Pain: Physiologic Background and Therapeutic Consequences

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Abstract

The perception of harmful or near harmful threats is expressed and recognized by a feeling of extreme discomfort that is pain. Starting with simple reflex circuits such as contraction or flight to avoid further harm in primitive organisms, evolution provided higher organisms with a complex structure of receptors, transmitters, hormones and nervous systems in order to not only detect harmful or near harmful threat, but also to discriminate quality and intensity of a threatening event as well as to learn how to avoid further exposure and how to behave in order to allow healing and recovery after getting injured. The variety of receptors and transmitters as well as the transmitting inter-neuronal networks are extremely complex and are still not completely elucidated. What is already known of this “nociceptive networks” on the other hand, allows better understanding of the progress and treatment of pathological pain states. By analyzing structure - action relation a variety of new drugs such as receptor agonists and antagonists as well as ion-channel blockers primarily not designed for pain treatment have been proven to be useful in the control of complex pain syndromes. A better understanding of the mechanisms involved in perception and transmission of nociceptive inputs as well as peripheral and central downregulating feedback may help to create individualize analgesic treatment regimens for complicated pain syndromes.

Keywords: Pain, physiologic, therapeutic

Introduction

Pain is an essential somatic and affective discomfort with the evolutionary goal of warning and protection to maintain integrity of the body, and as a result, the survival of animals and humans, respectively. On the other hand, exposure to life-threatening molecules of plants and animals as well, provided natural selection by evolutional adaptation. As has been shown in a recent publication the resistance of the monarch butterfly (Danaus plexippus) against a cardiac glycoside incorporated in milkweed leaves is an evolutionary advantage in several aspects. Not only the butterfly but also the caterpillar of the monarch is resistant against the plant poison; but, in addition, after consumption, the glycoside is stored in the body and is poisonous for birds who would eat the butterfly or caterpillar of the monarch species. Of course birds might learn by hard way which insects are unpalatable, by trial and error. However, many poisonous insects have alarming colours as another result of evolution which warns their enemies to eat them. Scientist were able to implant the responsible genome of this resistance by CRISPR from monarch butterflies into fruit flies (Drosophila melanogaster) by doing so the fruit flies became also resistant against this specific glycoside. [1] Interestingly glycoside containing plants are geographically, coevolutionary distributed by plant herbivore interactions. [2] This example proves, that interplay of exposure and adaption will result in resistance as well as learning to avoid dangerous experience. As stated above, pain and discomfort represents one of the most important affective warning and learning stimuli. Hence, it is not surprising, then noiception is transmitted by a complex interaction of receptors and synapses which not only recognises an exposure to a threatening or traumatic incident but in addition also allows a discrimination of intensity, quality, place and sequelae of a traumatic or nearly traumatic stress to somatic, visceral and nervous tissue. As we learned from the monarch butterfly experiment, individuals may adapt and even profit from exposures to plant or animal ‘poisons’ by evolutional selection. Hence it is not surprising that a huge amount of naturally available molecules contained within plants and...
even animals have been proven to be effective ligands for receptors and may have medical importance. Analyses of structure and effect on physiological and pathophysiological mechanisms allowed on one hand to understand structure and activity relationship of these molecules and on the other hand to analyse structure and sterical configuration of receptors. Besides this, knowledge allows to detect stereospecific modalities which are responsible for the activity on distinct receptors and as consequence to synthetically construct molecules mimicking this activity with less side effects and if possible better effectivity on receptors of medical interest. When looking on drugs on the medical market, at least 70% are of herbal or animal origin.\textsuperscript{[3]}

The purpose of this review is to provide insight into nociception from receptors to transmission until recognition and also downregulatory mechanisms of pain as well actual and future possible targets for pain therapy.

Nociception

Nociception starts with the detection of a traumatic or nearly traumatic stimulus by peripheral nociceptors which are able to discriminate chemical irritants, heat, cold, touch and inflammatory mediators. The cell bodies of nociceptors are located in the dorsal root and trigeminal ganglia and have a specific morphology which is called pseudounipolar, i.e. their dendrites with peripheral- and central-directed endings arise from a common axonal trunk. Since both endings receive proteins synthesised in the cell bodies of the dorsal root ganglia via axonal transport, they are biochemically equivalent and hence the nociceptor is able to receive and send signals to the central- and peripheral-directed nerve ending as well. Only the peripheral nerve endings are equipped with specific receptors which enable discrimination of external stimuli, both nerve endings are however receptive to endogenous molecules such as lipids neurotransmitters and pH changes. Nociceptors have two different types of axons: A\textsubscript{\alpha}-fibres, which are myelinated and allow a transmission speed of about 20 m/s and C-fibres which are unmyelinated and allow a transmission speed of about 1.5 m/s (also nerve fibre types in Table 1).

When activated by noxious peripheral stimuli, afferent fibres send signals to the dorsal horn for further transmission.

\textbf{Table 1: Types of nerve fibres (Erlanger-Gasser definition)}

| Fibre type | Myeline sheath | Diameter (µm) | Transmission speed | a=afferent, e=efferent |
|------------|----------------|---------------|--------------------|------------------------|
| A\textsubscript{\alpha} | Yes | 15 | 60-120 m/s | a=Muscle spindles, e=Motor neurones |
| A\textsubscript{\beta} | Yes | 8 | 30-60 m/s | a=Mecchanoreceptors |
| A\textsubscript{\gamma} | Yes | 5 | 2-30 m/s | e=Muscle spindles |
| A\textsubscript{\delta} | Yes | 3 | 2-30 m/s | a=Thermo-nociceptive fast |
| B | Yes | <3 | 3-15 m/s | e=Sympathetic preganglionic |
| C | No | 1 | 0.25-1.5 m/s | a=Thermo-nociceptive slow, e=Sympathetic postganglionic |

\textbf{The Dorsal Horn}

The dorsal horn is structured into 6 anatomically and functionally different layers in which interneurons represent the majority of neurons [Figure 1].

The neuronal components of laminae I–II are primary afferents, interneurons and projection neurons. Interneurons are either excitatory or inhibitory; their dendrites spread locally within the spinal cord and thus regulate transmission to local projection neurons and reflex pathways in that they augment or diminish the intensity of the primary input. Excitative neurons use glutamate as their principle transmitter whereas inhibitory neurons representing about 30\%-40\% of dorsal horn neurons use GABA as principal transmitter and a significant proportion of those use additionally glycine as cotransmitter.\textsuperscript{[4]}

Projection neurons transfer information to distinct areas of the brain and are most densely present in lamina I and also but in lesser density in lamina II–VI.\textsuperscript{[5]} About 80\% of the lamina I projection neurons express the NK1 receptor which reacts to Substance P (SP) which is released by peripheral afferents. Another population of projection neurons in lamina III/IV has also NK1 receptors and receives SP transmitted signals of primary afferent neurons. Both types of projection neurons receive descendent serotonergic (inhibitory) axons from the medullary raphe nuclei. Besides the lamina III/IV projection, neurons receive synapses from local inhibitory interneurons containing GABA and neuropeptide Y\textsuperscript{[5]} [Figure 1].

Projection neurons dependent on their target region in the brain translate nociceptive inputs to cardiovascular responses (caudal ventrolateral medulla), reflex tachycardia (nucleus of the solitary tract), emotional and autonomic reactions (lateral parabrachial area), descending modulation of pain (periaqueductal grey matter), sensory discriminative and affective aspects (via thalamus).\textsuperscript{[6]}

As shown in Figure 1, primary afferents follow a specific termination within the dorsal horn laminae in that myelinated low-threshold mecanohoreceptive spread in lamina III–VI and nociceptive and thermo-receptive A\textsubscript{\delta}- and C-afferents terminate in lamina I an even more in lamina II. There are C-fibres that contain neuropeptides, for example, SP innervating deeper regions of the skin as well as various other tissues and non-peptidergic C-fibres which innervate the superficial areas of the skin. All primary afferents use glutamate as fast transmitter and hence will act excitatory on their postsynaptic recipient. Nonpeptidergic C-fibres and A\textsubscript{\delta}-hair follicle afferents can form complex communication systems in that they are presynaptic to several dendrites and additionally postsynaptic at axoaxonic synapses. Peptidergic primary afferents on the other hand have a simpler communication in that they receive only few axoaxonic synapses. Depending on the expression of receptor types such as transient receptor potential V1 (TRPV1) and Transient Receptor Potential A (TRPA1) on C-fibres expression of kinases, for example, protein kinase C (PKC) by excitatory neurons and synaptic transmitters GABA, glycine by synapses, the neurons may communicate either axially or
Radially within the layers of the dorsal horn. The complex system of neuronal components and their synaptic circuits allow communication between primary afferents as well as excitatory and inhibitory interneurons and because of its complexity is still under intense investigation. Phenomena such as primary and secondary hyperalgesia, allostynia and secondary tactile hypoesthesia are the result of interneuronal communication in the dorsal horn.

