C025 Evaluation of antibiotic prescribing in upper respiratory tract infections R Vasilyev, W Al-Naeem, F Al- Omarri Sultan Qaboos University, Muscat, Oman

The purpose of this study was to evaluate trends in antibiotic prescribing at Family and Community Health Clinics. Males, females and children who attended for the treatment of upper respiratory tract infections from November to April were included. A data collection form that included question items on type of antibiotic, duration of treatment, medical conditions, other medications, performance of culture tests, sensitivity results, number of antibiotics and duration, adverse drug reactions and use of generic versus trade names. The number of prescriptions was 300. SPSS was used for statistical analysis. Forty three percent who received antibiotics was less than 18 years of age, 20% were between 18–30 years and 16%, were above 30 years. Males were 41% and females 47%. Most patients had one course of antibiotics (94%), 6% had two courses. There was no documentation of adverse drug reactions. In 45% of prescriptions generic names were used and 55% used trade names. The duration of treatment for 66% of patients was five days, 7 days for (21%) and less than 5 days in (9%). No data on antibiotic sensitivity was available. Co-amoxiclav accounted for (62%), followed by amoxicillin 37% and amoxicillin + clavulanate 12%. Antibiotics were largely prescribed to children. Antibiotics ranked 5th highest prescribed drugs in infants (Al Khaja et al., 2006). In our study most prescriptions were empirical. More co-amoxiclav prescribing compared with amoxicillin indicates resistance. The trend of prescribing beta lactam antibiotics for upper respiratory tract infections by primary care physicians has also been observed in other parts of the Arabian Gulf. (Al Khaja et al., 2008).

References:
Al Khaja KA et al. P J Trop Pediatr. 2006 Dec; 52 (6): 390–393.
Al Khaja KA et al. Pharmacoeconomic Drug Saf. 2008 Mar 5.

C026 Oral antibody to interferon gamma in ultra low doses: clinical efficacy and interferon inhibition in patients with upper respiratory viral infections S Tarasov, J Dugina, S Sergeeva, O Epstein R Vaishnav, W Al-Naeem, F Al-Omrani Sultan Qaboos University, Muscat, Oman

The data represent the mean values ± SE; *P < 0.001 vs. placebo.

| IFN production | Placebo, n = 46 | Placebo, n = 46 |
|----------------|----------------|----------------|
|                | baseline       | 2–3 days       | baseline       | 2–3 days       |
| Serum IFN-α, pg/mL | 44.9 ± 3.5 | 66.3 ± 2.3* | 40.8 ± 4.7 | 34.5 ± 4.6 |
| Serum IFN-β, pg/mL | 59.6 ± 2.9 | 78.9 ± 3.6* | 54.4 ± 4.7 | 54.0 ± 5.4 |
| Spontaneous IFN production, pg/mL | 77.4 ± 4.5 | 91.6 ± 4.6* | 73.9 ± 5.9 | 73.7 ± 7.3 |
| Spontaneous IFN production, pg/mL | 44.9 ± 2.5 | 65.5 ± 2.9* | 43.3 ± 4.1 | 43.3 ± 3.8 |
| Mitogen-induced IFN production, pg/mL | 103.5 ± 6.0 | 143.2 ± 8.3* | 107.9 ± 8.9 | 97.0 ± 11.7 |
| Mitogen-induced IFN production, pg/mL | 91.2 ± 4.6 | 162.2 ± 22.0* | 84.6 ± 6.5 | 78.9 ± 5.9 |

The data represent the mean values ± SE; *P < 0.001 vs. placebo.

C027 Effect of etanercept in a strain-dependent mouse model of allergen-induced airway hyperresponsiveness and lung inflammation N. N. Gujar, K. Tomlinson, L. Berry, M. Airey, S. Shaw, R. Foulkes, N. Gomard, P. Pfallmann

The data represent the mean values ± SE; *P < 0.001 vs. placebo.

Oral Communications
Tuesday 15 July
Respiratory and pulmonary pharmacology
(14.30–15.30)
ET-1 was measured by ELISA.

