.2 beats/min per 1 l of hypercapnic VT. The higher HR response in hypoxia is therefore attributable to the function of intact baroreceptor reflexes nor to the pulmonary reflexes activated by lung hyperinflation. We conclude that supraspinal influence on HR is dispensable for the tachycardic response to chemical stimuli.

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#### HEART RATE RESPONSES TO CHEMOSENSORY STIMULI IN THE CORD INJURED

M. Pokorski, Y. Sakakibara, A. Masuda, T. Morikawa, B. Ahn, S. Takei, P-E. Pauley, and Y. Honda

The inspiratory peak activity in sympathetic nerves is usually followed by a brief silent period during the postinspiratory (PI) phase. PI activity slowly declines. This mechanism of this silent period in sympathetic nerves during the PI phase, however, is poorly understood. In chloralose-anesthetized and artificially ventilated cats sympathetic activity in the cardiac (CR) and recurrent laryngeal nerve (RLN) were recorded during hypoxia and superior laryngeal nerve (SLN) stimulation. Pretreatment with a2-antagonist RO 10-100 had no effect on the inspiratory and a marked PI component; hypoxia silenced PN and abolished the inspiratory, but not the PI activity in RLN and caused the appearance of a prominent expiratory component in RLN. CN activity loses its inspiratory peak component during hypoxia and became more irregular. Although PI activity was still present in RLN during hypoxia, the silent period in CN activity during PI phase was either abolished or greatly reduced. SLN stimulation (20-50Hz) silenced PN completely, but resulted in a tonic activity in RLN without any respiratory modulation. Prolonged SLN stimulation (up to 1-2 min) caused rapid burst increases in RLN, but not PN activity at irregular intervals. When the muscle relaxans were off it was verified that this RLN burst activity coincided with the cat swallowing. SLN stimulation abolished the respiratory modulation of CN activity and, in addition, caused slight decrease in PI activity depending on the stimulation parameters. During swallowing, when SLN stimulation caused a burst increase in RLN activity, CN activity was completely abolished, the effect being less pronounced or abolished during hypoxia.

Pathological neuronal activity during hypoxia and superior laryngeal nerve (SLN) stimulation were recorded in sympathetic preganglionic neurones in the spinal cord. The abolition of CN activity during swallowing is due to spinal post-synaptic inhibition.

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#### ALPHA2-BINDING SITES IN THE MEDULLA OBLONGATA OF MAMMALS

G. Flügge and E. Fuchs

In the medulla oblongata (MO), alpha2-adrenoceptors appear to be involved in the mechanisms regulating blood pressure since in this brain area, hypertensive rats show different alpha2-adrenoceptor characteristics from normotensive rats. To investigate whether there are species differences in the patterns and characteristics of alpha2-adrenoceptors we visualized and quantified binding of the alpha2-antagonist [3H]RAUW 67598 in the MO of the mammal monkey, the rat, and the tree shrew (Tupaia belangeri) by in vitro-autoradiography.

In tree shrews and marmosets, the n. dorsalis nervi vagi (nX), the n. tractus solitarii (NTS), the n. nervi hypoglossi, part of the n. reticularis parvocellularis, and the area of the adrenergic cell group C1 were distinctly labelled, which is in clear contrast to the rat, where binding of [3H]RAUW was only quantifiable in the NTS and nX. Scatchard analysis revealed one type of high affinity binding sites in each nucleus of the tree shrew, except the NTS where high and low affinity binding was measured. Competition experiments demonstrated that [3H]RAUW bound specifically to alpha2-adrenoceptors in all investigated areas. In the nXII and the area of group C1, [3H]RAUW binding sites also interacted with the alpha2-antagonist propranolol.

In summary, there is no species as well as regional differences with respect to the patterns of pharmacological properties of alpha2-binding sites in the MO of the mammals investigated. These different types of receptors may be related to different physiological mechanisms in the distinct medullary regions.

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#### SYMPATHETIC AND RESPIRATORY NERVE ACTIVITIES IN THE CAT DURING HYPOXIA AND SUPERIOR LARYNGEAL NERVE STIMULATION

K. Dembowsky, S. König, J. Czachurski

Pathological neuronal activity at the border of cerebral infarcts has recently gained increasing attention. As a simple model for focal brain damage we produced a small heat lesion (1 mm diameter) in the cat visual cortex. Visually evoked responses and spontaneous activity of single cells were recorded with tungsten-in-glass electrodes at different distances (0.5-2.5 mm) from the center of the lesions on postoperative days 1, 2, 7 and 30 under halothane/\( \text{N}_2\text{O}/\text{O}_2 \) anesthesia. Physiological parameters were continuously monitored throughout the experiments. No single cell activity was obtained within the lesions. The neuronal responses in the surrounding included spontaneously inactive cells only weakly responding to maximal stimuli (IS), inactive cells with spontaneous or stimulus evoked high frequency (500-1000 spikes per second) bursts [IB], and cells with maintain high frequency activity modulated by the visual stimulus (HM). Normal responses [N] were always present far from the lesion. Epileptic discharges have not been observed. During the first post-lesion day IW cells were found at 1.0 mm distance, IB and IS at 1.5 mm and N cells at 2.0 mm. A similar pattern was observed on the second day but in addition to N cells, IS and IW cells were still found at 2.5 mm. After 7d IW cells were observed at 1.0 mm, IB responses at 1.5 mm and N cells again at 2.0 mm, and 30d IW cells were observed at 0.5 mm, IS and IS cells at 1.5 mm. Histological and immunohistochemical analysis of the lesioned cortex revealed a demarcated area of coagulative necrosis accompanied by various signs of neuronal damage and glial reactions. Vasogenic edema surrounding the lesion, involvement of the underlying white matter. Interestingly, the border region of the focal cortical lesion was characterized by cells with reduced excitability as well as hyperactive cells. (Supported by DFG Ey 7/14-1).

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#### PATHOLOGICAL NEURONAL RESPONSES IN THE CAT VISUAL CORTEX AFTER FOCAL HEAT LESIONS

U.T. Eyssel and R. Schmidt-Kastner

Pathological neuronal activity at the border of cerebral infarcts has recently gained increasing attention. As a simple model for focal brain damage we produced a small heat lesion (1 mm diameter) in the cat visual cortex. Visually evoked responses and spontaneous activity of single cells were recorded with tungsten-in-glass electrodes at different distances (0.5-2.5 mm) from the center of the lesions on postoperative days 1, 2, 7 and 30 under halothane/\( \text{N}_2\text{O}/\text{O}_2 \) anesthesia. Physiological parameters were continuously monitored throughout the experiments. No single cell activity was obtained within the lesions. The neuronal responses in the surrounding included spontaneously inactive cells only weakly responding to maximal stimuli (IS), inactive cells with spontaneous or stimulus evoked high frequency (500-1000 spikes per second) bursts [IB], and cells with maintain high frequency activity modulated by the visual stimulus (HM). Normal responses [N] were always present far from the lesion. Epileptic discharges have not been observed. During the first post-lesion day IW cells were found at 1.0 mm distance, IB and IS at 1.5 mm and N cells at 2.0 mm. A similar pattern was observed on the second day but in addition to N cells, IS and IW cells were still found at 2.5 mm. After 7d IW cells were observed at 1.0 mm, IB responses at 1.5 mm and N cells again at 2.0 mm, and 30d IW cells were observed at 0.5 mm, IS and IS cells at 1.5 mm. Histological and immunohistochemical analysis of the lesioned cortex revealed a demarcated area of coagulative necrosis accompanied by various signs of neuronal damage and glial reactions. Vasogenic edema surrounding the lesion, involvement of the underlying white matter. Interestingly, the border region of the focal cortical lesion was characterized by cells with reduced excitability as well as hyperactive cells. (Supported by DFG Ey 7/14-1).

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MONOCULARLY AND BINOCULARLY EVOKED POTENTIAL FIELD
TOPOGRAPHY: EFFECTS OF RETINAL STIMULUS LOCATION AND
SPATIAL FREQUENCY
W. Schandies

Most neurones of the primate visual cortex receive input from both eyes, and we investigated electrophysiological correlates of differences between monocularly and binocularly elicited brain activity related to stimulus parameters like spatial frequency and retinal location. Electric brain activity was recorded in eighteen healthy adults from an array of 21 electrodes covering the occipital areas. Vertical black and white grating patterns of different spatial frequency were presented in the center, or lateralized to the left or right hemiretina. Computation of "global field power" determined component latency independent of the reference electrode, and topographic characteristics were examined at individual component latency using statistical comparisons between experimental conditions.

We found no effects on component latency while the strength of the potential fields was significantly larger with binocular stimuli. Significant differences occurred in the potential field distribution of brain electric activity. With 2.5 c/d topographic differences between monocularly and binocularly evoked activity were obtained showing more anterior and more lateralized potential fields with binocular stimuli. In addition, when the gratings were presented binocularly significant differences in topography were observed when low and high spatial frequency stimuli were compared confirming our earlier observations on topography of VEFs evoked by stimuli of different spatial frequency (Brain Topography, 1988, 1, 107-116). These data show that the topographical relation of evoked components to retinal location and spatial frequency is different for monocular and binocular stimuli giving further evidence that binocular information processing triggers different neuronal processes in the human visual cortex.

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EFFECTS OF DOPAMINE ON THE KINETICS OF AMINO ACID
SENSITIVE ION CHANNELS IN RETINAL HORIZONTAL CELLS OF
THE WHITE PERCH (Roccus amercianus)
K.-F. Schmidt, A. G. Knapp and J. E. Dowling

Two effects of dopamine on cone horizontal cells, both mediated by a-CaP-dependent, phosphodiesterase (PDE) inhibition, have been demonstrated: an uncoupling of the electrical junctions of the cells and a reduction in the light responsiveness. This reduction can be explained by an enhancement of the cells' response to the proposed photoreceptor transmitter, l-glutamate and kainate (Knapp & Dowling, Nature 325, 437, 1987). We have sought to determine which physiological properties of horizontal cell glutamate receptors are modified by dopamine. In single-channel recordings from cells attached patches with agonist in the patch pipette, the frequency of 5-10 pS unitary events, but not their amplitude, increased by as much as 150 % following application of dopamine. The duration of channel openings (0.7-1 ms) also increased by 20-30 %. Analysis of whole-cell current recorded during slow superfusion of voltage clamped horizontal cells with agonist with and without dopamine also indicated that dopamine increased the channel open probability but not the amplitude (6-8 pS) of unitary events. In the case of kainate, noise analysis additionally demonstrated that dopamine did not alter the number of functional channels. The power spectra of whole-cell currents elicited by glutamate or kainate consisted of two Lorentzian components with time constants near 1 and 9 ms. The contribution of the slower component increased following dopamine treatment, suggesting a change in the open-time kinetics of the channels. This effect was more pronounced for currents induced by glutamate than for those induced by kainate. We conclude that dopamine potentiates the activity of horizontal cell glutamate receptors by altering the kinetics of the ion channel so as to favor the open state.

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EFFECT OF LUMINANCE AND CONTRAST ON THE THRESHOLD OF
BINOCULAR DEPTH PERCEPTION
T. Geib and C. Baumann

Stereoscopic acuity was investigated in 11 healthy volunteers. The three-rod arrangement of Halmihitz was employed in which three equally-spaced rods are used, with the middle one made movable in and out of the plane of the other two. The middle rod was shown in one of nine different positions and the presentations were repeated in random order until each of the positions had been presented to the subject 20 times. A psychometric curve was fitted to the data by the method of probits. Thresholds were expressed as angular disparities and referred to 75 % correct responses. Points of subjective equality were also determined. Both the luminance of the rods and that of the background could be adjusted independently. This allowed fixing the contrast when the effect of luminance was studied or fixing the luminance when the effect of contrast was investigated. Observation distance was 40 cm.