**Receptor Types**

**Transient receptor potential receptors**

This type of receptors represents nonselective cation channels consisting of six transmembrane subunits and a pore forming region between the fifth and sixth domain. The pores are formed by four homomeric or heteromeric complexes of the mentioned six transmembrane subunits [Figure 2].

TRP receptors may be activated by a wide range of physical and chemical stimuli such as heat and cold, ultraviolet-radiation, mechanical stimuli and a variety of exogenous ligands by opening the channel and allow transmembrane flux of cations such as Na⁺, K⁺ and Ca²⁺. Interestingly also endogenous lipids such as arachidonyl-glycerol-amide and arachidonylethanolamide (anandamide) well known as endogenous cannabinoids (CBs) and other eicosanoids 12-hydroxy-eicosanoic acid (12-HETE) and in addition to also various natural molecules such as capsaicin, resinitoxiferin and other hydrophobic molecules act as agonists at these receptors. Depending on their specificity, these channels are responsible for detection of external stimuli such as light, temperature, taste and pain. It is suggested that there are a lot of further functions regulated by these channels, which are however not elucidated until now. Six families of TRP-receptors are classified and further divided into subfamilies by number TRPC (1–7), TRPV (1–6), Transient Receptor Potential M (TRPM) (1–8) and TRPA (1–?), TRPP (1–3) TRPML (1–3).

Relevant for pain and temperature detection are:

- **TRPV1**: also known as vanilloid receptor (VR1); this channel type is expressed in ‘free nerve endings’ which act as nociceptors. The TRPV1 is activated by ligands such as capsaicin (Chili) and piperin (pepper) as well as high temperature and arachidonic acid metabolites such as anandamide, arachidonyl-glycerol-amide and 12-HETE giving the feeling of ‘hot’ or ‘burning.’ The channel transports Na⁺, K⁺ and Ca²⁺ as well as protons.
- **TRPV2**: this channel type is also present in nociceptors and reacts to ‘vulnerable heat’ above 50° centigrades giving the feeling of ‘intense pain and burning’
- **TRPV4**: responses to mechanical and osmotic stimuli and is accountable to induce hyperalgesia as reaction to inflammation and nerve damage
- **Transient Receptor Potential M (TRPM3)**: Form nonselective but Ca²⁺- permeable channels which may be activated by steroids such as Pregnonolone sulphate as well as heat. This heat sensation acts independently to TRPV1 receptors. Interaction with the G protein subunit of activated GPCRs by morphine or baclofen will block the responsiveness to heat. On the other hand, the opioid antagonist naloxone will increase the heat responsiveness of TRPM3 receptors. Like other TRP receptors, TRPM3 activity is also positively regulated by phosphatidylinositol phosphates which provides a potential link between TRPM3 receptors and metabotropic neurokinin receptors (NKR) such as for

![Figure 1: Unmyelinated peptidergic (orange) terminate in lamina one and connect together with myelinated Aδ-nociceptors and connect to projection neurons (red) as well as interneurons (green) located in the superficial part of lamina II. Unmyelinated nonpeptidergic nociceptors target interneurons in the deeper parts of lamina II. Finally Myelinated Aδ and Aβ-fibres terminate in another set of projection neurons (red) in lamina V. Descending serotonin-ergic denticles (blue) communicate with interneurons (green) and projection neurons (red) in lamina I and II, respectively](image1)

![Figure 2: (a) the monomers of TRP channels consists of six transmembrane monomers which in detail are different in TRPV, TRPC, TRPM and TRPA channels as far as it concerns length and form of N- and C-terminals (b) the schematic insertion of four transmembrane monomers forming a central channel](image2)
bradykinine and histamine which are essentially involved in inflammation and nociception[14]

- TRPM8: Its role as molecular transducer of cold somatosensation in humans, this receptor type is mainly expressed in a subpopulation of primary afferent neurons from the dorsal root, the Gasserian ganglion and in the nodose and geniculate ganglion of the peripheral nervous system. Responses to ligands such as menthol induce the feeling of cold thereby counteracting the transition of TRPV1 and hence is subject for development of selective ligands to use this receptor to downregulate pain
- TRPA1: Until now, the only channel type of this family reacting to various chemical irritants as well as mechanical and osmotic stimuli and hence is classified as chemical nociceptor. Dipyrdone, pyrazolone and derivatives are able to block this channels which explains at least in part the analgesic properties of these substances. [15]

**Neurokinin receptors**

These receptors are G-protein coupled (GPCR) and consist of seven loops of transmembrane helices which inside the cell are connected to the G-protein complex entailing an α-, β- and γ-subunit. The main ligands are SP and various neurokinins [Table 1]. On ligand binding, the GCPR complex dissociates into a G and a G βγ subunit through activation by guanosine triphosphate binding (GTP) [Figure 3].

G can activate various second messengers such as cyclic adenosine monophosphate (cAMP), phospholipase C (PLC), Rho GDP, etc., G βγ can activate G-protein-regulated inward rectifying potassium (K) -channels, P/Q- and N-type voltage-gated channels, phosphoinositide triphosphatkinase isoforms, PLC-isoforms and adenyl cyclase isoforms. GPCRs may also function independently of G-proteins via GPCR kinase, β-arrestin and Srcs. [16,17] In other words, depending on agonists and receptor type, these receptors have a wide range of actions. As it concerns pain and inflammation, noxious stimuli to peripheral tissue induce release or production of various factors that derive from circulation, immune cells and epithelial tissues. These factors include proteases (i.e. mast cell tryptase), nerve growth factors (NGF), peptides (bradykinin) lipids can (prostaglandins), amines (5-hydroxytryptamine [5-HT]), purines, adenosine triphosphate [ATP]), ions (protons), pressure and elevated temperature. These factors can activate several classes of receptors and channels expressed by peptidergic nociceptors, including GPCRs, TRP channels and receptor tyrosine kinases (RTKs). When activated, the nociceptors release SP and neurokinin A activating NK,Rs on endothelial cells at the capillary end stream resulting in plasma extravasation, attraction and invasion of granulocytes and calcitonin gene-related peptide (CGRP) release. The latter reacts with calcitonin like receptor (CLR), thereby dilating the arterioles with the result of hyperperfusion as a typical sign of inflammation. Nociceptive dorsal root ganglia neurons release SP, which mediates neurogenic inflammation and pain. Another SP mechanism is activation of NK,R on dorsal root ganglia and sensitising of nearby TRPV1, leading to hyperalgesia but not spontaneous pain, [18] Further, it has been shown that NK,R are activated in pain processing areas of patients with irritable bowel syndrome and irritable bowel disease; hence, NK,Rs and their ligands play an important role in the development of hyperalgesia. [19] Endocytosed NK,R marked with neurotoxins have been used for ablation NK,R expressing dorsal root ganglia to determine their contribution to nociception. [20] As a result of these findings, NKR and their activating ligands, respectively, are of increasing interest as it concerns the development of antagonists to treat inflammatory diseases of various tissues as well as acute and chronic pain. Some of these are already in clinical use [Table 2].

**Two pore domain (tandem pore) K⁺- channels**

Tandem pore K⁺-channels play an important role in maintaining resting membrane potentials in cells. They allow the passive K⁺ transport through cell membranes and thus control K⁺-homeostasis and cell volume. Physiological functions which are associated with changes of the membrane potential such as release of transmitters and hormones as well as neuronal and muscular excitability are modulated by these channels. [21] Contrary to other ion-transfer channels, they are not voltage dependent. There exist about 15 subtypes divided in several families of these channels that provide ‘background’ or ‘leak’ currents, thereby controlling cellular excitability by allowing inward or outward rectifying K⁺-flux. The channels are regulated by a variety of factors such as pH, temperature, hypoxia, poly-unsaturated fatty acids, anionic membrane crenators, general anesthetics as well as mechanical and osmotic stretch.