For gene therapy experiments, small isolated pulmonary arteries (100–150 μm) were mounted on a microvessel myograph to assess ET-1-induced constriction. Heterodimerization of both receptors was observed. Western blotting confirmed that each AS reduced protein expression of its targeted receptor. The use of ET B-AS significantly increased the vascular sensitivity (Sauvageau et al., 2007). In conclusion, suppression of ET B receptors reduced vascular sensitivity (Mitchell, JA. et al. Biochem Soc Trans. 2007; 35(Pt 6): 1449–1452.

Our results show that infection of HPASM cells with RSV leads to marked release of IP-10 (CXCL10) release following viral sensing by pulmonary artery cells-role of Toll like receptors. These observations confirm the functional importance of ET B receptors in ET-1-induced vasconstriction of small pulmonary arteries.

Reference:
Sauvageau, S et al. J Vasc Res. 2007; 44: 375–381.

Figure 1 for Abstract C030.
IP-10 from HPASM cells following treatment with viral PAMPs

Poly(I:C) & Lyovec 10µg/mL

RSV infection activates human pulmonary artery smooth muscle cells

Figure 1 for Abstract C032.

Figure 2 for Abstract C032.
C033 Role of key transmembrane residues in the pharmacological actions of isoprenaline and CGP 12177 at the human β1-adrenoceptor
J Baker, R Proudmam, N Hawley, S Hill
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Functional studies with catecholamines and CGP 12177 at the human beta-1-adrenoceptor have provided evidence for two different agonist conformations that have markedly different pharmacological properties (Baker et al., 2003). Here, key transmembrane (TM) residues in TM 3, 5 and 7 have been mutated to provide structural insights into the nature of these two conformations. Mutations (D138A, D138S, S228A, S229A, S232A and N363A) were generated using the Strategene QuickChange mutagenesis kit. These constructs were then transfected into a CHO cell line stably expressing a CRE-SAP reporter gene. The cells were selected for 3 weeks using G418 for the receptor and hygromycin for the CRE-SAP reporter. These stable mixed populations were then used in H1-CGP 12177 whole cell binding and CRE-SAP reporter assays as previously described (Baker et al., 2003). Responses to isoprenaline and CGP 12177 were greatly reduced. Mutations in TM6 (N341A and F343A) slightly reduced the binding affinity of isoprenaline and CGP 12177 but had little effect on their agonist actions. Antagonists by CGP 20712A of these two responses was similar (~log Kd values = −7.6 and −7.6 for F343A: −8.1 and −8.2 for N344A with isoprenaline and CGP 12177 as agonist respectively). This suggests that CGP 20712A was no longer able to discriminate between the two agonist conformations of the receptor. These studies suggest that F343 and N344 may have important roles in defining the two conformations of the β1-adrenoceptor.

References:
Baker JG et al. Mol Pharmacol. 2003; 63: 1312–1321.
Koblikia B Biochim Biophys Acta. 2007; 1768: 794–807.