Lowest thresholds (2.65 ± 0.67 sec of arc) were found for a moderate contrast of 0.5 whereas low (0.05) and high (0.95) contrast both produced higher thresholds (luminance 250 cd/m²). The differences were statistically significant. Altering the field luminance (50, 250, 1600 cd/m²) under constant contrast conditions (0.95) did not measurably influence stereoscopic acuity.

The threshold-raising effect of low contrast is attributable to a diminished visual acuity while that of high contrast may have to do with the central processing of depth signals.

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VISCUS EVOKED RESPONSES TO MOTION-REVERSAL
STIMULATION
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Direction changes of motion of a patterned visual stimulus (motion-reversal) produce visual evoked responses (VERs) which were described to be similar to the pattern-appearence VERs but they were classified as "genuine responses to changes in the motion as such" (P.G.H. Clarke, Exp Brain Res 31:156-164, 1972).

In our group of 20 repeatedly examined persons (recordings from lead O2, A1 and symmetrical lateral occipital leads 5 cm from 02) the distinct motion-reversal VERs were obtained in all cases. The average amplitude of 2 Hz horizontal motion-reversal VERs (motion velocity 23 deg/s) was significantly larger (13.1 ± 5.1 µV) than the amplitude of comparable 2 Hz pattern-reversal VERs - 8.2 ± 4.9 µV (black-white checkerboard, check size 25 ', contrast 0.95, average luminance 25 cd/m², stimulated area - central 20°). However, the shape of curves of these VERs was interindividually very different (the opposite polarities of the waves in some cases). Besides, using a low contrast non-structured stimulus (variant of random dots) the shape of the VERs to motion-reversal was changed in some persons to the form of motion-onset VERs (dominant negative peak with a latency of about 170 ms) and the maximum of response was localized over the right occipital area (as it was the case in our previous experiments with motion-onset VERs). We suppose the existence of different types of motion reversal VERs with predominance of either motion or pattern-on/off related components.

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The spatial contrast transfer function of the visual system of the pigeon was determined by recording from the optic tectum evoked potentials or the extracellular unit responses to a pattern stimulus. The spatial contrast transfer function, determined as “response function”, describes the relationship between the contrast of the pattern, whose intensity varies sinusoidally with position, and the amplitude of the response at various spatial frequencies (c/deg). The transfer function supplies an estimate of the high frequency limit, which is a measure visual resolving power.

The highest spatial frequency detectable in a visual system is limited by many factors, in particular the diffraction of light at the pupil and the anatomical spacing of the photoreceptors. The pupil factor can be controlled in experiments in a suitable way. In this paper the electrophysiologically determined high-frequency limit was compared with the theoretical resolution limit imposed by the photoreceptor mosaic. The experimental results show that the visual system of the pigeon has a high frequency limit at spatial frequency of 15.5 c/deg, which corresponds to a visual acuity of 1.9 min of arc. My attempts to relate visual acuity in the pigeon to the anatomical spacing of the photoreceptors show that the Nyquist frequency of the photoreceptor mosaic, the theoretical upper bound of the spatial resolution, agrees with measurement.

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**The High Frequency Limits in the Spatial Vision of the Pigeons.**

M. A. Pak

The specific effects of various anions that have been used for intracellular perfusion of excitable cells are poorly understood at present. Here we report the effect of anions on the dark voltage of retinal rods of the frog Rana esculenta. By means of the whole-cell patch-clamp technique we tested the effects of a number of potassium salts on the recording pipette containing a medium with potassium as the principle cation and chloride as the principle anion, a slow spontaneous hyperpolarization of the receptor potential due to a diffusional loss of cGMP and GTP was observed (Schmidt et al., Visual Neurosci. 1, 101-106, 1989). When 80% of 100 mM chloride was replaced by organic anions the speed of the hyperpolarization was diminished to 78% by acetate (mol.wt 59), to 58% by L-aspartate (mol.wt. 135), to 43% by gluconate (mol.wt. 196) or to 15% by D-aspartate. As these data indicate, the pure size of the anions may contribute to its stabilizing effects, but the different effects of L- and D-aspartate suggest an additional influence on the cell’s metabolism. To investigate this further we used various anions in combination with 1mM GTP, the precursor of the photoreceptor’s intracellular transmitter, which by itself slows down the speed of hyperpolarization to about 30%. When L-aspartate or acetate were used together with GTP, the stabilizing effects of anions and nucleotides on the dark currents were almost additive while acetate, on the other hand, diminished the stabilizing effect of GTP. Beside the effect on the dark voltage, aspartate had a profound effect on the configuration of the light responses. Replacement of the normally used HEPES-buffer by 10 mM phosphate-buffer reduced the speed of the hyperpolarization to 53%, because phosphate appears to stimulate phosphate synthesis. Accordingly, the application of GTP was less effective with than without phosphate.

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**Intracellularly Applied Organic Anions on the Dark Voltage of Isolated Retinal Rods.**

P. Jacobi, K.-F. Schmidt, G. Nöll and Ch. Baumann

In ectotherms the pineal organ contributes, in addition to its photoneuroendocrine function, to the regulation of body temperature by thermal thermoregulation. So far little is known how the pineal organ receives and transmits environment information about temperature. We report here on the effect of temperature on the neural activity of a photosensitive pineal organ maintained in flow-through organ culture conditions. Single dark-adapted achronmic ganglion cells were extracellularly recorded from the isolated pineal organ of the rainbow trout, Salmo gairdneri, while the temperature was changed between 5°C and 37°C at a rate of 1°C per min. At 5°C in darkness all ganglion cells showed spike discharge frequencies below 6/s, an irregular firing interval and the lowest spike amplitude. At increasing temperatures the impulse frequency increased almost linearly, the discharge pattern became more regular and the amplitude larger. However, increase of temperature above a certain range caused a sudden reduction, followed by an interruption of firing. Ganglion cells could be classified into two types according to this critical temperature range. The first type showed a reduction of spike activity at 20-22°C, with inhibition of firing at 24-25°C. The second type showed inhibition of firing at 29-30°C and 35-36°C, respectively. Decrease of temperature resulted in a reappearance of spike activity. The Q_{10} values for spike activity ranged between 1.8 and 2.2. The intensity-response relation displayed a distinct intensity-dependent inhibitory effect of light within an intermediate temperature range approx. between 10°C and 20°C. Measurement of spectral sensitivity at 10°C indicated slight higher values (0.4 log units) than at 25°C and the k shifted about 10nm toward longer wavelengths. The findings indicate that environmental temperature influences underlying neural activity of the photosensitive pineal organ in fishes.

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**Temperature Regulates Neural Activities in the Photoreceptive Pineal Organ of the Trout.**

M. Šabata, Ch. Martin and M. Meissl

In ectotherms the pineal organ contributes, in addition to its photoneuroendocrine function, to the regulation of body temperature by thermal thermoregulation. So far little is known how the pineal organ receives and transmits environmental information about temperature. We report here on the effect of temperature on the neural activity of a photosensitive pineal organ maintained in flow-through organ culture conditions. Single dark-adapted achronmic ganglion cells were extracellularly recorded from the isolated pineal organ of the rainbow trout, Salmo gairdneri, while the temperature was changed between 5°C and 37°C at a rate of 1°C per min. At 5°C in darkness all ganglion cells showed spike discharge frequencies below 6/s, an irregular firing interval and the lowest spike amplitude. At increasing temperatures the impulse frequency increased almost linearly, the discharge pattern became more regular and the amplitude larger. However, increase of temperature above a certain range caused a sudden reduction, followed by an interruption of firing. Ganglion cells could be classified into two types according to this critical temperature range. The first type showed a reduction of spike activity at 20-22°C, with inhibition of firing at 24-25°C. The second type showed inhibition of firing at 29-30°C and 35-36°C, respectively. Decrease of temperature resulted in a reappearance of spike activity. The Q_{10} values for spike activity ranged between 1.8 and 2.2. The intensity-response relation displayed a distinct intensity-dependent inhibitory effect of light within an intermediate temperature range approx. between 10°C and 20°C. Measurement of spectral sensitivity at 10°C indicated slight higher values (0.4 log units) than at 25°C and the k shifted about 10nm toward longer wavelengths. The findings indicate that environmental temperature influences underlying neural activity of the photosensitive pineal organ in fishes.

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P. Jacobi, K.-F. Schmidt, G. Nöll and Ch. Baumann

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A classical description of the Abney effect says that the hues of spectral lights change when white light is added to them. By means of a visual tristimulus colorimeter (GUILD-BECHSTEIN), the two chromaticity loci were determined on which the perceptual criteria "neither green nor red" or "neither blue nor yellow" held for normal trichromatic observers. Additionally, chromaticity loci were determined which obey the abstract criterion "neither positively bright nor negatively bright". Experimentally, these latter loci were measured from vectorial differences of colours that obey the perceptual criterion "heterochromatically equally bright". The three loci form curves that deviate more or less from straight lines, deviations being an expression of the Abney effect. By linearising two of them and piece-wise linearising one of them, opponent-colour triangles were obtained, with which corresponding opponent-colour spaces are associated. By construction, such opponent-colour spaces are, first of all, related to the instrumental colour space defined by the colorimeter used. By introducing a fundamental colour space, physiologically relevant spectral opponent-colour functions were derived that take some features of the Abney Effect into account.

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The vestibular sensory cells (hair cells) in the mammalian inner ear transduce acceleration of the head or acceleration due to gravity into bioelectrical and biochemical signals. A vestibular hair cell (VHC) responds to acceleration due to mechanical displacement of its sensory hair bundle. Vestibular hair cells (VHC) were isolated from the guinea pig inner ear. Using the whole cell variant of the patch recording technique a cell potential of $-63.1 \pm 5.9$ mV was measured in macular hair cells.

The angle of hair bundle displacement during VHC stimulation is thought to be determined by the energy of the stimulus and the passive micromechanical properties of the VHC and its accessory structures (e.g. endolymph fluid, otolith, cupula). They can be modelled by a mass-spring-damper system. Thus, the prevailing view is that VHCs have a passive micromechanical role in vestibular transduction with the stereocilia serving as passive plungers. By isolating living VHCs from the guinea pig inner ear, however, we have found that the cells produce spontaneous shape changes. Furthermore, depolarizing conditions evoke reversible motile responses in solitary vestibular sensory cells.

VHCs were gradually depolarized when subjected to increasing extracellular [K+]e > 25 mM K+ gluconate or K+Cl (25 - 137 mM). A decrease of [K+]e to the normal value of 5.7 mM induced a repolarization of the sensory cells. Under these conditions in 44% of the cells visible active mechanical responses were evoked. Both, type I (46%) and type II (40%) VHCs responded with a shortening when depolarized and responded with a subsequent elongation, when repolarized. The response amplitude produced by type II VHCs appeared to be smaller than that by type I cells. After elongation a cell could be restimulated to shorten. Individual VHCs were able to undergo several cycles of shortening and elongation when subjected to cycles of de- and repolarization. Detachment of the kinocilium from the hair bundle had no influence on the motile responses. Pretreatment (30 min) of VHCs in the presence of cytochalasin B or colchicine (100 gM) did not inhibit the evoked motile VHC responses.

The evoked active process, presently demonstrated, might contribute to regulate the proprioception of the hair bundle position. It could actively damp the angular motion near the equilibrium position of the displaced hair bundle for a given acceleration. Moreover an active mechanical mechanism could prevent damage to hair bundles and accessory structures by inhibiting ciliary displacements following profound stimuli.