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**Figure 3:** Schematic function of a G-protein coupled receptors: (a) An agonist binds to the extracellular part of the receptor and couples the G-protein complex to the transmembrane helices. (b) Guanosine triphosphate is released and exchanged by guanosine triphosphate binding which releases the βγ-complex. (c) The βγ-complex couples to a ‘effector protein in case of this figure to an ion-transport-channel.
Further PKA or PKC phosphorylation following stimulation of GPCRs may also modulate some of these channels. Moreover, agents that insert in one of the leaflets of the membrane and that modify the cell shape cause modification of the activity of these channels in a way that anionic or neutral carriers open and kationic cup formers of the bilayer inhibit basal and stimulated activities.\[21] Eight of this until now from 15 isolated channels are involved in the modulation of nociception. In dorsal root ganglia as well as trigeminal ganglia, transient weak inward rectifying K\+ channel, Twik RElated K\+ channel (TREK1, TREK2), Twik RElated spinal cord K\+ channel (TRESK), Twik RElated arachidonic acid stimulated K\+ channel (TRAALK), Twik RElated acid sensitive K\+ channel have been isolated. As to their function on pain perception, TREK1, TREK2, TRAAK and TRESK represent therapeutic targets for the treatment of pain.\[22]

Degenerin/ENa channels
The name degenerin (DEG) comes from the cellular phenotype induced by mutations of the deg-1 gene and other related genes that result in selective degeneration of sensory neurons involved in touch sensitivity.\[23] In mammalian epithelia, ENaC are involved in Na\+ and water transport to provide osmotic and ionic homeostasis and thus regulating blood pressure and viscosity of pulmonary mucosal excretion. In the nervous system, these channels are involved in sensory transduction\[24,25] and are composed of several subtypes of mechanoproteins (MEC-1-10)\[26] and if composed by MEC4, MEC-10 and DEG-1 subunits are responsible to transmit mechanical and painful stimuli by mechanical loads or shear stress.\[27] This and other related ion channels of the same family have been found to be pH sensitive and renamed as acid sensitive channels (ASICs). Although the involvement of ASICs in modulation and modification of sensory neurons has been underlined in a conceptual framework,\[28] there is still uncertainty in as much ASICs, besides being modulated by pH changes, are involved in mechanosensation.\[29] ASIC levels are increased in the spinal cord during inflammation, besides brain derived nerve growth factor (BDNF) promotes ASIC expression at the cell surface; hence, it is obvious that ASICs are involved in pain processing.\[30] Besides proton activation, ASICs have been proven to react to the endogenous protein agmatine which can bind to ASICs and cause pain. On the other hand, ASICs are blocked by the mamba snail toxin mam-algin-1 with the result of reduced pain to thermal and inflammatory stimuli, which offers the possibility to analyse the structural activity of this poison for development of synthetic analgesics.\[31]

Voltage-gated sodium channels
Are transmembrane pores important for generation and conduction of action potentials. Their involvement in nociception as well as peripheral and central sensitisation of neurons has been already detected by Wall et al. in 1974\[32,33] There are nine subtypes of these channels (Na 1.1–1.9) expressed in the dorsal root ganglion of which Na 1.7 and Na 1.8 have been described to be part of neural plasticity and hence of impact in the development of sensitisation and chronification of pain.\[34] Whereas Na 1.7 channels can be blocked by tetrodotoxin, Na 1.9 channels are resistant and hence can be distinguished in experimental setups. Both channel types are 4 highly expressed in nociceptors, dorsal root ganglions and trigeminal ganglions as well. As to pathological states, Na 1.7 channels have been observed in high concentration at blind ending axons of human neuromas.\[34] Besides the levels of p38 mitogen activated protein kinase (MAPK) and extracellular signal-related kinase (ERK1/2) were also increased in these neuromas. Hence it is suggested, that NaV1.7 are modulated by MAPKs, are responsible for ectopic discharges and hence are involved in the development of neuropathic pain. Further increased levels of ERK1/2 are discussed to modulate NaV1.7 in a way, that subthreshold inputs are amplified and leading to increased neuronal sensitivity and ectopic discharge and as a result contribute to central sensitisation.

P2X-receptor channels
Are purinergic receptors which consist of seven different subunits (P2X1-7) are located at the cell surface and form ionotrophic channels. Interestingly three of such subunits can lump together to build up a central nonselective cationic pore.

Purinergic receptor channel encoded by P2RX3 gene (P2X3): The involvement of the P2X3 channel type in neural plasticity ‘increasing the gain in pain’ has been already described 2000 by Woolf and Salter.\[30] Meanwhile there is more knowledge about structure, function and pharmacological targets of these channel types. Since channels of the P2X3 homomers and P2X2/3 heteromers have been detected predominately in primary sensory unmyelinated C-fibre afferents it is strongly considered that these ATP-gated channels sensitize C-fibres and facilitate pain transmission by membrane depolarisation and increasing the Ca\+ entry. However, these channels have not only sensitising properties at the nociceptive nerve ending but also in the central terminals of dorsal root ganglia as well as trigeminal ganglia located in the lamina II of the dorsal horn of the spinal cord [Figure 1]. Here, these P2X 3 receptors are located presynaptically and facilitate release of glutamate release at the first synapse of the nociceptive system.\[35] There is evidence that upregulation of P2X3 and increase of extracellular ATP are contributing to chronic pain, but it has also been shown that also other receptors are coexpressed at C-fibres and interact functionally with P2X3 Receptors. TRPV1 receptors have facilitatory interaction with P2X3 in a
way that the latter induce phosphorylation of serine residues of the former. In order to allow receptor crosstalks, the localisation of P2X3 receptors is influenced by the co-active partner. For instance, activation of the natriuretic peptide receptor-A will suppress P2X2 function whereas sustained inactivation of the BNP/TPR-A pathway will induce migraine. The membrane location of P2X3 is also regulated by CGRP another player in trigeminal nociceptive neurons and migraine. In inflammation models, the protease-activated receptor-2 will increase P2X3 activity in DRGs by translocation of P2X3 receptors to the cell surface via PKA and PKC pathways. An important member of the ‘inflammatory soup’, PGE, modulates P2X3 activity in sensory neurons by prostanoid receptor 3 (EP3) receptor triggered cAMP/PKA pathways. As to protein kinase interaction specifically the PKC-pathway gained interest with both PKCα- and PKCε-isofoms as implicated in P2X3 regulation and hence are possible pharmacological targets for inflammatory pain.[36] The metabotropic purinergic receptors P2Y1 and P2Y2 are coexpressed with P2X3-receptors in DRGs and have also been shown to modulate receptor activity via a GPC-pathway in an inhibitory manner and thus provide a homostasis by supressing excessive Ca++ overload.

P2X4: After nerve injury microglia in the dorsal horn undergo activation not only by changing morphology, gene expression and cell number, but also increased expression of P2X4 receptors.

Cannabinoid receptors cannabidiol1 and cannabidiol2
Cannabinoid receptors are GPCR a with an orthosteric coupling site for agonists and antagonists and allosteric site for allosteric modulators.[37]

The CB1 receptor is mainly expressed in the central nervous system (CNS)[38] but also in hematopoietic cells such as lymphocytes, splenocytes and T-cells.[39] The main endogenous ligands are anandamide (N-arachidonoylthanolamid [AEA]) and 2-arachidonoylglycerol (2-AG) besides there are further endocannabinoids such as 2-arachidonyl-glycerol ether, o-arachidonoyl-ethanolamin and N-arachidonoyl-dopamine. AEA and 2-AG are of main interest since their discovery as it concerns regulation of attenuation of synaptic transmission and psychoactivity. Besides AEA and 2-AG have been shown to be involved in many physiological functions as well as pathological disorders.[39]

CB2 receptors are primarily expressed in myeloid, macrophage, lymphoid and mast cells;[40] the principle ligand for CB2 is 2-AG. According to its expression, CB2 receptors are important players in the regulation of inflammation contrary to previous beliefs there is evidence that CB2 receptors are also expressed in the CNS such as the microglia.[41]

Inflammatory soup
The inflammatory soup consists of various molecules which are released after tissue damage and represent inductors of the inflammatory cascade including hyperperfusion, capillary leakage, release of reactive oxygen species (ROS), attraction of white blood cells, changes of regional pH value, primary and secondary hyperalgesia, necrotising of damaged cells and finally also tissue repair. The impact of the various contents of the inflammatory soup as it concerns nociception in acute and persistent pain specifically has been extensively and repeatedly discussed.[7,41] A typical representative of localised and easily accessible ‘inflammatory soup’ is the content of burn blisters.[42] As to their structure, the contents of the inflammatory soup can be divided in various groups:

Neuropeptides
Bradykinin, an endogenous peptide, is a ligand for two receptors, B1 and B2. Whereas B2 represent constitutive GPC-receptors for physiological processes such as the renin-angiotensin system and the kinin–kallikrein system, the B1 receptors are expressed only under pathological conditions such as trauma, burns, shock and allergy and also endotoxins and cytokines. When activated, they increase cytosolic Ca++ levels finally resulting in acute inflammatory reaction. As result, pain sensitivity is increased by presynaptic glutamate release SP release and activation of N-methyl-D-aspartate (NMDA) receptors.