C034 Long and short distance movements of β2-adrenoceptor in cell membrane assessed by photoconvertible fluorescent protein dendra- β2-adrenoceptor fusion
A Kayo, O Altuntas, O Ugur, O Onaran
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In the presented the following questions: 1) Is the local diffusion of the receptor in the cell membrane restricted to small domains?, 2) if the long distance diffusion of the receptor in the membrane is not macroscopically bounded in local domains, can local diffusion explain this large scale diffusion quantitatively?, 3) what is the nature of the compartmental connectivity in the cell in terms of receptor trafficking, when the cellular distribution of receptor is in the steady-state?, 4) We used [35S]methionine and used various human β2-adrenoceptor fused to the N-terminal of the dendra (a GFP-like fluorescent protein that undergoes an irreversible spectral conversion from {Ex. 488, Em. 507 nm} to {Ex. 558, Em. 575 nm} upon subsecond irradiation with 488 nm ~1 W/cm2 argon laser) as a model system. This construct allowed us to locally (and instantaneously) label the receptors in a small region of the membranes in living cells. We used a confocal microscope (Lecia) equipped with appropriate lasers, thermostatic baths and software for data collection. Solutions of two-dimensional diffusion coefficient (analytical or numerical) for appropriate boundary and initial conditions were used for quantitative evaluation of the data. We found that 1) functional integrity of the dendra-tagged receptor remains intact (as assessed by agonist stimulation of cAMP production or radioligand binding experiments), 2) inward or outward flux of the receptor to, or from a small membrane patch (~ 4 micron-sq) can be symmetrically explained by the same simple diffusion process with a diffusion coefficient of ~0.1 micron-sq/s (with an average mobile fraction of 85%), 3) this process is found to be independent of the activity state of the receptor, as assessed by using constitutively active mutants of the receptor, 4) only a part of the large scale movement of the receptor in the membrane can be explained by the same local diffusion process, implying the presence of large-scales diffusion barriers in the membrane, 5) cell-wide re-distribution of the receptor protein in the membranes is driven by the same local diffusion process (i.e. the entire cell membrane is apparently available to the receptor molecules, and 6) all the visible compartments (within the spatial resolution of the microscope) in the cell are inter-connected within a time frame of hours. These are the first experiments that demonstrate these points.

C035 Role of key residues in TM2 and TM6 in the two agonist conformations of the human β1-adrenoceptor
J Baker, R Proudmam, N Hawley, S Hill
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Functional studies with CGP 12177 at the human β1-adrenoceptor have provided evidence for two different agonist conformations that have markedly different pharmacological properties (Baker et al., 2003). In the β2-adrenoceptor Asp79 in TM2 and residues in TM6 (Asn 293 and Phe290) have been reported to interact with receptor mediated by agonists (Kobilka, 2007). Here, the equivalent residues in the β1-adrenoceptor have been mutated to investigate the contribution of these residues to the binding and CRE-SAP reporter assays as previously described (Baker et al., 2003). Responses to isoprenaline and CGP 12177 were inhibited in the WT receptor by CGP 20712A to give a log Kd values of ~8.65 and ~7.26 respectively, indicative of the two conformations of the β1-adrenoceptor. Mutations of Asp104 had very little effect on the affinity of ligands however functionally CGP 20712A was no longer able to discriminate between the two agonist conformations of the receptor. These studies suggest that F341 and N344 may have important roles in defining the two conformations of the β1-adrenoceptor.

References:
Baker JG et al. Mol Pharmacol. 2003; 63: 1312–1321.
Koblikia B Biochim Biophys Acta. 2007; 1768: 794–807.

C036 Regulation of G-protein-coupled receptor kinase 2 (GRK2) by calmodulin and protein kinase C
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G-protein-coupled receptor kinases (GRKs) mediate the first step of homologous desensitisation of G-protein-coupled receptors, namely phosphorylation of agonist-activated receptors. It has been previously shown that one of the [35S]methionine and a calmodulin reporter. We have now investigated this regulatory mechanism further. To directly assess GRK2-calmodulin interaction, we covalently labelled calmodulin with dansyl chloride and investigated its interaction with various concentrations of GRK2 (GRK2(1–53) and GRK2(552–689) by dansyl fluorescence at 486 nm. Fluorescence increases in a saturable manner when calmodulin interacts with increasing concentrations of a binding partner. GRK2 activity was assessed in the presence and absence of calmodulin by inhibition of forskolin-stimulated adenyl cyclase (Suppl. 2) 37–45