The cellular mechanisms underlying the observed active VHC motility remain unknown. The experiments, however, give some information that the mechanical responses are controlled by the intracellular potential or related intracellular parameters. Hence, they might be induced by the receptor potential and could be electromechanically operated processes.

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DUAL RESPONSE OF XENOPUS OLFACTORY RECEPTOR CELLS UPON APPLICATION OF CAMP AND NATURAL STIMULI
D. Schild, J.A. DeSimone, S. Hallwig

It has been suggested that these channels form a complex in CAMP, at least in the ganglion cell layer. However, recent work has shown that there are two distinct types of CAMP channel, one of which is sensitive to sensory activity but the other is not. A possible explanation for this is that the two types of CAMP channel are differentially regulated by sensory input. Further studies are needed to determine whether these results can be extended to other preparations.

Mechanosensitive primary afferents innervating the urinary bladder are activated by increases in intravesical pressure evoked by both isotonic distention and isovolumetric contractions of the bladder. However, it is thought that the stimulation response curves differ under both conditions and that the excitability of afferents is modulated by parasympathetic efferents, thereby changing the bladder tone. Here, using standard techniques, we have quantitatively compared both the stimulation response of single fibres that were recorded from the dorsal root ganglion afferents of anesthetized and paralyzed cats. Intravesical pressure was continuously recorded through an apical catheter, and the bladder was either slowly filled with an infusion pump (2ml/min) or distended using a pressure reservoir. Myelinated afferent fibres had no resting activity and thresholds below 25 mmHg. At an intravesical pressure of 50 mmHg they discharged at 1.2-19.1 Hz during filling and 2.2-17.3 Hz during distension. In 22/28 units the response curves for both type of stimuli were virtually identical and only in 6 units did slow filling produce a slightly steeper curve than isometric distension. After normalization of all 28 stimulation response curves, using the discharge at 50 mmHg as a reference, there was neither a significant difference between the two groups of curves nor between the threshold distribution in both stimulation protocols. To exclude the reflex contraction of the detrusor muscle that accompanies intravesical pressure increases, 8 units were studied prior and after a complete transaction of all spinal roots distal to L6. However, this did not significantly change the average response curve of afferents with respect to the intravesical pressure, although the volume pressure curve of the bladder was flatter after rhizotomy. Only following distended vesical afferents were activated by the pressure stimulus applied. They were not spontaneously active and had significantly higher thresholds than myelinated units. Removal of stimulus tested there was no clear difference in the stimulation response curves during filling or distension. In conclusion, mechanosensitive afferents innervating the urinary bladder encode the actual intravesical pressure regardless of the initiating type of stimulus used and regardless whether the efferent parasympathetic innervation of the bladder is intact or not.

ACETYLCHOLINE- AND GABA-RECEPTORS IN OUTER HAIR CELLS OF THE GUINEA-PIG COCHLEA
A.H. Gitter, F.K. Plinkert and H.P. Zenner

In the mammalian inner-ear cochlea active mechanical processes give rise to depolarization and a sharp rising of the sound-induced vibrations of the cochlear partition. Effort nerve endings at the basal end of one type of auditory receptor cells, outer hair cells (OHC), may participate in the mediation of these processes. There is evidence for putative neurotransmitters, acetylcholine (ACh) and y-aminobutyric acid (GABA), but direct demonstration of receptor molecules was absent so far. Monoclonal antibodies allowed us to visualize epitopes of postsynaptic ACh receptors and GABA/benzodiazepine receptors at the basal end of guinea-pig OHC of all cochlear turns, but the local distribution of the receptors types was different. GABA-receptor immunoreactivity was higher in apical than in basal turns of the cochlea, whereas ACh-receptor immunoreactivity was more pronounced in basal turns. However, in isolated living OHC application of ACh or other agonists did not induce the significant depolarization expected from nicotinic ACh receptors. By contrast, simultaneous application of GABA and flunitrazepam elicited significant hyperpolarization of OHC membranes, which decreased when the drugs were removed. Whole-cell recordings with different chloride concentrations in the pipette showed an inwardly rectified chloride conductance as electrochemical basis for the observed cell membrane hyperpolarization.

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DYNAMIC COMPONENTS IN SENSORY TRANSDUCTION OF THERMAL AND ELECTRICAL STIMULI
H. Wissig, K. Schäfer and H.A. Braun

The frequency-stimulus-response of sensory receptors is generally described as a proportional-derivative (PD) transfer characteristic. A rapid step-like stimulus induces a dynamic frequency overshoot or undershoot (D) which is followed by adaptation to a new steady-state (P). In thermosensitive afferents, however, the initial dynamic phase is often followed by a second dynamic component of inverse direction. Such a biphasic response has particularly been observed in two of the most sensitive receptor populations: in cold sensitive afferents of the ampullae of Lorenzini (dog fish) and in warm afferents of boa constrictor. We examined the dynamic responses of these receptors using ramp-shaped instead of step-like stimuli. For comparison we stimulated the ampullary electroreceptors with linear current ramps. The onset of a slow temperature ramp induced a fast initial frequency change, but the receptors already adapted during the ongoing ramp. The end of the ramp evoked a second dynamic component of inverse direction. The occurrence of a second dynamic component depended on stimulus conditions and was generally more pronounced in ampullary cold afferents. It is therefore of particular interest that electrical ramps never induced a second dynamic component.

Sensory transduction of temperature stimuli obviously induced specific membrane mechanisms which are not activated by electrical stimuli. These mechanisms enable the receptor to detect only absolute temperature and temperature changes but also temperature acceleration.

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396 SOMATOSENSORY PATHWAYS FOR FLIGHT CONTROL IN BIRDS
R. Necker

It is well known that stimulation of cutaneous receptors is important for flight control in birds (M. Naegele and H. Wolke, Z. Tierpsychol. 47:293, 1978; D. Bilo and A. Bilo, Naturwiss. 65:161, 1978). Interactions between sensory and motor systems occur both at the spinal and at brainstem levels (vestibular system, cerebellum). Possible pathways of the mechanosensory system in the pigeon were studied both with electrophysiological and anatomical methods (tracer technique).

Feather follicles are richly supplied with mechanoreceptors (Herbst corpuscles, Merkel cells). In the spinal cord cutaneous mechanoreception is processed in the n. proprius (mainly lamina IV) of the dorsal horn where a distinct somatotopy could be demonstrated both with electrophysiological and with anatomical studies. N. proprius neurons of the cervical cord (wing) project to the dorsal column nuclei in the brainstem which otherwise receive a direct input from primary afferent fibers. However, some proprius neurones have descending projections also. Furthermore, lumbar n. proprius neurons (leg) seem to terminate largely in the cervical cord. This suggests that n. proprius neurones may be involved in coordination of limb movements during walk (legs) and flight (wings). There is no evidence of a direct cutaneous input to vestibular nuclei. However, the dorsal column nuclei project to the vestibular nucleus via the inferior olive thus reaching supraspinal motor systems.

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397 DISTRIBUTION OF SOMATOSTATIN IN PRIMARY ARTICULAR AFFERENTS IN CAT
U. Hanesch, B. Heppelmann, and R. Schmidt

Somatostatin (SOM), a recently isolated and characterized neuropeptide, has been demonstrated immunohistochemically in afferent nerve fibers of various tissues and predominantly in small dorsal root ganglion cells giving probably rise to unmyelinated and thinly myelinated fibers. The knee joint of the cat is also mainly innervated by fine afferent nerve fibers: in the medial articular nerve (MAN) 70% belong to Group IV and 21% to Group III (Heppelmann et al, Somatosens Res 5, 273-281, 1988). It could be shown that a portion of MAN neurons contains the peptides substance P and calcitonin gene-related peptide (CGRP) (Hanesch et al, Europ J Neurosci Suppl 2, 69, 1989). The aim of this study was to investigate whether SOM also occurs in primary afferent perikarya of the MAN.

For retrograde tracing the MAN was cut and exposed to the fluorescent dye Fast blue. After 4 days the corresponding dorsal root ganglia were treated with colchicin. After a further day the cats were fixed by perfusion, the ganglia were cut in 50 #m sections and fluorescent cell bodies were photographed. After visualizing SOM-like immunoreactivity with the PAP-method, the percentage of peptide containing articular neurons was determined by comparing the photographs of labelled perikarya with the immunopositive cells. A morphometrical analysis followed.

After exposure of the MAN with Fast blue, labelled perikarya were found in dorsal root ganglia of segments L5 and L6. Their soma size were in a range from 26 to 88 #m. About 21% of these perikarya showed a SOM-like immunoreactivity. Their diameter ranged from 30 to 50 #m.

Conclusion. Somatostatin has been shown to be present in dorsal root ganglia cells of the MAN. Their diameter distribution suggests that SOM is nearly exclusively contained in small sized neurons, which are thought to give rise to group III and IV nerve fibers. In addition to the neuropeptides substance P and CGRP, SOM was shown to be a further candidate probably acting as a neurotransmitter or -modulator in articular afferent neurons.

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398 INFLUENCE OF LEUKOTRIENE D4 ON THE DISCHARGES OF SLOWLY CONDUCTING AFFERENT UNITS FROM NORMAL AND INFLAMED MUSCLE IN THE RAT
S. Mense and U. Hoheisel

Previous experiments have shown that in carrageenan-inflamed muscle of cats and rats, many group III and IV muscle receptors exhibit signs of a sensitization (increase in resting discharge and/or decrease in mechanical threshold). Among other mediators the leukotrienes (LTs) are currently discussed as possible candidate substances that might elicit these changes.

In the present study, the impulse activity of single muscle receptors with group III and IV afferent fibers (conduction velocities 0.5-5.4 m/s) was recorded in anaesthetized SD rats. All the afferent units had mechanosensitive receptive fields (RFs) in the gastrocnemius-soleus muscle. The RFs were stimulated with quantitative mechanical stimuli and the response magnitudes determined before and after infiltration of the RF with 100 ng/L LTD4 in 0.1 ml Tyrode solution.

Changes in the resting discharge of the units were not observed following LTD4 application. However, receptors in normal muscle exhibited a decrease in response magnitude under the influence of LTD4. The LTD4 effect became significant 30 min after injection of LTD4 and was present in both low- and high-threshold mechanosensitive units. In the mechanically induced responses of the receptors in inflamed muscle such changes were not observed. The results suggest that LTD4 has a desensitizing rather than a sensitizing action on muscle receptors.

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STIMULATION OF UNMYELINATED PRIMARY AFFERENTS INCREASES BLOOD FLOW IN SKIN AND SPINAL CORD

M. Kollenburg*, G.P. Lewis, S.B. McMahon

Activation of unmyelinated primary afferents produces vasodilatation and plasma extravasation in the skin. Here, we have asked whether C-fibre stimulation precipitates the same phenomena in the spinal cord. In anaesthetized (1.25 g/kg urethane, i.p.) rats, the sciatic nerve was stimulated supramaximally (4mA, 1ms). Rat spinal cord and skin blood flow increased in response to C-fibre stimulation by 6.7+3.1#g/g. The systemic blood pressure and the contralateral skin temperature remained constant. These responses were almost totally abolished by topical application of capsaicin (10^-5M) or TTX (10^-7M). The results demonstrate that C-fibres evoke a sensitization to heat. Interactions between pH and those mediators remain to be elucidated.