Substance P (SP) is a ligand of the NK1 receptor and thus induces neurogenic inflammation, PGE2 production, plasma leakage and sensitises NMDA receptors. It has been the first detected member of the tachykinin peptides and hence be called as ‘pioneering neuropeptide’. As also other neuropeptides SP is involved in a wide range of physiological signalling and specifically in pro-inflammatory and inflammatory processes such as p38 activation, generation of pro-inflammatory cytokines, activation of NF-xB leading via PKCβ to formation of interleukin (IL)-6 IL-8 and tumor necrosis factor-α (TNF-α) hence is an essential player in inflammation and pain processing.[18] When released by neurons of the dorsal horn, SP triggers endocytosis of NK,R,[43] this mechanism has been used to deliver toxins into NK,R-expressing dorsal horn neurons and thus suppressing hyperalgesia.[20]

CGRP: is primarily localised to C and Aδ sensory fibres which have a dual role in nociceptive afferent and vasodilative efferent function. CGRP is contained in perivascular nerves and provides thus a major link to the cardiovascular system. In patients suffering on migraine CGRP blood levels are increased also in pulmonary excretions of COPD patients.[44]

Neurotrophins
NGF is a basic neurotropic factor in embryogenesis, in the adult NGF is produced as result of tissue injury and upregulates expression of TRPV1, SP, CGRP, Nav1.8 and Nav1.9 thereby promoting nociception and hyperalgesia.

BDNF also a pronociceptive factor and content of the inflammatory soup upregulates NMDA-receptor subunit 2 B phosphorylation and inhibits K⁺-Cl⁻- Cotransporter and GABAergic inhibition.[45]

Cytokines
Cytokines are made by various cell populations but mainly by T-helper cells and macrophages with the purpose to allow
interaction and communication between cells. Dependent on the releasing cells cytokines can be further divided into lymphokines (of lymphocytes), monokines (of monocytes), chemokines (with chemotactic properties), ILs (allow interaction between leucocytes). Cytokines although different may induce similar functions dependent on the receptor cells and on the other hand may also act synergistically and antagonistically as well. They are often be produced in a cascade as one cytokine stimulates a target cell to produce a further type of cytokine. Besides cytokines may act on the producing cell itself (autocrine) or on neighbouring cells (paracrine) and in distinct circumstances also on distant cells (endocrine). Concerning pathological pain development IL-1β, IL-6 and TNF-α as contents of the inflammatory soup, have been shown to play a pivotal role.\textsuperscript{[46]}

Chemokines

There are constitutive chemokines which serve as chemo-attractants for leucocytes, generating basal leucocyte migration and inducible chemokines, which are expressed by inflammatory stimulation by IL-1, TNF-α, lipopolysaccharides or viruses. Chemokines are responsible for attraction of immune cells to the site of trauma. Chemokins react with GPCR on the surface of leucocytes and other cells and activate PLC which in further steps activates second messengers such as DAG and IP3.\textsuperscript{[47]} Hence are important players in pain processing. On the other hand, it has been shown by analysis of burn blisters contents that chemokines besides inducing and maintaining pain are also important to induce wound healing.\textsuperscript{[42]} Besides other chemokines, MIP-1α (monocyte inflammatory protein), MCP-1 (monocyte chemoattractant protein) and IL-8 are upregulated in neuroinflammation and CNS trauma.\textsuperscript{[46]}

Reactive oxygen species

ROS have strong oxidating capabilities and dependent on their tissue concentration they serve as signalling molecules that regulate cell growth adhesion between cells differentiation and apoptosis. In higher concentrations however damage proteins, cell-bilayer-constituents and DNA. The main ROS molecules are H2O2, OH-, HO2-, HOCl- and super oxide O2-. ROS are important for the clearance of pathogens and hence are produced by phagocytic cells during inflammation. If ROS are not controlled as it may happen in escalating inflammation they may lead to inflammatory tissue injury.\textsuperscript{[48]}

Neurotransmitters

Serotonin (5-HT), depending on the site of action and receptors, respectively, serotonin has excitatory (hyperalgesic) and inhibitory (analgesic) action. Serotonin generally is classified as the ‘good mood’\textsuperscript{[49,50]} and the importance in pain regulation is widely underestimated.

Glutamate is the main transmitter in the nociceptive system at peripheral inflammatory sites, besides an important transmitter in the CNS. Glutamate is an agonist for AMPA, NMDA and Kainate receptors when released by low- and high-threshold sensory afferents to activate C-fibres the glutamate receptors increase their ion-transport capacity in a self-stimulating mechanism by switching from a closed still closed but ‘released safety catch’ to a small, a middle and finally a large conductance dependent on how many of the channel homomers are reacting with the agonist.\textsuperscript{[51,52]} Kainate receptors are not involved in acute pain but facilitate development of chronic pain and hyperalgesia.\textsuperscript{[53]}

ATP is immediately released in damaged tissues and stimulates ionotropic purinoceptors (P2X1-6). Most important for nociception are the P2X3 receptors which are expressed in the small Ci-fibre nociceptors and allow Na+ inward flow and as a sequence activate various intracellular Ca++ sensitive processes which result in pain sensitisation and hyperalgesia. At presynaptic located receptors ATP induces release of glutamate. After dephosphorylation to adenosine, this may bind to Gi-PCRs for inhibitory action or to Gs-PCRs which are located peripherally and centrally as well Norepinephrine (NE) is synthesised from phenylalanine in nerve endings. It is the main transmitter of the adrenergic pathways and also like various other transmitters is stored in vesicles at nerve endings. NE receptors include α1-Gqα,-, α2-Giα,-, β-Gsα-protein coupled receptors. α1-G0α,-, β-Gis-α-receptors are predominantly located on postsynaptic neurons and when activated stimulate the cAMP/PKA and PLC/PAK pathways. α2-Gi-α-receptors are presynaptically expressed and are responsible to inhibit Ca++-influx and thus perform a retrograde inhibition of presynaptic NE release. This mechanism is an important factor in physiological downregulation of pain.\textsuperscript{[54]}

GABA (γ-amino-butyric-acid) is thee inhibitory transmitter in the CNS. GABA binds to ionotropic (GABA_A) and metabotropic (GABA_B) receptors, which are expressed in great numbers within the nervous system, the former more in the CNS and the latter more at peripheral nerve endings. When binding to the ionotropic receptors, GABA induces Cl− influx into neurons and thus reduces the membrane potential thereby hindering depolarisation with the result of an inhibitory effect. When binding to the metabotropic receptors GABA_B, the intracellular formation of cAMP is suppressed and thus phosphorylation-dependent pathways (of nociception) are inhibited.\textsuperscript{[49]}

Although dopamine is not a member of inflammatory soup contents, this neurotransmitter plays a central role in the control of systemic inflammation and sequelae to systemic inflammation. This neurotransmitter is released in response to microbial infection and endogenous danger signals and suppresses the activation of inflammmasomes which promote the maturation and release of pro-inflammatory cytokines such as IL-1B and IL-18. On the other hand when depleted together with 5-HT and NE as response to ongoing inflammatory processes, the decreased levels of dopamine will result in mood disorders such as depression.\textsuperscript{[56-58]} Hence, it seems worthwhile to mention dopamine in the list of transmitters and modulators of inflammation and pain.

Eicosanoids

Are metabolites of arachidonic acid (AA) which contains 20 carbon atoms (in greek \( \varepsilon \kappa \alpha \tau \zeta \) means 20). As for nociceptive
processing prostaglandins, thromboxanes, leucotrienes and eicosanoic acids are important players and subject of pharmacological targeting as well [Figure 3].

Prostaglandins are AA-metabolites of the cyclo-oxygenase pathway (COX1, COX-2). PGE, and PGI, are main players in the sensitisation of nociception. For PGE2 there are 4 different receptors EP1 a G-protein-coupled receptor which induces a PLC/IP3 and DAG/PKA signalling, EP2, 4 an IP are G-protein-coupled receptors which promote AC/cAMP/PKA signalling an EP, a G-protein-coupled receptor act inhibitory. Prostaglandins enhance the effects of other mediators such as 5-HT and bradykinin in a back-coupling way as well as the release of SP and CGRP. Hence, the prostaglandin pathway is until of main interest in drug development.[59]

Leukotrienes are also inflammatory mediators and are produced inside leukocytes by oxidation of AA and eicosapentaenoic acid (EPA) by arachidonate 5-lipoxigenase. As it concerns pain mediation leukotriene B4 is important for promoting the production of cytokines and besides attracting neutrophils to the site of trauma. Besides, LTB4 can cause sensitisation of nociceptors by increasing cAMP/PKA activities.[60]