References:
Brockmann A, et al. Mol Pharmacol. 2003; 63: 1312–1321.
Koblikia B Biochim Biophys Acta. 2007; 1768: 794–807.
these need to be checked against models derived from aminoacid sequences. The showing areas (or pockets) in the receptor with which the drugs may interact but paired. From the geometry of the drugs it is possible to construct crude models binding is associated with changes in entropy, even though the compounds are water. Cumulative frequency curves for the effects of temperature on affinity for have only small apparent molal volumes it is likely to involve interactions with the group which can be accommodated. For hydroxyl groups and others which different areas of binding. Groups can have positive effects in some pairs of effects, analyzed from their cumulative frequency curves, in some instances fit a numbers (24–89) of pairs with and without a particular group. Values of group colocalization with arrestin, which regulates P2Y12 receptor entry into CCPs was may be multiple populations of clathrin-coated pits (CCPs) available to selectively sort GPCR cargo. In this present study we examined the structural determinants present in the COOH tail of the P2Y12 receptor that regulate their internalization into CCPs. These studies were undertaken either in CHO cells stably transfected with receptor constructs or in HEK293 cells. N-terminal tagged (either HA or FLAG) receptor constructs were co-transfected into cells using Lipofectamine. Surface receptor surface loss was measured by ELISA and cellular distribution of HA-tagged or FLAG-tagged receptor examined by immunofluorescence microscopy as previously described (Mundell et al., 2006). Our initial studies confirmed that deletion of the last four amino acids (E118-stop), a PIDZ motif (ETPM), of the P2Y12 receptor attenuated receptor internalization. Interestingly mutation of P341 to alanine in this motif also attenuated receptor internalization. Subsequent immunofluorescent studies revealed that both P341A and E118-stop trafficked to clathrin-coated pits but did not colocalize with full length P2Y12 receptor at these sites. In addition colocalization with arrestin, which regulates P2Y12 receptor entry into CCPs was not evident for E118-stop. Subsequent experiments revealed that following internalization both E118-stop and P341A did not traffic normally back to the cell surface but were retained in an as yet uncharacterised post-endosomal compartment. This intracellular retention attenuated receptor resensitization. In conclusion this study demonstrates that the presence and integrity of a PIDZ motif on the P2Y12 receptor is essential for correct targeting to distinct populations of CCPs and subsequent receptor internalization and traffic.

References:
Mundell et al. Traffic. 2006; 7(10): 1420–1411. Puthenveedu and von Zastrow Cell. 2006; 127(1): 113–124.

C039
Effects of rosiglitazone and sumatriptan in human isolated small and large coronary arteries
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Recently, the FDA expressed concerns over a potential increased risk for heart attacks in relation to rosiglitazone (www.fda.gov/bbs/topics/NEWS/2007/NBV01636.html) (PPARγ agonist used in diabetes management). Coronary vasoconstriction, a known side effect of some drugs such as triptans, has been demonstrated in human isolated tissues (Maussemassen, et al., 1998). Therefore, in the current study, coronary constrictor potential of rosiglitazone was compared to sumatriptan and 5-HT in human isolated small and large coronary arteries (HCA). HCA were taken from different diseased hearts obtained from ethically approved organ procurement organisations. Small HCA (SHCA, 0.3–0.5 mm internal diameter (i.d.)) and large HCA (LHCA, 1–2 mm i.d.) rings were dissected out, suspended in organ baths containing Krebs physiological salt solution, gassed with 95% O2/5% CO2 and maintained at 37°C. After initial viability assessments with KCl, compounds were assessed either under basal or endothelin-1 (ET-1) pre-contracted conditions (LHCA only). Subsequently, function of the endothelium was assessed pharmacologically. In rings from all three donors, KCl (30–100 mM), PGF2α (1 μM) and ET-1 (0.1–10 nM) caused contraction, whereas sodium nitroprusside (100 μM) caused relaxation. Substance P (1–10 nM) caused relaxation in the majority of rings tested demonstrating a functional endothelium. Sumatriptan and 5-HT but not rosiglitazone (1 nM–10 μM) caused concentration-dependent contractions that were similar in both SHCA and LHCA. Compared to sumatriptan, 5-HT was ~3–10-fold more potent and caused approximately double the contraction magnitude. Vehicle (DMSO) caused small but variable relaxation. In conclusion, rosiglitazone did not contract HCA in these preliminary studies. Further studies are ongoing to understand the significance of these findings in relation to the reported clinical side effects.

References:
Maussemassen, et al. Circulation 1998; (98): 25–30. www.fda.gov/bbs/topics/NEWS/2007/NBV01636.html.