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PATIENTS

We have previously demonstrated an inability of atopic dermatitis (AD) patients to distinguish different levels of intradermally applied histamine concentrations as shown by their diminished vascular reactions and itch responses. Since the results indicated an impaired function of small cutaneous nerve fibers, we conducted a further study on skin reactions and on sensations of itch and burning pain after intradermal injection of substance P (SP) and topical application of mustard oil (MU) in 20 AD patients and 20 healthy controls. As in the previous study we measured the change in skin blood flow with a Laser Doppler Flowmeter (LDFM), quantified the sizes of wheel and flare responses and assessed itch and pain sensations by verbal report on a category partitioning scale. SP evoked dose-dependent wheel and flare reactions. Stronger SP stimuli elicited smaller flares and weaker itch ratings compared to those of the controls. However, identical wheel reactions in both groups indicate undisturbed direct plasma-extravasation due to SP in AD. In contrast, the indirect effect of SP, release of histamine from cutaneous mast cells, which induces flare and itch sensations, is diminished in AD. In both groups MU elicited similar inflammatory reactions, seen as erythema and increase of skin blood flow by LDFM measurements. However, burning pain sensations were significantly delayed in AD patients, although these patients show a decreased skin barrier function. The observed diminished flare, itch, and burning pain responses may result from histamine receptor down-regulation in AD patients impeding the "cascade reaction".

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This study was supported by DFG grants Ha 831/8 and He 1576/1

SENSITIZATION AND SELECTIVE EXCITATION BY PROTONS OF NOCICEPTIVE NERVE ENDDINGS IN RAT SKIN, IN VITRO

K.H. Steen, F. Anson, P.W. Reeh and H.O. Handwerker

In ischemic and inflamed regions pH levels down to 5.4 have been measured (Höber: Archiv f. klin. Chirurgie 156, 1929, 20–42) and this may explain the increased sensitivity of pain and hyperalgesia in these regions. In a skin biopsy of human temporal nerve in vitro preparation, receptive fields of identified primary afferents were superfused at the corium side with CO₂ saturated synthetic interstitial fluid (pH 6.1). During a period of superfusion with a CO₂ phosphate buffered carbogen gassed SIF at acid pH levels down to 4.2 (duration 5 min; interval 10 min). Mechanical thresholds were repeatedly measured by von Frey hair stimulation.

Mechano-sensitive Aβ (n=12) and Aδ fibres (n=11) were not excited nor sensitized by acid pH levels. In 16 out of 58 mechano-heat sensitive (MH), "polymodal" fibres and 8 out of 70 mechano-heat sensitive C-fibres irregular low frequency discharge was induced. No significant difference was detected between the ipsi- and contralateral side of the lumbar spinal cord. The blood flow increases in the spinal cord are probably a passive effect following the corresponding changes of the systemic blood pressure. However, prolonged or repeated application of CO₂ or low pH induced a marked and lasting decrease (+50%) of the v.Frey thresholds in almost all C-MH fibres tested (n=16; mean before 34.8±10.9 N, after 15.9±5.3 N, p<0.001 Wilcoxon) and this occurred whether or not a fibre was excited by the acid pH. The more pronounced the higher the v.Frey thresholds had previously been (0.75 rank correlation).

This is so far the only chemical condition which in our preparation produced a sensitization to mechanical stimulation, since previously, even an extensive combination of inflammatory mediators only induced a sensitization to heat. Interactions between pH and those mediators remain to be elucidated.

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Sensitization to heat. Interactions between pH and those mediators remain to be elucidated.

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In ischemic and inflamed regions pH levels down to 5.4 have been measured (Höber: Archiv f. klin. Chirurgie 156, 1929, 20–42) and this may explain the increased sensitivity of pain and hyperalgesia in these regions. In a skin biopsy of human temporal nerve in vitro preparation, receptive fields of identified primary afferents were superfused at the corium side with CO₂ saturated synthetic interstitial fluid (pH 6.1). During a period of superfusion with a CO₂ phosphate buffered carbogen gassed SIF at acid pH levels down to 4.2 (duration 5 min; interval 10 min). Mechanical thresholds were repeatedly measured by von Frey hair stimulation.

Mechano-sensitive Aβ (n=12) and Aδ fibres (n=11) were not excited nor sensitized by acid pH levels. In 16 out of 58 mechano-heat sensitive (MH), "polymodal" fibres and 8 out of 70 mechano-heat sensitive C-fibres irregular low frequency discharge was induced. No significant difference was detected between the ipsi- and contralateral side of the lumbar spinal cord. The blood flow increases in the spinal cord are probably a passive effect following the corresponding changes of the systemic blood pressure. However, prolonged or repeated application of CO₂ or low pH induced a marked and lasting decrease (+50%) of the v.Frey thresholds in almost all C-MH fibres tested (n=16; mean before 34.8±10.9 N, after 15.9±5.3 N, p<0.001 Wilcoxon) and this occurred whether or not a fibre was excited by the acid pH. The more pronounced the higher the v.Frey thresholds had previously been (0.75 rank correlation).

This is so far the only chemical condition which in our preparation produced a sensitization to mechanical stimulation, since previously, even an extensive combination of inflammatory mediators only induced a sensitization to heat. Interactions between pH and those mediators remain to be elucidated.

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PAIN EVOKED BY INTRAVASCULAR ELECTROSTIMULATION OF SUPERFICIAL VEINS OF MAN
J.G. Arndt and W. Klement

Veins, that are innervated afferently by uniform free nerve endings, are said to respond to thermal, mechanical, and chemical stimuli. We explored what kind of sensation could be evoked by intravenous electrostimulation.

Electrical stimuli (bipolar, constant current, intensity 0 - 10 mA, pulse width 0.1 - 20 msec) were applied within a vascularity isolated segment of superficial hand veins in man (N informed and consenting physicians). We valued the subjects sensation, thresholds, and tolerance maxima of pain, and also the blocking concentration of intravenously administered procaine.

Painful sensations described as sharp and tearing at the site of stimulation had thresholds at 1.5 mA and tolerance maxima at 2.5 mA at 20 msec pulse width. These sensations were blocked by intravenous procaine concentrations of 0.6 g/ml within 5 min.

Intraluminal electrostimulation of superficial veins evokes pain in man which can be abolished by intravenous concentration of 0.8 g/ml procaine.

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CHEMOSENSITIVITY OF AFFERENT FIBRES INNERVATING THE GUINEA-PIG'S URETER IN VITRO
H. Sann, K. Hammer and Fr.-K. Pieau

The guinea-pig ureter provides a good model to study visceral afferent fibres involved in nociception. It is innervated by two types of mechanosensitive afferents: U-I units (<10%) have low thresholds and respond to contractions, whereas high threshold U-2 units (90%) are not excited by contractions (Cervero & Sann, J. Physiol. 412:245-256, 1989).

To study the chemosensitivity of these afferents electrophysiological recordings were made from small branches of the hypogastric nerve using an in vitro model. The cannulated ureter was placed into an organ bath and perfused externally and internally with oxygenated artificial interstitial fluid at 37 °C. Most mechanosensitive U-2 units were excited by intraluminal (1.3) application of at least one of the chemicals used (bradykinin: 0.1-10 µM; capsaicin: 0.03-33 µM or mustard oil). Some of these units expressed only weak response to high 1.1. pressure (>40 mmHg) but could be repeatedly excited by strong probing. The response thresholds of the units for bradykinin (0.1-1 µM) and capsaicin (<0.03 µM) were lower than those necessary to induce contractions. During chemical stimulation, however, the discharge pattern appeared to be modulated by ureteric contractions. Repeated application of 3-33 µM capsaicin resulted in a desensitization of its contractory effect and a reduction of the evoked discharges. In contrast, the chemically induced responses of the few U-1 units studied, appeared to be predominantly evoked by contractions.

The results demonstrate that like in other visceral organs most of the urine afferent fibres can be classified as polymodal receptors. In addition, chemical stimulation appears to sensitize U-2 units to mechanical stimulation.

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SERAING AND LOCAL INFLAMMATORY RESPONSES INDUCED BY APPLICATION OF CARBACHOL, DOPAMINE, 5-HT, HISTAMINE AND MUSTARD OIL TO THE SKIN IN HUMANS
W. Maserl, G. Grämer and H.O. Handwerker

Mediators of inflammation (histamine and 5-HT) and substances related to the action of prostaglandin sympathetic nerves (dopamine and the acetylcholine analog carbachol (carnamoycholine)) were compared to the effects of 20 % mustard oil in the skin of human subjects. The polar substances were insthosphoresed into the skin (4, 20 and 80 mcg for 5-HT, dopamine and carbachol, and 1 mcg for histamine, respectively) by means of a constant current stimulator (WPI A 360), the apolar mustard oil was applied in a standardized soaked filter paper to the skin surface. Inflammatory responses were measured as wheal, flare and degree of erythema (flux). The latter was measured by Laser Doppler flowmetry. 5-HT, dopamine and carbachol were equipotent. All substances induced dose-dependent inflammatory reactions and sensations. However, the qualities of sensations were quite different. The substances could be ranked from itching to burning in the order of histamine, 5-HT, dopamine and mustard oil.

Histamine predominantly induced itching and mustard oil predominantly induced burning. All other substances significantly elicited more burning/less itching than histamine and less burning/more itching than mustard oil. It is concluded that skin nociceptors may belong to groups of differential chemosensitivity, respective activation of which elicits different sensory perceptions. Tissue hormones released from sympathetic postganglionic nerve endings may possibly contribute to the early stages of inflammation in the skin.

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PROSTAGLANDIN I2 ENHANCES THE MECHANOSENSITIVITY OF FINE AFFERENTS FROM THE KNEE JOINT OF THE CAT
K. Schepelman, K. Mellingher, H.-G. Schaible and R.F. Schmidt

In the cat, an experimental arthritis of the knee joint as well as close arterial injection of inflammatory mediators, in particular Prostaglandin E2, lead to ongoing activity and sensitization to passive movements of slowly conducting articular afferents of the medial articular nerve.1

In order to study the effects of Prostaglandin I2 (PGI2), a further mediator of inflammation, extracellular single unit recordings were made from units of the medial articular nerve in cats anesthetized with xemal-chloralose. We recorded from 48 units with low conduction velocities (CV, 30 belonging to group III (CV 2.5-20 m/s) and 18 to group IV (CV below 2.5 m/s) and monitored their responses to passive movements of the knee joint. PGI2 was applied intraarterially close to the joint in concentrations from 3.0 to 30 µg per bolus injection.

We observed an excitatory effect in 50% of the group III and in 50% of the group IV units. A sensitization to passive movements occurred in 66% of the group III and 44% of the group IV units. In 9 of the units studied, a sensitizing effect took place without an excitation and 2 were excited without any sign of sensitization. We conclude that PGI2 increases the mechanosensitivity in a large proportion of articular afferents in a similar way to an artificial arthritis. Thus, PGI2 may be another important mediator of inflammation which plays a major role in generating arthritic pain.

1Schaible,H.-G. and Schmidt,R.F., J.Neurophysiol. 54 (1985): 1109; 2Schaible,H.-G. and Schmidt,R.F., J.Physiol. 403 (1988): 91.

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PRIMARY NOCICEPTIVE AFFERENTS IN MONKEY
THAT MAY SIGNAL FIRST PAIN
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First pain to a stepped heat stimulus is perceived within 0.5 s of stimulus onset and is characterized by a sharp pricking sensation. The myelinated fibers that signal first pain sensation should therefore have a short activation latency and respond with a high discharge rate at the stimulus onset.

We studied the response properties of 39 A-fiber nociceptors (AMHs) that were sensitive to mechanical and to heat stimuli. Standard teased-fiber techniques were used to record from single AMHs that innervated the hairy skin of pentobarbital anesthetized monkey. Radiant heat stimuli with a rise time of 0.1 s were delivered to the receptive field by a laser thermal stimulator. AMHs were classified based on the response to a 30 s, 53°C stimulus. Two parameters were evaluated: the latency to first action potential and the latency to peak discharge.