**Cannabinoids the endocannabinoids**

**anandamide (arachidonylethanolamide AEA)** 
2-arachidonoylglycerol-amide (2-AG) are also products of the lipid breakdown of membrane derived AA. Cannabinoids bind to the -GPCR CB1 expressed in the CNS and supraspinal regions responsible for nociception and CB2 receptors distributed in the periphery on immune cells being a target for inflammatory pain processing. The activation of CB-Receptors inhibits the formation of cAMP and thus reduces the excitatory energy within neurons besides the activation of CB2 receptors prevent mast-cell degranulation and thus release of neuroinflammatory mediators. Both actions will suppress pain sensation.[61]

**Acidosis**

**Protons**

The value of interstitial protons is increased in pathological stress such as inflammation, expansive tumour growth and after and during hypoxia respectively. Acidosis is the result of glycolytic cell metabolism accompanied by hindered removal of acidic derivatives. Acid sensing ion-transport channels such as ASICs and VR1 are modulated by low pH values and thus the reaction to receptor typical triggers such as heat. As it is typical for the contents of the inflammatory soup, the presence of BK, PGE2 and histamine at the site of an injury will further increase intracellular Ca influx with the result of enhanced expression of VR1 and SNS-Na channels. As sequelae the rise of Na influx forces the development of action potentials causing sensitisation and even modulation of afferent nerve fibers (Woolf and Salter). Besides Protons are also modulating the inflammatory program of monocytes and macrophages in a way that the release of MCP-1 and TNF-α is reduced and COX-2 as well as IL-6 where increased. In macrophages on the other hand, the regulation of inflammatory markers by acidosis depends on the activation state but not for the release of MCP-1 and TNF-α.[62]

**Opioids**

Opioids such as the endogenous released representatives endorphin and dynorphin inhibit the release of excitatory transmitters form afferent nerve terminals. They are agonists of specific G-protein-coupled receptors which when activated inhibit the AC/cAMP activity thereby reducing the excitability of excitatory neurons. There are three receptors classified as opioid receptors these are µ-, δ- and κ-receptors. However, there are also further receptors reacting to opioids but lack the typical properties of opioid receptors these are δ-, ε-, and ζ-receptors which are now classified as opioid-like receptors.[63] Details will be discussed later on. It has to be pointed out, that as reaction to inflammation, opioid receptors are not only expressed in peripheral and CNS but also in peripheral tissues as a response to inflammation.[64]

**Peripheral nerve endings of nociceptors**

To exactly discriminate the peripheral noxious stimulus into either mechanical, chemical or thermal quality, the nociceptive neurons express specific receptors at their peripheral nerve endings. Which are receptive to heat, cold or are polymodal in a way that discrimination is not only limited to quality but also to intensity of the external stimulus, i.e. innocuous or noxious. There are certainly various subtypes of nociceptors with specific arrangements of receptors for distinct stimulus modalities but for better understanding this may be simplified to three basic representants of C-fibre endings.

**Heat**

There are specific heat nociceptors which express TRP receptors such as TRPV1-4 which are activated when the temperature reaches a specific ‘set point’. These set points are 41°C for TRPV1, 52°C for TRPV2, 33°C for TRPV3 and 27°C for TRPV 4.[65]

**Cold**

The menthol sensitive TRPM8 receptors are the main population in cold receptors; but, to differentiate various gradients of cold, it needs supportive presence and activation of additional receptor types such as NaV1.8 and 1.9 as well as potassium channels TRAAK, TREK-1[66] and TRP channels like TRPA1[59,87] and TRPC5.[86] Only this diversity of receptors grant the possibility of discrimination between cold and noxious cold.

**Polymodal**

Nonpeptidergic C-fibres have nerve endings with mechanotransduction channels of the DEG/ENaC family as well as TRAAK and TREK-1 channels.

Peptidergic C-fibres are equipped with non TRPV1 heat sensitive channels, DEG/ENaC channels as well as TRAAK, TRPV1, TRPA1 and TREK-1 channels and thus are able to differentiate incoming stimuli more selective.

**Acute pain**

When nociceptors receive a stimulus of noxious or nearly noxious intensity, this information is transferred via the already
Ilias: Pain, physiology

described anatomical and microchemical processes to the dorsal horn and further to the brain. At this pathway important physiological reactions such as withdrawal reflexes, cognitive analysis and engramming take place, in order to remember circumstances under which the painful stimulus threatened the integrity of the body. The latter is an important evolutionary step which secures the individual to avoid situations which had caused this threatening.

The cascades of neuronal processing as response to such stressors start already at the level of the dorsal horn. The results of axial and vertical neuronal communication between HT-WDR and interneurons are phenomena like primary and secondary hyperalgesia which teach the individual to avoid renewal of a painful trigger. Interestingly, the area corresponding to the direct point of the noxious event, due to inter-neuronal sensitisation will develop primary analgesia which turns up as mechanical and temperature hypersensitivity, whereas neighbouring areas as result of communication between WDR and interneurons will develop hypersensitivity against mechanical stimuli representing secondary hyperalgesia.[9] Besides, regional expression of inflammatory mediators described as inflammatory soup will induce typical signs of inflammation such as hyper-perfusion, capillary leakage, increase of regional temperature, attraction of blood cells and release of glycosaminoglycans to ensure tissue reconstruction and wound healing.[8,9] In other words, acute pain is a physiologic response to stressful events of harmful or nearly harmful intensity. Under certain conditions, however, such as in the embossing phase of children, also less harmful events such as heel lancinating or circumcision in neonates will result in long-term hypersensitivity to painful events.[10] But also in adults prolonged exposition against pain may induce ongoing hypersensitivity against painful stimuli and if accompanied by life-threatening circumstances even in posttraumatic stress disorder.[11]

The first step of sensitisation is modulation of nociceptors. This occurs as response to prolonged painful triggers, in that contents of the ‘inflammatory soup’ such as PGE2, 5-HT, Bradykinin BK, epinephrine EP, adenosine and neurotrophic factors (NGF) released during tissue damage, sensitize the nerve endings and hence the amount of depolarisation necessary to induce an action potential is dramatically reduced. These changes are under physiological circumstances reversible. A prolonged exposure to a painful stimulus however and/or damage of nerve fibre may induce modification of the nociceptors in that not only constitutive but also novel genes are expressed and lead to marked changes in the expression of transmitters, synaptic neuromodulators, ion channels, GPCRs, and growth associated and structural proteins. These changes are more permanent, and the possibility of reversal is drastically decreased or impossible.[26]

As a consequence, acute pain treatment is the most important factor in preventing a transition from physiologic to pathological manifestations of pain. Of course one of the easiest approaches is the perioperative management of pain in a surrounding of postoperative and intermediate care.[72]

**Chronic pain**

Contrary to acute pain, which under physiological conditions will disappear after successful reconstruction of damaged tissue, chronic pain represents an ongoing debilitating condition although the initial tissue damage, that initially triggered the onset of pain, seems to be resolved. Definitions of chronic pain such as pain that last longer than 3–6 months, or longer than 12 months after first appearance or pain that persist longer than experienced after healing[71] may help to describe a certain clinical condition but are not really helpful when it comes to diagnose the underlying problem. Hence, patients suffering on chronic pain states have usually a long ‘patient career’ with multiple contacts to medical inpatient and outpatient services, doctors, other healthcare providers and nonmedical health providers. The need to direct these patients into a multidisciplinary diagnostic and therapeutic treatment has been discussed since years[74] but until now even in countries with high levelled healthcare systems, patients who suffer on chronic pain need up to 2.2 years until receiving adequate diagnosis and treatment as well.[75] Possible mechanisms of chronic pain are multifold and can be divided into ‘pathological modifications’ of the nociceptive system such as collateral sprouting of nerve endings after axonal damage, invasion of dorsal root ganglia by sympathetic postganglionic fibres, unmasking of silent nociceptors in the peripheral system as well as hyperexcitability of central nervous neurons and removal of descending inhibitory activity and into causal not treatable conditions such as failed back surgery syndrome, multiple neurofibromas (M. Recklinghausen), post stroke pain, paraplegia, and erythromelalgia. Also pain as accompanying condition of malignant tumours has to be categorised as chronic pain with pain inducing factors associated with compression of nerves, vessels and visceral organs as well as side effect of chemotherapeutics.[76] Besides with pain as such, chronic pain strains the individual with an ongoing exhaustion of transmitters of suppressing systems such as endorphins, GABA, serotonin, nociception, glycine and dopamine,[57] resulting in depressive mood disorder [Figure 4].[77]