Table 1. Effect of rosiglitazone, sumatriptan and 5-HT in human isolated coronary arteries

| Treatment | CEC | Treatment | CEC | Treatment | CEC |
|-----------|-----|-----------|-----|-----------|-----|
|           | Small HCA | Large HCA |       | Small HCA | Large HCA |       |
|           | Basal tone | Basal tone | ET-1 elevated tone | Basal tone | ET-1 elevated tone | Basal tone | ET-1 elevated tone |
| pEC50 | pEC50 | IC50 | IC50 | IC50 | IC50 |
| Rosiglitazone | 8.3 ± 0.8 | 8.3 ± 0.8 | 21.6 ± 15.3 | 21.6 ± 15.3 | 9.8 ± 3.8 | 9.8 ± 3.8 |
| Sumatriptan | 6.4 ± 0.4 | 6.5 ± 0.1 | 45.8 ± 17.4 | 62 ± 0.1 | 31.9 ± 9.9 |
| 5-HT | 7.4 (7.1–7.6) | 6.9 ± 0.1 | 89.6 ± 13.3 | 6.96 ± 5.73 | 8.5 ± 2.5 |
| Vehicle | not tested | not tested | not tested | not tested | -19.1 ± 13.4 | -19.1 ± 13.4 |

Data are mean ± s.e.mean (n = 3–6 rings, 3 donors), or range (n = 2 rings, 2 donors); EC50 = % KCl 100 mM
t-carnitine protects neuroblastoma (SH-SY5Y) cells from oxidative stress by mitochondria, stress response proteins and GADD genes

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Oxidative stress plays an important role in neurodegenerative disorders and H₂O₂-induced oxidative toxicity is a well-described model of oxidative stress-induced neurodegeneration. t-carnitine (LC) is an endogenous mitochondrial membrane compound and some studies have reported that LC could effectively protect various functions of mitochondria and cells against oxidative injury both in vitro and in vivo. However, the exact molecular mechanism of LC on oxidative stress in neurodegeneration is unclear. In the present study we used the human neuroblastoma SH-SY5Y cell line as an in vitro model and assessed the effect of t-carnitine on hydrogen peroxide (H₂O₂)-mediated oxidative stress and neurotoxicity. Cells in culture were treated for 24 h with 100, 200, 300, 400, 500 μM H₂O₂ alone or pretreated with 0.1, 1, 10, 100, 300 and 1000 μM t-carnitine. H₂O₂ produced a dose-related decrease in cell viability as measured by Trypan blue exclusion assays, a reduction in the mitochondrial metabolism of 5-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and an increase in DNA fragmentation analysis. Pretreatment with LC 2 h inhibited H₂O₂-induced cell death in a concentration-dependent manner. And analysis of DNA fragmentation analysis showed that LC could prevent H₂O₂-induced DNA damage and apoptosis. Western blot analysis showed that LC could inhibit the release of cytochrome c from mitochondria and up-regulated the expression of heat shock proteins (HSPs). This evidences support the pharmacological potential of LC in the management of oxidative stress, neurotoxicity and neurodegenerative diseases.

Carnitine protects neuroblastoma (SH-SY5Y) cells from oxidative stress by mitochondria, stress response proteins and GADD genes

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Hydrogen peroxide (H₂O₂), which can be produced as a byproduct during metabolic activity, is a significant source of oxidative stress and is a major cellular toxin. The current study aimed to investigate the protective effects of LC against oxidative stress in SH-SY5Y cells. LC was demonstrated to have significant protective effects against oxidative stress in part by protecting mitochondria and up-regulating the levels of endogenous anti-oxidant defense components and stress proteins, GADD genes (growth arrest and DNA damage-inducible (GADD) mRNA, GADD45 and GADD153), were observed at the early phase during cell death in 400 μM H₂O₂-treated control groups and LC could elevate the expression of GADD genes to protect DNA from oxidative damage. Taken together, these results demonstrate that LC exerts protective effects against oxidative stress in part by protecting mitochondria and up-regulating the levels of endogenous anti-oxidant defense components and stress proteins, GADD genes and HSPs. This evidences support the pharmacological potential of LC in the management of oxidative stress, neurotoxicity and neurodegenerative diseases.