Three distinct types of responses were observed: One group of AMHs had a mean activation latency of 124 ms, a peak discharge rate of 1000 spikes per s, a mean conduction velocity in the slow Aδ range (16 ± 3 m/s, mean and s.e.m., n=12). Another group (n=14) had longer activation latencies (370 ms) and a peak discharge close to the end of the stimulus. The third group did not respond to short (1 s) heat stimuli up to 53°C. Activation latencies to the 30 s, 53°C stimulus were between 5 and 29 s, and the mean conduction velocity was 28 ± 4 m/s (n=11). This group may more appropriately be considered as high-threshold mechanoreceptors that sensitize to heat.

In conclusion, AMHs can be classified into three distinct groups based on their heat response properties. One group (previously termed type 2 AMHs) has properties expected of fibers that signal first pain sensation.

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FORCED CHOICE TESTING AND METHOD OF LIMITS - IS THERE A SUBSTANTIAL DIFFERENCE IN MEASURING RESULTS?
D. Claus, B. Neundörfer

Thermal testing was carried out in 55 healthy subjects in order to establish normal results. A modified Marstock thermode was used and tests were repeated on three consecutive days in order to investigate reproducibility of warm and cold thresholds. The variability of different testing procedures was investigated in 33 patients with diabetes mellitus but without disabling polyneuropathy. Measuring procedures were carried out at the right ankle in random order.

Forced choice testing takes 6 times longer than the method of limits; however, the reliability and variability of the results are not considerably different between forced choice and method of limits. When performed in diabetics, forced choice testing is not more sensitive (pathological results m.limits/forced choice, warm: 7/8; cold: 12/10).

It is thought that the forced choice algorithm does not provide a method appropriate for clinical routine investigations. The limitations of the assessment of perception thresholds are not affected by painstaking forced choice testing.

This work was supported by the Marohn foundation.

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NEUROGENIC INFLAMMATION IN THE PIG'S SKIN: CAPSAICIN EFFECT AND INTERACTION WITH RUTHENIUM RED
R. Ernst, Fr.-K. Pierau and H. Sann

The pig's skin appears to be the only animal model in which the neurogenic inflammation in response to local irritants resembles the triple response reaction in humans. As in the human skin intradermal electrical stimulation produces antidromic vasodilation and flare reactions which can be measured with the laser Doppler technique and planimetry of the flare area recorded on transparent foil. The magnitude and duration of the vasodilation is directly related to the number of impulses but demonstrates a frequency optimum at about 1 Hz. The reaction depends on capsaicin sensitive peptidergic afferents since repeated previous painting with 1% capsaicin solution prevents the neurogenic vasoreaction. While the flare response to local injections (20 μl) of tachykinins and calcitonin gene-related peptides (CGRP) is only small (maximal concentration 100 μM and 10 μM, respectively) capsaicin produces dose dependent flare reactions in a threshold concentration of 300 nM, attaining areas of 10 to 20 cm² at a concentration of 3 μM, lasting for 15-30 min.

It has been demonstrated in the rat urinary bladder that ruthenium red specifically interacts with the capsaicin induced activation and desensitization of sensory nerves (Maggi et al. Neurosc Lett 88:201, 1988). Constant perfusion of an experimental blister (cantharidin, 0.4%) in the pig's skin with ruthenium red (5 μM) protects the desensitization to repeated capsaicin perfusions. The vasoreaction to capsaicin is blocked by a ruthenium red concentration of 10 μM. The results suggest that the mechanism responsible for the local effector function of different nerves containing sensory peptides is similar in different organs and species.

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PROCESSING IN AN AFFERENT THERMAL PATHWAY: RESPONSES OF COLD RECEPTORS AND HUMAN SENSATION INDUCED BY RAMP-SHAPED SPOT-LIKE STIMULI
A.M. Tick-Waider, K. Schöfer and H.A. Braun

Identical thermal stimuli were applied either to receptive fields of single facial receptors in the rat or to human single cold spots of the forearm by means of an electronically controlled thermode of 4 mm tip diameter. The stimuli were linear temperature changes of either 0.05, 0.1 or 0.5 °C/s from 34 °C adapting temperature to 24 °C and back. A single neuronal action was recorded from the infraorbital nerve; human thermal sensation was recorded continuously in arbitrary units by setting of a gliding bar.

Cold receptors responded with excitation followed by inhibition, which was only complete during warming with 0.5 °C/s. In no case the dynamic responses showed a sign of the presence of a second order component, i.e. the responses consisted only between forced choice and method of limits. When performed in diabetics, forced choice testing was not more sensitive (pathological results m.limits/forced choice, warm: 7/8; cold: 12/10).

It is thought that the forced choice algorithm does not provide a method appropriate for clinical routine investigations. The limitations of the assessment of perception thresholds are not affected by painstaking forced choice testing.

This work was supported by the Marohn foundation.

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The response properties of ampullary electroreceptors to sinusoidal electrical stimulation were studied in the eel (Ancistrurus) at constant solutions containing 10^{-4} to 10^{-2} M ouabain, which in all receptors induced excitatory responses. These consisted of a short vigorous burst immediately following the pump inhibition, which in all cases was followed by a recovery period during which background activity to temperature was obtained. There were no modifications of the burst frequency and the number of impulses per burst. During the response, both parameters persisted for a maximum of 30 seconds. The recovery period indicated that these pumps are temperature-dependent and that they may be controlled independently. The oscillation frequency attained values that increased monotonically with warmer temperatures and which were considerably greater than the control values. However, the intervals within impulse groups (bursts) were always shorter than the burst period, indicating that the process of impulse generation is not the limiting factor for the oscillation frequency. These data allow the conclusion that an electrogenic Na-K pump in fact contributes to the transducer process of cold receptors, and that this pump evidently gives rise to a depolarizing imbalance of the membrane potential, accelerating the oscillation frequency to a maximal value. Thus the oscillation frequency seems to be controlled by temperature and by membrane potential in cold receptors.

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The study was designed to find out whether somata of different size or function are distributed differentially within a spinal ganglion. Two sets of data were sampled, one concerning the size distribution of the somata along a medio-lateral axis, and another one concerning the intraganglionic location of single cells connected to hair follicle receptors and muscle spindle primary endings, respectively. The impulse activity of the latter cells was recorded intracellularly with glass micropipettes filled with Lucifer Yellow. The dye was iontophoretically injected into the cells. The somata were tested for the presence of calcitonin-gene related peptide-immunoreactivity (CGRP-IR) using the indirect PAP method. The medio-lateral size distribution showed clear differences in that the medial and lateral thirds of the ganglion contained a significantly higher proportion of large cells than the centre. The proportion of cells exhibiting CGRP-IR was smallest in the medial third.

The somata of the hair follicle receptors and muscle spindle primary endings did not show a preferred location within the ganglion. There was likewise no topographical relationship between the location of the soma within the ganglion and the location of the receptive ending in the hind-limb. Units showing CGRP-IR were missing among the cells connected to muscle spindle primary endings and were underrepresented among the hair follicle receptors.
The effects of repeated cold exposures on the relationship between skin and core temperatures (Tskin, Tcore) in control of metabolic rate (MR) were investigated in 119 experiments on three conscious goats. Tskin was manipulated by a rapidly circulating shower bath, while Tcore was controlled by intracoronary heat exchangers in a chronic arterio-venous shunt. After the relationship between Tskin, Tcore and MR had been determined by a first standard test, the animals were exposed to combinations of low Tcore and high Tskin, and vice versa. These counteracting stimuli maintained MR close to resting values. In spite of a definite cold load imposed on either the skin or the body core, after 10 to 15 exposures the standard test was repeated. The thresholds of Tcore, at which MR began to increase, were found to be significantly lowered. The second standard test was followed by 10 to 15 experiments with exposures to severe cold stress, in which Tskin and Tcore were simultaneously lowered to induce major and long lasting increases of MR. Two exposures were performed per day. No systematic differences between the responses to the first and the second exposure were observed. The subsequent third standard test revealed no significant change in threshold temperatures relative to tests I and II. The slope of MR over Tcore, however, decreased. It is concluded that repeated cold exposures without manifest shivering can induce tolerance adaptation to cold.

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CORE-TEMPERATURE RHYTHMS IN THE SHEEP AND THEIR POSSIBLE RELATIONSHIPS TO ORGANIC FUNCTIONS

E. Mohr and H. Krzywanek

Regarding the time course of core temperature during daytime, it is remarkable that the basic circadian rhythm is superimposed by oscillations of much higher frequencies (ultradian rhythms). Furthermore, the variations of these ultradian rhythms let us presume that the intensity of these rhythms is changing within daytime. Because internal as well as external factors could modify these temperature variations, different origins for the various rhythms seem to be possible.

To examine potential relationships between several rhythms and organic functions, thermoprobes were implanted at various sites in the abdomen of sheep; sheep as animals large enough to expect a temperature gradient between the different points of measurement. To minimise any influences due to measurement or experimental conditions, a telemetric system was used for registration and all animals were kept without restraint. With regard to the various intensities of the rhythms, long-time temperature records were analysed by Fast-Fourier-Transformation. Shifting the beginning of the analysis, the changes which occur during daytime could be taken into consideration.

Independent from the point of measurement and the time of day, ultradian rhythms with wave-lengths of about 140 and about 90 min were found. For these cycles, an oscillator located in the central nervous system can be supposed. Because other rhythms were not stable in time and occurred at the different points of measurement with unequal intensities, they seemed to be triggered by local processes. Especially for rhythms with wave-lengths in the range between 12- and 2.5-hours, a comparison with the used feeding-schedule points to intestinal activity as a possible origin for their variability. (Supported by DFG Mo 450/1/1)

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DIURNAL THERMAL AND ELECTROMYOGRAPHIC RESPONSES IN HUMMING-BIRDS (AMAZILIA Tzacatl, COLIBRI CORIASCANS) TO CHANGING TEMPERATURES

G. Warncke, K.-L. Schuchmann, W. Linow and F.W. Klußmann

In two neotropical hummingbird species (Amazilia tzacatl, Colibri coriascans), average body mass, 3.9 g, Colibri coriascans; 7.9 g) core temperature (Tco), electromyographical activities (EMG) and respiratory frequency were simultaneously and continuously recorded under laboratory conditions (LD 12:12, ambient temperatures (Ta): day time, 22-24°C; night time, 6-8°C; food ad lib.) over 7 to 14 consecutive days. Both trochilids revealed a taxonomic and/or body mass related regulatory pattern in all parameters studied. During the day Amazilia tzacatl exhibited average electromyographical activities of 390 μV (max. amplitude). In the larger Colibri coriascans, these values reached 470 μV during light regimes. Amazilia tzacatl did not enter torpor at night, a 'nunatak'-like state, indicated by a relatively high core temperature of about 36.2°C and by continuous muscle activity (bursts) reaching up to 280 μV. Whereas, the heavier Colibri coriascans, underwent torpor at night. Prior to this energy saving state, core body temperature dropped in this species from 42.1 to 36.3°C (shallow sleep), and decreased steadily to 8.2°C (time span required to drop from maximum to minimum core temperature: 2.5 h). This lowest temperature value was regulated by means of a sporadically appearing but then low and continuous EMG of 40 μV. During the nocturnal stages from shallow sleep to torpor Colibri coriascans lowered the respiration frequency from 120-min⁻¹ to 30-min⁻¹. Arousal from torpor to shallow sleep phase was accompanied by strong shivering (4200 μV, time span 1.5 h). Finally awaking from sleep was induced by light, after which daytime EMG-activity was resumed by bird (470 μV).