**Ascending pathway**

The nociceptive cascade starts with a noxious stimulus activating receptors at the peripheral nerve endings. A majority of these are cationic channels. In a next step of the cascade bradykinin activates phospholipases which release arachidonic acid from the cell-membrane-bilayer providing the substrate for prostaglandin and thromboxane synthesis by COX-1 and -2 as well as lipoxygenase (LOX) for synthesis of a variety of EAs, in the dorsal horn glutamate is released by nerve endings to provide further transmission via spinothalamic tract to the CNS, besides dependent on the intensity and duration of the input activates NMDA receptors by release of SP thereby augmenting transmembrane ion flux and intracellular phosphorylation cascades. At the presynaptic region, opioid receptors control transmembrane ion-transport and by doing so
Figure 4: Ascending pathway: The nociceptive cascade starts with a noxious stimulus activating receptors at the peripheral nerve endings. A majority of these are cationic channels. In a next step of the cascade, bradykinin activates phospholipases which release arachidonic acid from the cell-membrane-bilayer providing the substrate for prostaglandin and thromboxane synthesis by COX-1 and -2 as well as LOX for synthesis of a variety of EAs, in the dorsal horn glutamate is released by nerve endings to provide further transmission via spinothalamic tract to the CNS, besides dependent on the intensity and duration of the input activates NMDA receptors by release of SP thereby augmenting transmembrane ion flux and intracellular phosphorylation cascades. At the presynaptic region, opioid receptors control transmembrane ion-transport and by doing so are able to reduce presynaptic glutamate when reacting with the endogenous ligands dynorphin and endorphin. Cannabinoids such as anandamide and arachidonyl-glycerol-amid are released as response to glutamate receptor activation at the postsynaptic membrane and by binding at presynaptic CB receptors retrogradly reduce glutamate release. On the other hand, activation of CCKR by tachykinins and activators of NMDA receptors are augmenting factors of nociception. The arrows on the left show substances which are used to inhibit the nociceptive pathway at certain steps. Arrows at the right show substance which are used to inhibit distinct augmenting paces at the nociceptive pathway.

Descending pathway: Transmitters such as 5HT, NE, GABA, Glycine, Nociceptin and adenosine are important at the descending pathway of pain control, in that they counteract excessive membrane depolarization which otherwise will end up in modifications of the nociceptive system with the result of pathological pain states. Arrows at the left show substances such as antidepressants with selective 5HT and or NE reuptake inhibition, specific opioid such as tramadol, tapentadol and pethidine which act not only as opioid but also as reuptake inhibitors. Arrows at the right show substances such as the opioid buprenorphine which besides acting at OPRs acts also at the opioid-like nociception receptor. Glycine may be used by intrathecal routes, SCS releases adenosine and GABA at dorsal horn neurons.
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NC, alias Orphanin FQ, has been recently identified as the endogenous ligand of the opioid receptor-like 1 receptor (OP (4)). This new NC/OP (4) receptor system belongs to the opioid family and has been characterised pharmacologically with functional and binding assays on native (mouse, rat, guinea-pig) and recombinant (human) receptors, by using specific and selective agonists and a pure and competitive antagonist. The similar order of potency of agonists and affinity values of the antagonist indicate that the same receptor is present in the four species. OP (4) is expressed in neurons, where it reduces activation of adenylyl cyclase and Ca++ channels while activating K+ channels in a manner similar to opioids. In this way, OP (4) mediates inhibitory effects in the autonomic nervous system, but its activities in the central nervous system can be either similar or opposite to those of opioids. In vivo experiments have demonstrated that NC modulates a variety of biological functions ranging from nociception to food intake, from memory processes to cardiovascular and renal functions, from spontaneous locomotor activity to gastrointestinal motility, from anxiety to the control of neurotransmitter release at peripheral and central sites. These actions have been demonstrated using NC and various pharmacological tools, as antisense oligonucleotides targeting OP (4) or the peptide precursor genes, antibodies against NC, an OP (4) receptor selective antagonist and with data obtained from animals in which the receptor or the peptide precursor genes were knocked out. These new advances have contributed to better understanding of the pathophysiological role of the NC/OP (4) system and ultimately will help to identify the therapeutic potential of new OP (4) receptor ligands.

Various NMDA antagonists have been proven to attenuate development of neuropathic pain by intraplantar injection of dextrorphan, ketamine or memantine attenuates formalin-induced behaviors, although a meta-analysis did not show satisfactory results to generally conclude efficacy of NMDA-Antagonists in chronic neuropathic pain. Stress-induced development of Allodynia (Cortisol and NMDA induction) could be prevented by memantine application before nerve ligation and stress exposure in the rat.

Pharmacological pain treatment
Non-steroidal anti-inflammatory drugs
Nonsteroidal anti-inflammatory agents are the most often used first step analgesics. They act by blocking the COX – enzymes and hence interfere with prostaglandin synthesis. Besides non-steroidal anti-inflammatory drugs (NSAIDs) in blocking the AA oxidation by COX-1 and COX-2 direct the metabolism of AA in the LOX and peroxynase pathway and hence force the creation of hydroxy-peroxy-eicasoenoic-acids such as 12HPTE and 5HPTE by 12 LOX and 5 LOX, respectively. Not only there will be a decrease in the pain producing prostaglandins by inhibiting cyclooxygenase, but there will be increased production of pain-relieving LOX metabolites such as 5-HPTE and 12-HPTE, which are activated by opioids. So contrary to old believes that NSAIDs act only as ‘peripheral’ analogies, this proves that NSAIDs indirectly induce also a central analgesic effect.

Because of side effects such as gastrointestinal bleedings, kidney injury and cardiovascular complications, these drugs have to be used with care in that patients should be treated as short as possible and monitored as close as possible. Although COX-2 selective agents had been designed with the aim to induce less side effects specifically at the gastrointestinal tract and the kidney, their overall risk incidence was not lower than with nonselective COX-1-inhibitors because of increased cardiovascular side effects. On the other hand, NSAIDs are still the drugs of choice in treatment of pain due to inflammatory reactions as it happens in acute pain and osteoarthritis. There are also individual differences as it concerns development of serious side effects of either the gastrointestinal or the cardiovascular system or both. Besides in suppressing PGI production, NSAIDs may increase blood pressure and bronchoconstriction. In prolonged use also NSAIDs elevate hepatic enzymes but usually this is reversible when discontinued. Risk factors are age greater than 65 years, history of peptic ulcer or gastrointestinal bleeding, previous gastric irritation with NSAID use, use of multiple NSAIDs or COX-2 inhibitors, and concomitant use of corticosteroids, anticoagulants and SSRIs. The risk of peptic ulcers can be decreased by adjuvant use of proton pump inhibitors as well as misoprostol. Interestingly, adjuvant therapy of misoprostol may also reduce the risk of cardiovascular side effects. NSAIDs with shorter elimination half-life are of lesser risk to induce gastrointestinal side effects besides have the tendency to accumulate at the site of inflammation such as the synovial fluid. Hence NSAIDs with short elimination half-life should be preferred. Another possibility to reduce risk of side effects are topical NSAIDs which have been assessed in clinical studies in the treatment of arthritis and sports injuries. The outcomes in patient pain and oedema between systemic and topical treated patients were similar and topical treatments generated reduced instances of GI-related side effects.

Opioids
Opioids act on presynaptic receptors which belong to the GPCR-group and by interacting with transmembrane ion channels reduce the presynaptic release of glutamate. Opioid receptors have endogenous ligands such as β-endorphin the preferred ligand for µ-opioid-receptors, dynorphin the preferred ligand for δ-opioid-receptors and enkephalin for...
Finally, opioids are the preferred first choice drugs for intrathecal pain therapy.\[108\]

**Sodium channel blockers**

Carbamazepin has been traditionally used for treatment of neuropathic pain states such as trigeminal neuralgia and postherpetic neuralgia newer drugs such as oxcarbazepine eslicarbazepin belong to the same type of sodium channel blockers and although primarily are used as antiepileptics are effective in the management of neuropathic pain. The same has been proven for other sodium channel blockers such as mexiletine\[109\] and tolperison\[101\] as well as systemically applied local anaesthetics.\[111\] Unfortunately, as has been stated in a recent review the effectivity of this class of drugs is widely underestimated.\[112\]

**Calcium channel blockers**

Gabapentin and pregabalin both act as presynaptic neurotransmitter release inhibitors by binding to the \( \alpha_2\delta \) subunit of voltage gated N/P/Q-type calcium channels reduce \( Ca^{++} \)-influx which results in decreased transmitter release. Belonging also to the class of antiepileptics they are very effective in the treatment of neuropathic pain.\[113\] Ziconotide a sea snail toxin of conus magus, is also a presynaptic N-type \( Ca^{++} \)-channel blocker and has been proven to be a very potent analgesic in various pain states. Since it can be used only by intrathecal application via implantable pain pumps and because of a narrow effect to side-effect relation which needs a peculiarly observed dose finding, its use is not very popular.