Rosiglitazone inhibits GPVI-stimulated platelet activation through non-genomic signalling

C041
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Rosiglitazone, the most potent of the currently marketed thiazolidinediones, acts by activation of the peroxisome proliferator-activated receptor-γ (PPARγ). The PPARγ are members of the nuclear hormone receptors that heterodimerise with the retinoid X receptor (RXR) and then modulate transcription of many target genes (Moraes et al., 2005). Although platelets are anucleate cells, recent reports demonstrate that they express the intracellular receptors PPARγ (Akbiyik, 2004), PPARβ/δ (Ali 2006), GR (Moraes et al., 2005) and the RXR (Moraes et al., 2007). Previous work has demonstrated that the treatment of diabetes with PPARγ agonists is associated with a reduced risk of some cardiovascular complications. We have, therefore, examined the effects of PPARγ agonists on platelet function. Washed platelets were stimulated with PPARγ ligands and collagen-induced aggregation was measured using optical aggregometry. Calcium levels were measured by spectrofluorimetry in Fura-2/AM loaded platelets. Tyrosine phosphorylation levels of early signalling components of the GPVI signalling pathway were measured using immunoblot analysis. The role of PPARγ agonists in thrombus formation was assessed using an in vitro flow system, where fluorescently labelled whole blood was perfused through a collagen coated capillary at a shear rate of 1000/s in the presence or absence of PPARγ agonists. Thrombus volume was quantified by confocal microscopy using Leica SP2 software. In this study, we report that PPARγ ligand rosiglitazone inhibits collagen-stimulated platelet aggregation. This was accompanied by a reduction in collagen-stimulated intracellular calcium mobilization and reduction of thrombus formation on immobilised collagen under arterial flow conditions in a concentration-dependent manner. This was accompanied by inhibition of collagen-stimulated tyrosine phosphorylation of phospholipase Cγ2. We propose that the PPARγ ligands may have beneficial clinical actions through inhibition of platelet activation. Furthermore, our results demonstrate a novel non-genomic mode for nuclear receptor action, and functional cross-talk between the collagen receptor GPVI and nuclear receptor signalling families in platelets.

Identification of the anti-apoptosis activity of nerve growth factor on cardiac myocytes

C042
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Neurotrophins (NTs) control the survival and regeneration of neurons. Recent research showed that NTs possess cardiovascular actions. In this study, we investigated the hypothesis that the NT nerve growth factor (NGF) prevents cardiomyocyte apoptosis. We demonstrated that cultured rat neonatal cardiomyocytes (RNCMs) produce NGF and express its trkA receptor. RNCMs given a neutralising antibody for NGF or the trkA inhibitor K252a underwent apoptosis, thus suggesting that NGF is an endogenous pro-survival factor for cardiomyocytes. Recombinant NGF induced trkA phosphorylation, followed by Ser473-phosphorylation and nuclear translocation of Akt in RNCMs. In response to Akt activation, Forkhead transcription factors Foxo-3a and Foxo-1 were phosphorylated and excluded from the nucleus. Adenosin (Ad)-mediated NGF over-expression RNCMs protected RNCMs apoptosis induced by either hypoxia/reoxygenation or angiotensin II. Inhibitory approaches using K252a, LY294002 (a pan-phosphatidyl inositol 3-kinase -PI3K- inhibitor), and adenosinovires carrying a dominant negative mutant form of Akt (Ad.DN.Akt) or an Akt-resistant FoxO-1 (Ad.AAA-Foxo-1a) demonstrated that the pathway encompassing trkA, PI3K-Akt, and Foxo is essential for the pro-survival effect of NGF. The anti-apoptosis action of NGF was confirmed in adult mouse cardiomyocytes extracted from the mouse heart, which were submitted to the angiotensin II apoptosis test in the presence of recombinant NGF. Finally, intramyocardial NGF gene transfer prevented cardiomyocyte apoptosis in a mouse model of myocardial infarction.
Communicative methods of teaching: A structuralistic model of the construction of knowledge in problem based learning