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An increasing oxidative phosphorylation coupling in rat liver and heart during the early phase of burn injury

Wang Yuemin, Chen Kaiming, Shi Ying and Shi Hanping

We have recently found an initial increase in oxidative phosphorylative coupling in succinate respiratory chain in rat-liver mitochondria during the early phase of burn injury (1).

Using glutamate + malate and d-ketoglutarate as substrates, the respiratory control ratio of liver mitochondria from rats with full skin thickness burns covering 20 per cent of body surface area were increased at 45, 60, 75 and 90 min after burn, the peak being at 75 min post-burn. The ADP/O ratio and the rate of ATP formation were also increased at about 75 min after burn and the ATP content in liver was increased at 120 min following burn. The ATP and creatine phosphate content in heart was increased at 105, 120 and 135 min post-burn respectively. A sham group acted as control. All results suggested an increased oxidative phosphorylation coupling in some organs during the early phase of burn injury.

(1) Wang X. M., Chen K. M. et al. (1986) Functional changes in rat-liver mitochondria during the early phase of burn injury. Burns 12, 461.

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Primary stenosing plaque tissue from 19 patients (age: 64 ± 12 years) and restenosing plaque tissue from 5 patients (age: 64 ± 9 years) was extracted either by a percutaneous suction technique from internal carotid arteries (extraction was done by Prof. Holting and Dr. Baueriedel, Klinikum Großhadern, Munich) or by thrombendarterectomy in case of coronary arteries (Prof. Unger, Dr. Hutter, Landesklinikum der Stadt Salzburg, Austria). The obtained plaque tissue was enzymatically disaggregated by a collagenase/elastase enzyme mixture. Isolated cells could be identified as smooth muscle cells (SMC) by positive reaction with antibodies against smooth muscle alpha-actin. Plaque derived cells were routinely cultivated in Dulbecco's Modified Eagle's medium (DMEM) and 15% fetal serum. Growth rates were determined by a cell analyzer system (CASY 1, Schärfe Systems, Reutlingen).

Growth Rates in Population Doublings/Day (PD/Day): The growth rates of SMC from primary stenosing plaque tissue (0.64 ± 0.15 PD/day) were highly increased in comparison to SMC from primary stenosing tissue (0.16 ± 0.04 PD/day, p<0.001).

The effects of Fibrinolytic Agents on SMC from Primary Stenosing Tissue: Streptokinase (Kabi), urokinase (mediac) and human plasminogen-activator (Thomae) were added 24 hours after cell seeding to SMC cultures of primary stenosing plaque tissue at the following concentrations: 1, 10, 100, and 1000 IE/mL. At each medium exchange the agents were added at the appropriate concentration. Control dishes were incubated without drugs. At clinically relevant concentrations (approximately 20 IE/mL for streptokinase and urokinase and approximately 100 IE/mL for tissue plasminogen-activator), streptokinase and urokinase had no significant effect on proliferation rates, whereas tissue plasminogen-activator caused 70% inhibition. At higher concentrations all three drugs reduced the cell proliferation rates significantly.

Conclusions: The highly increased growth rates of SMC from stenosing lesions might be the in vitro-equivalent to the rapid progression of restenosing events after angioplasty. Therefore, the influence of drugs on SMC proliferation is of clinical interest. As demonstrated by our results, all three drugs routinely used for a fibrinolytic therapy do not stimulate plaque SMC proliferation and, thus, may not be a unspecific stimulus for restenoses.

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MICHENVIRONMENT, CELL PROLIFERATION AND NECROSIS IN TUMOR SPHEROIDS
U. Karbach, S. Walenta, M. W. Grossu, M. Müller-Klieser

Multicellular tumor spheroids, cultured in vitro in stirred media, are cell aggregates that mimic the in vivo situation in malignant tumors with regard to nutrition, oxygen supply, proliferation, and differentiation (Mueller-Klieser: J. Cancer Res. Clin. Oncol. 113: 101, 1987). Histological sections show central necrotic zones surrounded by a cell rim of 150 to 250 μm thickness depending on the cell line and the growth condition. The thymidine labeling index in EMT6 spheroids with diameters < 460 μm, i.e., before the onset of necrosis, decreases from 47.4 % in superficial cell layers to 16.3 % in inner regions at a radial distance from the surface of around 100 μm. PO2 profiles obtained in EMT6 spheroids by microelectrode measurements show values decreasing from average central values of 0 mmHg only in spheroids > 800 μm in diameter. At the onset of necrosis central PO2 values are often > 50 mmHg. Furthermore, the oxygen status of these spheroids can be characterized by 31P-mesonucleonucleotide labeling of cell regions with PO2 values < 10 mmHg. The local distribution of glucose, lactate, and ATP is obtained using a bioluminescence method (Mueller-Klieser et al.: J. Natl. Cancer Inst. 80: 609, 1988). Central ATP values during spheroid growth decrease from 1.1 mM to 0.1 mM, yet the emergence of necrosis precedes this drop in ATP to very low levels. The data summarized are indicative of pronounced changes in the microenvironment of tumor cells during spheroid growth. However, the specific metabolic milieu is apparently not directly related to proliferation arrest and massive cell death in the inner regions of the aggregates. For further study in this biosphere phenomena, serumfree culture techniques for spheroids and new spheroid models with differentiated tumor cell clones have been established. Supported by the Deutsche Forschungsgemeinschaft (Az. Mu 576/2-4)

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31p NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF MURINE TUMORS P. Vaupel*, P. Okunieff*

31p nuclear magnetic resonance spectroscopy (31p-NMR), a noninvasive and nondisturbing technique which allows repetitive measurements in nonanesthetized animals has been applied to subcutaneous murine tumors. In a first attempt, tumor size dependent changes in the bioenergetic status and in the apparent intracellular pH of murine fibrosarcomas (FSAII) and mammary adenocarcinomas (MaCaV) have been investigated. In a second series of experiments, tumor energy status and pH are evaluated upon therapeutic measures (e.g., irradiation, hyperthermia) and during modulation of the metabolic status (hyperoxia, hyperglycemia). Finally, 31p-NMR spectroscopy-derived parameters have been verified in murine tumors growing in an irradiated tissue and, thus, mimicking tumor regrowth after unsuccessful radiation therapy. From these experiments there is clear evidence that tumor bioenergetics reflect the efficiency of microcirculation in these malignancies. Furthermore, there is a close correlation between tissue oxygenation and energy status suggesting that the microcirculation in these tumors yields an O2-limited energy metabolism.

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MODIFICATION OF MICROCIRCULATORY FUNCTION AND ENERGY STATUS OF SUBCUTANEOUS TUMORS BY HEAT AND/OR GLUCOSE W.-K. Mayer#, M. Stohrer*, S. Walenta*, C. Schaefer*, W. Träger#, W. Müller-Klieser*, and P. Vaupel#

The effects of hyperglycemia (12.5 mg/g BW/2.5 h i.v., 40% glucose solution) and/or local water bath hyperthermia (tumor temperatures: 42.7-44.0°C for 2 h) on microcirculatory function and energy status of s.c. rat tumors (0.4 ± 0.20 mm) were studied using laser Doppler flowmetry, HPLC techniques, standard enzymatic assays for tissue extracts, and single photon imaging with quantitative bioluminescence. Control animals were given respective volumes of a 0.9% NaCl solution (tumor temperatures: 35.5-36.2°C).

Laser Doppler flow significantly decreased in all treated tumors to about 20% of initial values. During hyperglycemia (blood glucose level: 135 mM), estimated glucose availability and tumor tissue glucose levels were elevated. Tissue ATP concentrations were unaffected. During hyperthermia, glucose availability did not change during the first hour, then finally declined below starting level. Despite relatively constant glucose levels, tumor ATP significantly dropped upon heating, though some regions with "normal" ATP levels could still be detected. Following hyperthermia/hyperglycemia, glucose availability and tissue glucose levels were elevated. Tissue ATP concentrations were significantly lower than in untreated tumors. From the results it is concluded that hyperthermia alone or in combination with hyperglycemia leads to a significant ATP drop which cannot be compensated by an increased glucose supply.

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Peripheral nerves of patients with diabetes show an abnormal resistance to ischemia. The mechanisms of this alternation are not yet clear. We have now explored whether axonal depolarization is involved, as has been proposed by several authors. For this purpose axonal superexcitability was used as an indirect measure of membrane potential. This phenomenon is potential-dependent, i.e., depolarization abolishes superexcitability (Bostock H and Grafe P, J Physiol 1985; 365: 239-257). The peroneal nerve was stimulated at the popliteal fossa; compound muscle action potentials (CAP) were recorded from m. ext. dig. brev. Superexcitability was measured as an amplitude ratio: the change in the amplitude of a test potential (30% of max. CAP) without and with a supramaximal conditioning stimulus (distance 10 - 30 ms) was observed. The susceptibility to ischemia was determined by threshold tracking (Weigl P et al., EEG and Clin Neurophysiol 1989; 73: 369-371): the strength of stimulation (0.2 ms current pulse once/s) over the peroneal nerve was adjusted by feedback to maintain a constant CAP (90% of maximum), and recorded.

Our data reveal a striking resistance to ischemia (10 min period) of the peroneal nerve in diabetic subjects (n= 11) compared with a control group (n= 14): 5 min after the onset of ischemia threshold current fell to 59.2 ± 7.1 % (mean ± S.D.) of pre-ischemic values in the control group and to 82.4 ± 7.9 % in diabetics (p < 0.001). Additionally, the post-ischemic increase of threshold current was in diabetics (117.6 ± 14.9 %) of control values significantly smaller (p < 0.001) than in the control group (200.6 ± 23.8 %). However, no difference in the post-stroke superexcitability was found between controls and diabetics. The post-stroke superexcitability (20 ms distance) was 133.5 ± 25.4 % in diabetics and 138.7 ± 26.8 % in controls (mean ± S.D.). This observation indicates that membrane depolarization is not involved in the resistance to ischemia of motor axons in diabetic subjects.

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FIRST AND SECOND PAIN IN POLYNEUROPATHY
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Cutaneous CO₂ laser stimuli (e.g. 20 ms duration, 5 mm diameter, 20 Watt) activate Aδ- and C-fibres. Aδ-fibre mediated first pain is reflected by late evoked potentials with latencies between 200 and 430 ms (EP; vertex vs. linked earlobes). These potentials were studied in 5 patients with polyneuropathy of various etiology with disturbances of mechanosensitivity and impairment of pain and temperature sense. In 4 cases the late EP was completely missing, if the laser pulses were applied to the most affected body site. In one patient the EP was missing and in 4 patients it was delayed. C-fibre mediated second pain is reflected by ultralate EP with latencies of more than 1000 ms. Since cerebral activity is focussed upon the first arriving information mediated by Aδ-fibres, in healthy men ultralate EP can be observed only with experimental A-fibre block. However, if illness has affected myelinated fibres, ultralate EP appear in (4 of 5 patients).

But, prolonged latency of late EP may only not be discussed in terms of selective disturbance of myelinated fibres, but also in terms of plasticity of spinal cord function. In conclusion, measures of EP in response to cutaneous heat pulses proved to be a sensitive tool to assess disturbances of pain and temperature sensitivity.