**Antidepressants**

Tricyclic depressants such as the tertiary amines amitryptilin, imipramine, clomipramine and the secondary amines desipramine, nortryptiline and maprotiline have been traditionally used in the treatment of pathological pain states. Their analgesic action had been primarily assigned to serotonin and norepinephrine reuptake inhibition, meanwhile there is evidence, that contrary to newer selective SNRI and SSRI, TCA have also blocking activity on \( \alpha \)-adrenergic, H1-histaminergic, mACh and NMDA receptors as well as sodium and calcium-channels.\[114\] Although there is evidence based support for venlafaxine, duloxetine and paroxetine as well as citalopram in the treatment of neuropathic pain, TCAs seem to be superior as to their manifold action on receptors and ion-channels involved in nociception.\[115\]

**Non-opioids**

**Acetaminophen (paracetamol)**

Is a widely used analgesic and antipyretic, contrary to NSAIDs paracetamol has no anti-inflammatory potential. The action of

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**Table 3: Certain opioids are acting on different receptors**

| Opioid       | \( \mu \) | \( \kappa \) | \( \delta \) | 5HT3 | a2 | NMDA | NOP/ORL1 |
|--------------|-----------|-------------|------------|------|---|------|---------|
| Morphine     | +         | –           | –          | –    | – | –    | –       |
| Fentanyl     | +         | –           | –          | –    | – | –    | –       |
| Hydromorphone| +         | –           | –          | –    | – | –    | –       |
| Sufentanyl   | +         | –           | –          | –    | – | –    | –       |
| Oxycodone    | +         | –           | –          | –    | – | –    | –       |
| Methadon     | +         | –           | –          | –    | – | –    | –       |
| Tramadol     | +         | –           | +          | –    | – | +    | –       |
| Pethidin     | +         | –           | +          | –    | – | +    | –       |
| Tapentadol   | +         | –           | –          | +    | – | –    | –       |
| Buprenorphine| +         | –           | –          | –    | – | –    | +       |
| Cebranopadol | +         | +           | +          | –    | – | –    | +       |
| Levorphanol  | +         | +           | –          | –    | – | –    | +       |

NMDA: N-Methyl-D-Aspartate, NOP/ORL1: Nociceptin-Opioid-Peptide/

Opioid Receptor Like (receptor) 1, +: Active; –: Non active

\( \kappa \)-receptors. Morphine isolated by Serthürner in 1804 has the longest tradition as ‘pain killer’ in modern medicine but as content of opium has been used also in ancient times as potent analgesic. Because of the risks of addiction to opioids, the use of opioids had been primarily recommended to the treatment of cancer pain in with a guide to availability by the WHO. The effectivity of the WHO recommendation for opioid use in cancer has been repeatedly put into question\[95-102\] thereby ignoring however, that the WHO recommendations had been also directed to the availability of opioids, which especially for strong opioids is not the case in all countries of the WHO members.\[103\] There is no question however, that opioids represent the most effective pain killers and can be used not only in cancer pain but in all pain conditions.\[104\] Besides opioids are available in various formulations such as injectibles, tablets, capsules, rectal applications, transdermal patches and transmucosal sprays, lollypops, buccal tablets, and soluble films offering possibilities for applications for all sorts of slow and fast release as well.\[105\] Of course opioids are stigmatised to induce addiction and dependency, which has been corroborated by recent reports concerning misuse of oxycontin. It has to be taken into account however, that any medication prescribed by physicians has to be controlled by the prescribing physician as it concerns efficacy, tolerance and side effects.

Besides formulations, which provide fast, immediate and slow release of opioids, the opioids available for pain therapy are also different in their action not only on opioid receptors but also on other receptors of the nociceptive pathway [Table 3]. Besides dependent on their pharmacokinetic properties, may also develop different elimination and cumulation profiles, which are however more relevant in continuous applications during anaesthesia and intensive care analgesia represented as so called context-sensitive half-life.\[106\] As it concerns pain therapy in acute and chronic pain, opioids may be applied via patient controlled electronic infusion pumps for postoperative pain control as well as fast release formulations for enteral applications. For chronic pain however a close controlled application of slow release tablets (capsules) and transdermal patches are recommended\[104\] in order to omit addiction, which is more adherent to fast release transmucosal and lipophilic opioids. It is recommended therefore to check the patients history as it concerns addictive behaviour such as smoking, binge drinking and/or consumption of other drugs.\[107\] Finally, opioids are the preferred first choice drugs for intrathecal pain therapy.\[108\]
paracetamol is not clearly elucidated it is well established that it crosses the blood–brain barrier within a short time and seems there to inhibit the activity of the COX pathway reducing the active form of COX rendering it catalytically insufficient. This mechanism is only present in tissues where the level peroxides is very low as it is the case in the brain. The lack of peripheral side effects on the gastrointestinal system is also explained by the lack of peripheral COX inhibition. Both analgesia and antipyresis occur through distinct mechanisms. Analgesia may involve an action on the endogenous cannabinoid system explaining the calming and relaxing effect of paracetamol which has been reported by patients as a feeling of well-being, relaxing and tranquilising. This effect may be the result of the de-acetylation to p-aminophenol and conjugation with AA by FAAH to N-arachidonyl-phenolamine in brain, dorsal-root ganglia and spinal cord. Having structural similarities to AEA and arachidonylethanolamid (anandamide) the central CB-receptor agonist may have agonistic properties on CB-receptors.[116] Acetaminophen is often used in combination with opioids[117] and NSAIDs.[118] A rare but dangerous side effect of severe overdose and or combination with alcohol intake is acute liver failure. Normally paracetamol is glucurononlated and or conjugated with sulphate and renally eliminated. Only a small amount undergoes oxidative metabolism by CYP450, particularly the CYP2E1, CYP1A2, CYP3A4 and CYP2A6 isoforms. The reactive intermediate of this metabolic pathway known as N-acetyl-p-benzoquinone-imine is normally neutralised by combination with reduced glutathione (GSH), which subsequently is converted to cysteine or mercapturic conjugates. These two metabolites are non-toxic and readily eliminated in urine and bile. If the admitted dose is too high or other toxic substances such as alcohol taken parallel, the GSH reserves may be exhausted with the result acute liver failure. In these cases intravenous GSH serves as an effective antidote.[119]

Metamizole (dipyrone)
Metamizole is a pro-drug, which spontaneously breaks down after oral administration to structurally related pyrazolone compounds. Apart from its analgesic effect, the medication is an antipyretic and spasmyloytic agent. In animals the analgesic action is attributed to blockade of COX-3 this enzyme has not been found in humans however.[120] Other mechanism are activation of the cannabinoid system as well as activation of the opioidergic system and seems to be similar to the mechanisms of paracetamol analgesia.[121] In some countries metamizole is banned of the market because of inducing agranulocytosis (United States, the United Kingdom, Sweden and most recently India). It seems that this side effect is endemically more or less distributed[122] and hence the incidence of this side effect varies dramatically i.e. an incidence of 0.2 per million person days and in Sweden 1:1439 prescriptions.[125] On the other hand metamizole (Dipyrone) is a very popular first line analgesic even in children in most European countries and in south America. It is used in acute and chronic pain such as migraine,[124] postoperative pain in children and adults[125,126] as well as in cancer related pain.[127] The combination of metamizole and opioids showed synergistic analgesic effects.[128]

Drugs acting at more the one site
Valproic acid
acts at presynaptic Na+-channels and post-synaptic T-type Ca2+-channels interferes with GABA reuptake and breakdown in GABA-ergic neurons and finally has NMDA-glutamate receptor antagonism. Effective pain control has been reported in cancer related neuropathy[129] and diabetic neuropathy.[130]

Orphenadrine
Primarily introduced as an anti-Parkinson drug, acts antagonistic on mACH-channels,[131] H1-receptors,[132] NMDA-receptors,[133] blocks norepinephrine reuptake,[134] Na+1.7, Na+1.8 and Na+1.9 sodium channels[135] and finally also HERG-potassium channels. It is rarely used as a monotherapy, combined with NSAIDS[136,137] or paracetamol;[138,139] however, it is well established in acute and chronic pain control. Since it has also blocking activity on HERG-potassium channels, ECG control of Q-T-time should be performed in regular intervals.[140]

Opioids
Some opioids act in addition to μ-opioid receptors also on 5HT3, NMDA, Nociceptin, δ-,κ- and α2-receptors [Table 3]. Hence, it seems worthwhile specifically in complex pain situations to test which opioid fits best to the demands. Besides, clinical signs such as serotonin syndrome as result of interactions with other drugs concomitantly used in pain patients, for example, antidepressants,[141,142] triptanes and orphenadrine have to be taken into account.[143]