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THE ROLE OF GLIBENCLAMIDE-SENSITIVE POTASSIUM CHANNELS IN REGULATING HYPOXIC AND POST-ISCHEMIC VASODILATION IN ISOLATED GUINEA-PIG HEART
N. v. Beckerath, W. Maier-Rudolph, G. Mehre, K. Günther and L. Goedel-Meinen and J. Daut

The coronary arteries of isolated guinea-pig hearts were perfused at a constant rate (5-10 ml/min) with bicarbonate-buffered physiological salt solution. Coronary perfusion pressure (CPP) and isovolumetric left ventricular pressure (LVP) were measured. When the P02 of the perfusate was changed from 500 to < 10 mm Hg a decrease of CPP by about 50% was observed within 30 s, indicating a change in coronary resistance. After a 30 s period of no-flow ischemia a transient change in coronary resistance of similar magnitude was found. Both hypoxic and post-ischemic vasodilation were completely abolished in the presence of 1 μM glibenclamide (n=20 hearts), a blocker of ATP-sensitive K⁺ channels. Application of 100-500 μM cromakalim (SRL 34915), an opener of ATP-sensitive K⁺ channels, produced the same reduction of CPP as hypoxia (n=30). The effect of cromakalim could also be blocked by glibenclamide. Substances known to lower intracellular ATP (200 μM cyanide or 50 μM 2,4- dinitrophenol) also produced a glibenclamide-sensitive vasodilation (n=7). On the other hand, the change of coronary resistance produced by the endothelium-dependent vasodilator bradykinin was not affected by glibenclamide. Our results suggest that early hypoxic vasodilation in guinea-pig heart is mediated by the opening of ATP-sensitive potassium channels in vascular smooth muscle cells. This leads (1) to a hyperpolarization, (2) to a reduction of Ca influx through potential-sensitive Ca channels, (3) to a reduction of intracellular Ca2+ and thus (4) to a relaxation of coronary resistance vessels.
The existence of various cell populations with different biological properties within tumors is associated with a large variability in the response of cancers to treatment. The detection of pathophysiological parameters that may characterize such heterogeneities in each individual malignancy may contribute to an "individualization" of strategies in cancer therapy. Therefore, a method using quantitative bioluminescence and single photon imaging has been developed in our laboratory allowing for the assessment of local ATP-, glucose-, and lactate-concentrations in absolute terms with high resolution. Unlike previous procedures, the new technique includes a glass slide with a flat casting-mold that has been filled with an enzyme cocktail being kept at -20 °C. The cocktail contains enzymes to link the substrate of interest to the bioluminescent reaction of luciferase. For measurement, a cover glass with a frozen tissue cryostat section adhered to its upper side is placed upside down upon the glass with the mold, and the enzyme reactions are started by raising the temperature of the sandwich above the melting point. The concomitant light emission is registered with a microscope and an imaging photon counting system (Hamamatsu Europe, Herrsching, FRG).

The application of the technique in multicellular spheroids demonstrated a spatial resolution at the single cell level (μm) determinable and valid after HPLC and resulted in a coefficient of variation for ATP measurements with bioluminescence of 5%.

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436 EFFECTS OF ADENOSINE AND ATP ON THE MEMBRANE POTENTIAL OF CULTURED CORONARY ENDOTHELIAL CELLS ISOLATED FROM GUINEA-PIG HEART

G. Mehrke and J. Daut

The release of adenosine from hypoxic cardiomyocytes is assumed to play an important role in mediating hypoxic and post-ischemic vasodilatation in the heart. Adenosine released into the perivascular space may modulate coronary smooth muscle cells and coronary endothelial cells. However, the relative importance of these two effects is not yet clear. We have measured the effects of adenosine and ATP on the membrane potential of confluent monolayers of cultured coronary endothelial cells. At 37 °C the monolayers had a membrane potential of -20 to -40 mV. Adenosine (0.2-2 μM) produced a transient hyperpolarization of up to 20 mV which, after 1-2 min, was followed by a depolarization of up to 12 mV. The transient hyperpolarization, but not the depolarization, could be inhibited by 10 μM theophylline, an antagonist of the P1 subtype of purinoceptors. Application of ATP (0.2-2 μM) which primarily acts on P2 purinoceptors, produced an even stronger transient hyperpolarization than adenosine, but no subsequent depolarization. The effects of ATP were not reduced by 10 μM theophylline.

The transient hyperpolarization is probably due to the phosphatidylserine-mediated release of intracellular Ca and the subsequent opening of Ca-activated K channels. Since the release of prostacyclin and nitric oxide from the endothelium appears to be always associated with a rise in intracellular Ca and a transient hyperpolarization, our results suggest that both adenosine and ATP can elicit release of vasoactive substances from coronary endothelium.

437 MITOCHONDRIAL ATP-SYNTHETASE ACTIVITY IN ANOXIC CARDIOMYOCYTES

R.M. Piper, A. Koop, T. Noll

It has been suggested that in the oxygen-deficient myocardial cell the (oligomycin-inhibitable) mitochondrial ATP-synthetase turns into an ATP-hydrolase and contributes to ATP depletion. To test this hypothesis, isolated cardiomyocytes from adult rats were incubated in substrate-free Tyrode's solution (37°C, pH 7.4) at defined lowered pO2 (0.1 torr) using the "oxystat" (Biochem J 236: 765, 1985). The oxygen consumption was halfmaximal at 0.5 torr, at 0.1 torr metabolism was anoxic. When oligomycin (18 μM) was added at 0.1 torr, ATP depletion of the cardiomyocytes was accelerated by 10% and did not slow down, as expected for a prevailing mitochondrial ATP-hydrolase activity.

Conclusion: In anoxic cardiomyocytes, (oligomycin-sensitive) mitochondrial ATPase activity does not contribute to energy exhaustion. Instead, mitochondrial ATP-Synthetase is still the source for a small amount of ATP. This may be due to electron flux through complex I with a coupling to ATP-synthetase is maintained, enabled by an inverted succinate dehydrogenase reaction.

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438 TUMOR ENERGY STATUS AND GLYCOLYTIC ACTIVITY EVALUATED BY 31p-NMR SPECTROSCOPY, HPLC, ENZYMIC ASSAYS, AND SINGLE PHOTON IMAGING WITH QUANTITATIVE BIOLUMINESCENCE

C. Schaefer*, P. Okunieff+, M. Stohrer*, W. Mueller-Klieser*, and F. Vaupe1*

Energy status and glycolytic activity of s.c. marine fibrosarcomas (FSaII) were evaluated as a function of tumor size (range: 50 - 350 mm³). Bioenergetic status was evaluated in vivo by 31p nuclear magnetic resonance spectroscopy (31p-NMR). In addition, mean tissue concentrations of adenosine nucleotides (ATP, ADP, AMP) on the membrane potential of confluent monolayers of cultured coronary endothelial cells. At 37 °C the monolayers had a membrane potential of -20 to -40 mV. Adenosine (0.2-2 μM) produced a transient hyperpolarization of up to 20 mV which, after 1-2 min, was followed by a depolarization of up to 12 mV. The transient hyperpolarization, but not the depolarization, could be inhibited by 10 μM theophylline, an antagonist of the P1 subtype of purinoceptors. Application of ATP (0.2-2 μM) which primarily acts on P2 purinoceptors, produced an even stronger transient hyperpolarization than adenosine, but no subsequent depolarization. The effects of ATP were not reduced by 10 μM theophylline.

The transient hyperpolarization is probably due to the phosphatidylserine-mediated release of intracellular Ca and the subsequent opening of Ca-activated K channels. Since the release of prostacyclin and nitric oxide from the endothelium appears to be always associated with a rise in intracellular Ca and a transient hyperpolarization, our results suggest that both adenosine and ATP can elicit release of vasoactive substances from coronary endothelium.

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MUCH MICROVASCULARIZATION AND WATER RETENTION DUE TO MECHANICAL VENTILATION WITH POSITIVE AIRWAY PRESSURE IN CONSCIOUS DOGS

M. Niemans, Gabriele Kaczmarczyk, Dinah Richter, Gabrielle Korte, J. Förther and H. W. Reinhardt.

Positive airway pressure (as used in intensive care) produces sodium and water retention. The inhibition of smooth muscle cell proliferation following balloon injury in the carotid artery by the calcium-calmodulin antagonist, fendiline.

Animals receiving only the vehicle (water) had an intimal DNA content of 4.07 ± 0.17 (n=13; mean ± SD) 14 days after surgery. Fendiline (given orally) at concentrations of 40, 20 and 10 mg/kg/day displayed a dose-dependent inhibition of intimal lesion formation; 2.01 ± 0.46, 2.30 ± 0.34 and 2.70 ± 0.21 (n=6), respectively. Even at the high concentration of 40 mg/kg/day no obvious toxic side-effects could be seen during the experiment.

Of interest, endothelial regeneration of the denuded vessel was induced by fendiline 28 days after balloon injury. Furthermore, collagen types I and IV were not reduced in the neo-intima while collagen types III remained unaffected.

In conclusion, fendiline was able to inhibit specifically SMC proliferation and its production of extracellular matrix components, suggesting that it may be useful to prevent restenosis after angioplasty and bypass surgery.
IMMUNOHISTOLOGICAL STUDY OF THE INDUCTION OF STROMELYSIN AND COLLAGENASE IV IN SMC IN VITRO

R. Schreiber, J. Rupp, G. Murphy*, E. Betz, Z. Fotev and J. Fingerle

The migration of smooth muscle cells (SMC) and their excessive production of extracellular matrix are important steps for the formation of intimal thickening and vessel stenosis. This may occur if the extracellular matrix in the media is re-organized by specific SMC proteinases. We have investigated the induction of stromelysin (strom) and collagenase IV (cova) in SMC cultures. Rabbit SMC were cultured in serum-free medium (SFM) containing insulin, transferrin and thyroglobulin, following a 48 hr adaption period in SFM, cultures were incubated with various stimulants. Proteinase induction was examined by immunofluorescence after 24 hr and 72 hr after incubation. Cells positive for both or cova were counted and expressed as percentage from total cell number.

| stimulant        | strom 24 hr positive | strom 72 hr positive |
|------------------|----------------------|----------------------|
| SFM (control)    | 0%                   | 0%                   |
| SFM + PDGF (B-B) | 25 ng/ml             | 10%                  |
| SFM + PMA        | 10 ng/ml 10*         | 4%                   |
| SFM + PMA        | 10 ng/ml 10*         | 2%                   |
| II-2, TNF-α, TGF-β | no effect           | 0%                   |

Endogenous PDGF (10 ng/ml) and PDGF stimulation (10 ng/ml, 1 ml in 15 s) increased the percentage of positive cells from 3% to 10%.

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NMR-SPECTROSCOPY AND DOPPLER FLOWMETRY OF RAT BRAIN AFTER GLOBAL ISCHEMIA

O. Klüber, T. Miyazawa, M. Höhn-Berlage and K.-A. Bossmann

The relationship between post-ischemic restoration of blood flow and recovery of energy metabolism was studied in 10 rats submitted to 30 min forebrain ischemia produced by 4-vessel occlusion. Blood flow and blood volume were measured in the parietal cortex by laser Doppler flowmetry, and energy metabolism by NMR-spectroscopy, using a surface coil and a 4.7 T horizontal magnet. Animals were anaesthetized with halothane, mechanically ventilated and thermostabilized at 37°C. Ischemia was induced in the magnet, and continuous measurements were carried out for 3 h into the recirculation period. After vascular occlusion blood flow completely ceased in all animals, ATP and creatine phosphate were depleted in less than 6 min, and pH declined within 4 min. Post-ischemic recirculation was successful in 9 out of 10 rats. Blood volume measured in 9 animals consistently returned to control level within a few seconds after opening of vessels. Return of blood flow to control, in contrast, depended on blood pressure and varied between 30 sec and 29 min. Recovery of energy metabolism was flow dependent and varied accordingly.

The results demonstrate that the limiting factor in this model for post-ischemic recovery is post-ischemic blood flow which, in turn, depends on post-ischemic blood pressure. Post-ischemic reanimation, in consequence, can be greatly accelerated by stabilizing post-ischemic systemic pressure.

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ENHANCEMENT OF K⁺ CONDUCTANCE IMPROVES IN VITRO THE CONTRACTION FORCE OF SKELETAL MUSCLE FROM PATIENTS WITH HYPO- OR HYPERKALEMIC PERIODIC PARALYSIS

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An abnormal ratio between Na⁺ and K⁺ conductances seems to be the cause for the depolarization and paralysis of skeletal muscle in hypo- and hyperkalemic periodic paralysis. Recently, we have shown that K⁺ channels 'openers' hyperpolarize mammalian skeletal muscle fibers (S. Quasthoff et al., Pflügers Arch. 414:S179, 1989; see Fig. A). Now, we studied the effects of such drugs on the twitch force of muscle biopsies from normal and diseased human skeletal muscle. Specimens of fiber segments (4-6 cm long) were dissected from biceps brachii, deltoid, vastus medialis or vastus lateralis muscles under local anesthesia. The preparations were superfused at 36°C in a perspex chamber and silver plates on both sides of the chamber were used for direct ("field") stimulation (2 ms, 100 V, 0.1 Hz). Cromakalim (100 μmol/l), EMD 52692 (10 μmol/l) and pinacidil (300 μmol/l) had little effect on the twitch force of normal muscle whereas these drugs strongly improved the contraction force of fibers from patients suffering from hypo- or hyperkalemic periodic paralysis (see Fig. B). These experiments were supplemented by measurements of intracellular K⁺ and Ca²⁺ activities. The results of these recordings are in accordance with the view that cromakalim, EMD 52692, and pinacidil enhance the membrane K⁺ conductance. Gibenidolamide (1 μmol/l) completely blocked mechanical and electrophysiological effects of the K⁺ channels'openers' indicating the involvement of ATP-sensitive K⁺ channels. The data show that enhancement of membrane K⁺ conductance may have a beneficial effect in hypo- and hyperkalemic periodic paralysis.

A B

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Egg and EP Recordings in the Students Laboratory Sessions: An Approach Combining a Demonstration with PC Based Data Analysis

M. Illert, H. Wiege and U. Wolfram

We developed a teaching program which supplements a demonstration of EEG and EP recordings. For the EEG and visual EP recordings a volunteer is seated in a Paracycle cage. An EEG and chart recorder is used. Visual EPs are evoked with a switching chequered pattern on a PC monitor. The EPs are on-line averaged and shown on a PC screen. To give all students an impression of the signals and of the ongoing experiment the analog traces of the recordings (from the chart recorder or the PC screen) are displayed with aid of a video projector onto a wall. In the second part of the laboratory session 16 students share six PCs to analyze EEG and EP records. These data from healthy subjects under ideal measuring conditions resemble the demonstration experiment. Additional data deal with the different sleep phases and with pathologic potentials of an epileptic patient. After A-D conversion the data had been stored on the hard disks of the PCs from where they are loaded during the execution of the teaching program.

The EEG analysis section offers the following experiments: pick up of remote physiological signals, analysis of alpha- and beta-waves, desynchronisation and habituation of cortex activity, different sleep phases and epilepsy. The EP analysis section offers the experiments: principle of the averaging method and visual EPs in different areas of the cerebral cortex.

The experiments can be started by selecting the particular item from the main menu. An oscilloscope (EP) or a chart recorder (EEG) are simulated on the terminal. For measuring frequencies, latencies and amplitudes of the different EEG or EP components a cursor function is offered which reads relative and absolute values.

This combined approach of recording EEG and EP data from a volunteer together with the possibility of a PC based data analysis --including sleep and epilepsy-- has proved very valuable for a thorough understanding of integrative processes in the central nervous system.

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Fluid Intake and the Sympatho-Adrenomedullary System in the Guinea Pig: Influence of New Housing Conditions

M. Fenske and N. Sachser

Guinea pigs show a strong reduction of fluid intake if exposed to new housing conditions. However, if allowed to adapt to the new situation, they exhibit a normal fluid intake, irrespectively of the spout size (1.4 or 0.7 mm) of the drinking bottle (M. Fenske, Versuchstierkd. 32:119, 1989). To test whether catecholamine (CA) plasma levels are changed during fluid deprivation, adult male guinea pigs (body weight: 700-900 g) were exposed to new housing conditions for three days, having access to drinking bottles with normal (1.4 mm: group A) or unfamiliar, reduced (0.7 mm, group B) spout size.

Table: Fluid Intake and CA Plasma Levels in Guinea Pigs

| Condition     | Day 1     | Day 2     | Day 3     |
|---------------|-----------|-----------|-----------|
| Body weight   | 619±90g   | 814±85g   | 820±83g   |
| Fluid Intake  | 53±9g     | 87±25g    | 119±44g   |
| Food Intake   | 48±26g    | 41±19g    | 54±24g    |
| Norepinephrine| 0.8±1.0g  | 0.8±1.0g  | 1.1±1.0g  |
| Epinephrine   | 0.3±0.5g  | 0.2±0.2g  | 0.3±0.2g  |
| Dopamine      | 1.1±1.3g  | 0.8±0.8g  | 0.7±1.0g  |

Grp A/B: *p<0.01, **p<0.001.

The table shows that a marked loss of body weight occurred in animals of group B (p<0.01) and that both fluid and food intake were reduced. On the other hand, an increase of hematocrit values was observed in animals of group B. Surprisingly, CA plasma levels were very similar in both experimental groups, indicating that CA levels do not serve as a sensitive index for stress induced by fluid deprivation.

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Dietary and Wall Composition of Mesometrial Arteries during Longterm Estradiol Treatment and Early Pregnancy in Guinea Pigs

E. Pohl, A. Nienartowicz, and W. Moll

During pregnancy the mesometrial arteries widen and undergo extensive changes in mass and composition in order to adjust placental blood flow to fetal growth. The controlling signals are not yet identified. We have tested, in guinea pigs, whether estradiol (E2) is capable of inducing the arterial changes seen in early pregnancy. Nonpregnant guinea pigs were treated with 250 µg E2 subcutaneously. After 1, 2, 3 and 4 weeks, the animals were killed and 20 arteries were excised from the fat-free mesometrium. External and internal diameters, weight, collagen, desmosin (a measure of elastin) and DNA content were determined. The same measurements were also made in pregnant guinea pigs in the first weeks of pregnancy. E2 plasma concentration rose from 10 pg/ml before treatment to around 80 pg/ml and fell with a half time of 4 weeks. During the first 3 weeks, internal diameter, weight, length and DNA content increased 2-times, as did uterine weight. Desmosin concentration fell, while collagen concentration remained constant. Apart from collagen concentration which decreased, similar findings were obtained in the first 3 weeks of pregnancy. In the 4th week, however, no further changes of the measured variables were observed under estradiol treatment while the changes in pregnant animals accelerated 10 times.

It is concluded that most changes seen in early pregnancy can be induced by estradiol. It must be doubted, however, that estradiol can account for the arterial changes seen in later pregnancy.

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ENDOCRINE OSMOREGULATION DURING EMBRYONIC DEVELOPMENT IN THE CHICK
M. Klempt, F. Eiendorff and R. Großmann

The aim of the present study was to investigate the development of the response of arginine-vasotocin (AVT)-system to osmotic stimulation during late embryonic life.

In a first experiment blood samples were collected by heart puncture or decapitation in chick embryos after 15 to 20 days of incubation (E15-E20) and in newly hatched chicks (D1). AVT measured by RIA (in collaboration with Prof. Simon, MPI, Bad Nauheim) is first detectable at E16 (5.9 ± 2.1 pg/ml; mean ± SEM; n=5) peaks at E19 (27.3 ± 4.2 pg/ml; n=11) and drops subsequently to 10.0 ± 1.0 pg/ml (n=13) at D1. Plasma osmolality increases continuously from 279.3 ± 5.6 mosmol/kg (n=5) at E15 to 301.3 ± 1.4 mosmol/kg (n=13) at D1.

In a second experiment E18 embryos and D1 chicks were anaesthetized with urethane (3 mg/kg BW i.p.) and catheterized into the jugular vein. Blood samples were taken before, and 15 and 30 min. after osmotic challenge (0.1 ml, 1.5 M NaCl; i.p.). Plasma osmolality was increased to similar extent (10.0 vs 9.2 mosmol/kg) in E18 and D1. However, plasma AVT concentrations exceed the basal values 10 fold in D1 (3.8 vs 35.4 pg/ml) and 1.5 fold in E18 (29.8 vs 45.3 pg/ml).

We conclude that the efficiency of osmoregulation in chicks rapidly matures during late embryonic development.

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DATA-ACQUISITION AND REAL TIME EXPERIMENT CONTROL WITH THE PERSONAL COMPUTER (AT)
K. Peper

Whereas abundant software is available for Data Analysis with the Personal Computer (AT), the hardware for quick Data acquisition and real time experiment control has become very sophisticated and expensive and its development is normally beyond the possibilities of a scientist. The reason is that the AT was developed as an office instrument and is, from its structure, mostly unsuitable for real time experiments. For utmost performance it is quite often necessary to build a dedicated interface between computer and experiment. However, the AT-Bus is not well designed for this purpose, and DMA and Interrupt techniques have very poor time responses. For this reason, real time applications have to be constructed in hardware rather than in software.

What is needed is a processor with an open, quick bus structure and a well designed real time structure. This is now available as the Harris RTX 2000. A rather cheap card with this RISC-Prozessor is on the market. Here the use of this (slightly changed) one plate computer is discussed for a general purpose real time system with a quick link to the AT. Data input and output cards can easily be constructed. The CPU has 10 Mips and is directly programmed in a higher level language: FORTH. By this preprocessor arrangement it appears much more easy to optimize the equipment to the experiment without tradeoffs.

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OXIDATIVE AND REDUCTIVE ACTIVITY OF 11B-HYDROXYSTEROID DEHYDROGENASE (11B-HSD) OBTAINED FROM HUMAN PLACENTA
S Blum, H Bühler, I Lichtenstein, A Novak, H Siebe and K Hierholzer

11-B-HSD catalyses the reaction: Corticosterone(B) --> 11-B-Dehydrocorticosterone (11-DHB) in many target tissues. It is a membrane bound protein localized in the endoplasmic reticulum. In all but one available studies it was observed that 11-B-HSD exerts only oxidative activity in the trophoblastic tissue of the human placenta. Expression of reductive activity is of interest since the 11-B-HSD might serve important biological functions in the placental barrier. Expression of only oxidative activity might point to the existence of two separate enzymes, an oxidase and a reductase (dual-enzyme-hypothesis Monder, Shackleton, 1989).

In slices and homogenates of term placentas high ox activity was found, but no significant red activity. Preparation of microsomes, solubilization with 1% CHAPS and detergent extraction revealed significant red activity (Fig.). The red/ox-ratio increased from 0.003 (microsomes) to 0.06 (solubilized enzyme + detergent) and 0.27 respectively (solubilized enzyme after removal of detergent).

Conclusions: 1) Under physiological conditions 11-B-HSD is active only as an oxidase and may, thus, control the activity of glucocorticosteroid passing from maternal to fetal circulation; 2) Red-activity of the intracellularly bound enzyme was virtually absent, however, 11B-HSD can be unmasked by solubilization as an oxidoreductase system (E.C.1.1.1.46).

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