Riluzole
Basically developed for the symptomatic treatment of amyotrophic lateral-sclerosis[144] riluzole gained interest as possible analgesic because of its antagonistic effects on NMDA-receptors, NaV1.7-channels,[145,146] TREK-1 potassium channels,[147] glutamate release and transport systems[148] as well as downregulation of P2X7R in experimental models. In animal models of neuropathic pain riluzole effectively suppressed development of allodynia.[149] Also in animal models, riluzole showed beneficial effect on sensorimotor and pain disorders, as well as related comorbidities, after repeated administration of oxaliplatin.[150] Contrary to the cited animal models, riluzole showed no effect in suppressing allodynia in a human pain model.[151] In patients with spinal cord injury, riluzole significantly improved the neurological outcome.[152]

N-methyl-D-aspartate-receptor antagonists
Primarily developed for the treatment of Alzheimer’s disease, memantine because of its NMDA-receptor antagonistic activity gained interest also for pain therapy. There are studies showing analgesic effects in prevention of postoperative pain,[153] in control of phantom limb pain[154,155] but no effect in diabetic neuropathy.[156] Amantadine like memantine chemically an aminoadamantane has also been tested in
neuropathic pain such as post-mastectomy pain\textsuperscript{[157]} and post-surgery pain in cancer patients.\textsuperscript{[159]} There are also studies for dexamethasone, a substance primarily used as antiinfluenza, which contrary to memantine showed effects in diabetic neuropathy,\textsuperscript{[156,159]} and chemotherapy induced pain\textsuperscript{[160]} as well as general neuropathic pain.\textsuperscript{[161]} Ketamine, a general anaesthetic proved to be effective in various neuropathic pain states and has been used intravenously, epidurally and orally subdural application may induce neural damage overall the use of ketamine for neuropathic pain is discussed controversially.\textsuperscript{[162]} As it concerns neuropathic pain, there are recommendations to use an intravenous ketamine test in order to prove a possible response to other NMDA antagonists such as dexamethasone or memantine.\textsuperscript{[163,164]} Overall, the data of NMDA antagonists as monotherapy in neuropathic pain are not convincing,\textsuperscript{[80,165]} hence, it may be recommended to use this substances as comedication with other drugs such as opioids\textsuperscript{[166]} and calcium-channel blockers.\textsuperscript{[167]} On the other hand combined regimens have a higher risk of interaction and side effects\textsuperscript{[167]} specifically as it concerns combination of NMDA antagonists and antidepressants.\textsuperscript{[168]}

**Cannabinoids**

Because of their regulatory action on transmitter release in the CNS, as well as on inflammatory response, exogenous cannabinoids such as $\Delta$-9-tetra-hydro-cannabinol (THC) and cannabidiol are of interest as mediators of pain. THC was the first structurally investigated natural occurring cannabinoid with analgesic properties. After analysis of structure activity relationship nabilone and ajulemic acid have been synthetically produced and underwent clinical testing. Cannabidiol a further natural compound of hemp acts anti-inflammatory and besides counteracts the psychotropic effects of THC. A mixture of THC and Cannabidiol (Sativex\textsuperscript{®}) is also available with the purpose to combine analgesic and anti-inflammatory effects and to supress psychotrophic effects of THC. Further analysis of hemp compounds such as flavonoids and terpenoids such as apigenin-inhibiting TNF-$\alpha$ or Cannflavin-inhibiting PGE2 thirty times more than aspirin as well as Mircene a terpenoid with analgesic properties which can be blocked by naloxone and finally $\beta$-sistoloster a phytosterol reducing inflammation are also important compounds of the hemp plant.\textsuperscript{[169]} In total, there are meanwhile more than 120 cannabinoids\textsuperscript{[170]} and more than 200 other substances such as terpenes, flavonoids, phytosterols and various phenylalsenes structurally analysed which have properties which are far beyond limitation to pain therapy and hence are from medical interest.\textsuperscript{[171]} As it concerns pain therapy there is still controversy about beneficial\textsuperscript{[172,173]} and detrimental effects of cannabinoid therapy.\textsuperscript{[174]}

**Fatty acid amide hydrolase inhibitors**

Since endocannabinoids are controlling transmitter release in various systems which are relevant for nociception, inflammation and mental function, there has been interest to develop substances which interfere with the metabolism of endocannabinoids such as fatty acid amide hydrolase inhibitor (FAAHI). Expecting that increasing the amount of endocannabinoids will have stronger action with less side effects than application of external cannabinoids.\textsuperscript{[175,176]} Although there have been good experimental results of various synthetically developed FAAHIs\textsuperscript{[175,176]}, severe central nervous side effects in a phase 1 study of BIA 10-2474 however elucidated that the multiple actions of endocannabinoids and other fatty acid amides are not transparent enough to interfere with the degradation of fatty acid amides.\textsuperscript{[177]}

**Targeting calcitonin gene related peptide**

Since CGRP levels are elevated in migraine patients\textsuperscript{[178]} it seemed worthwhile to interfere with the binding of CGRP to the CGRP-receptor (CGRPR). Although effective in the prophylaxis of migraine, two CGPR-antagonists olcegepant and telcagebart, because of liver toxicity are not in clinical use.\textsuperscript{[179]} Two other antibodies erenumab (Aimovig\textsuperscript{®}) blocking the CGPR\textsuperscript{[180]} and galcanezumab (Emgality\textsuperscript{®}) an antibody binding to CGRP itself are meanwhile at the market with good results in migraine prophylaxis.\textsuperscript{[181]}

**Targeting the NK1 receptor**

The NK1 receptor is fundamentally involved in various central-nervous mechanism such as development of secondary hyperalgesia\textsuperscript{[182]} but also in the gastrointestinal and respiratory system by targeting this it may expected that development of secondary hyperalgesia could be diminished until now however the only substance blocking this receptor in the CNS is the very effective antiemetis aprepitant.\textsuperscript{[183]}

**Nerve growth factor-antagonists**

Although NGF-antagonism is expected to be a highly effective therapeutic approach in many pain states, and to be free of the adverse effects of traditional analgesic drugs, the first drugs developed are only available for i. v. application and showed osteonecrotic side effects.\textsuperscript{[184]}

**Botulinumtoxine**

Besides inhibiting the release of acetylcholine from the skeletal and autonomic nervous system inducing flaccid muscle paralysis botulinum toxin interferes also with TRPV1 expression, glutamate release and SP secretion. Clinical studies have shown that Botulinum toxin is effective in treatment of central neuropathic pain states\textsuperscript{[185]} as well as non odontogenic orofacial pain\textsuperscript{[186]} and even painful scars.\textsuperscript{[187]} A preliminary study showed also optimistic results for subcutaneous treatment of postherpetic neuralgia\textsuperscript{[188]} and is meanwhile also recommended for the treatment other neuropathic pain conditions.\textsuperscript{[189]} Since there are various types of botulinum toxins defined it may be expected that by further analysis of the specific structure action relationships the design of substances with target specific action and less side effects may be possible.

**Bradykinin antagonists**

Bradykinin starts by binding to Bradikinin receptor B2 the activation of phospholipases and hence can be seen as primary inducing molecule of the inflammatory cascade. Hence it seemed logical to develop substances which are able to block the B2 receptor in order to interfere with vasodilation oedema.
and nociceptor sensation. The only drug available at the moment is icatibant with the indication for treatment of the hereditary angioedema.\[190\] There is no information yet how far other bradykinin antagonists are designed although there has been an optimistic few after the first long acting molecules had been synthesised.\[191\]

**COX/LOX inhibitors**

Since leucotrienes play a fundamental role in in the anti-inflammatory process and classical NSAIDs show various risk factors specifically in prolonged application it seemed worthwhile to target COX2 or LOX5 by drugs with specific inhibiting potential.\[192\] Though several molecules have been synthesised with this objective, their unfavourable toxicity profile prevented them from being used in clinics.\[193\]

**CONCLUSION**

Structures, and functions of receptors, ion-channels and feedback mechanisms involved in nociception have been elucidated mainly by commitment of naturally available toxins as well as multiple natural compounds of plants, animals, bacteria and fungi. By analysing their structure–action relationship, many of these compounds could be synthetically modified to improve their action and side effect profile and finally entered clinical practice. Besides treatment of other morbidities such as cancer and cardiovascular diseases,\[194,195\] this concerns also drugs used for the control of acute and chronic pain\[196\] and associated inflammation, respectively.\[5,197\] Despite the knowledge about a wide range of underlying mechanisms involved in pain perception, transmission and engramming, there are still deficits in providing adequate pain relief for all patients suffering on chronic pain. In this sense the meaning of adequate is not limited to ‘no pain or tolerable pain’, but includes freedom of side effects, for example, no or only weak interference with alertness, freedom from giddiness and sureness of step, undisturbed legal competence, undisturbed libido, no constipation and other unwanted side effects. Hence, further analysis of natural available receptor ligands will be necessary to create drugs with better action and no side effects.

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