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Although methodology of teaching has been developed extensively in the last years, teachers of science and biological sciences usually use a methodology of teaching, which is about 70-years-old. During the last few years, an effort has been made in medical, to use contemporary communicative methods of teaching, like problem based teaching. Nevertheless, it is obvious that lack of theoretical knowledge on the procedure of learning and on methodology of teaching restricts the application of communicative methods of teaching. The aim of this work is to present the theories of constructivism in learning, and to develop a new structuralistic model of the construction of knowledge in integrated methods of teaching, which may serve as the theoretical basis for problem based learning (PBL) and problem based teaching (PBT). The psychological constructivism of Piaget, the social constructivism of Durkheim and the radical constructivism of von Glaserfeld have given the bases for the development of communicative methods of teaching. Neo-Piagetian theories have developed models of learning process and have suggested new strategies for solving problems in teaching and learning. Understanding memory and its organization can make teaching and learning easier. Factors influencing memory and learning are: motivated attention, grade of familiarity with new information, the way of classifying and relating new information with old data, chunking of perceived information, repetition, and knowledge of rules of memory processing and data storing. Problem based teaching is a communicative, student oriented method of teaching, which offers strong motivation for learning and memorizing. In this work, as well as presenting the theoretical background, models of the construction of knowledge are presented briefly, as well as the new structuralistic model of the construction of knowledge in PBL, which has been developed by the author. All the data are presented in a simple, interactive and friendly to the audience manner, with a lot of images and in a way that provides strong motivation for participation of the audience. Structuralistic theories of cognitive development have given new tools in methodology of teaching, and lead to the development of communicative and very effective methods of teaching and learning, like PBL and PBT.

Preparation of list of essential cardiovascular drugs for medical school teaching

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An essential task facing those teaching medical pharmacology is to decide what drugs must be covered. This study aimed to prepare a list of essential cardiovascular drugs using evidence-based medicine. We first determined the CVS drugs in the top 200 drugs in 2006 used in hospitals and prescribed by trade or generic names in the US. There were marked differences in the drugs listed in these three categories. CVS drugs totalled about 70. We next listed all drugs included in Guidelines of the American College of Cardiology for management of hypertension, chronic & unstable angina, myocardial infarction, cardiac failure, atrial fibrillation, ventricular arrhythmias, and peripheral vascular disease. Because such Guidelines do not always name individual drugs but rather classes of drugs (e.g. ACE Is; ARBs; CCBs etc), we used the drugs listed in the top 200 drugs in 2006 in the US to determine members of such groups. This combined approach yielded a list of about 60–70 drugs. We next examined the number of these 60–70 drugs covered in latest editions of commonly used US textbooks. Major texts (e.g., Goodman & Gilman, 2005; Katzung, 2006 and Golan et al., 2008) covered essentially all the drugs. Shorter texts designed to be less comprehensive not surprisingly covered fewer of these drugs, the chief difference being the number of individual drugs in major classes covered. We next examined whether the number of drugs within such groups (e.g., ACE Is; ARBs; CCBs; beta blockers etc) could be reduced by comparing relative efficacies of such drugs using data from Evidence-based Practice Centers, listed by the Agency for Healthcare Research and Quality. However, with the exception of beta-blockers in cardiac failure, little or no significant differences in the efficacies of drugs within a given group have been found. The Consumers Reports Best Buy Drugs Program is distinguishing between such groups of drugs based on other factors (e.g., safety, ease of administration, cost etc). Using these combined approaches a list of about 30–55 essential cardiovascular drugs was prepared. Whenever possible USAN stems for drugs with similar mechanisms of action (e.g.-olol for beta blockers; -pril for ACE Is; -sartan for ARBs; -azosin for alpha1 antagonists; -vastatin for HMG-CoA inhibitors) should be pointed out to students to simplify their study of CVS pharmacology and therapeutics.
C045
Lipopolysaccharide augments the contractions of rat prostatic vas deferens via mechanisms that do not involve the endothelin receptors
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Lipopolysaccharide (LPS)-induced hyporesponsiveness of isolated smooth muscles to vasoconstrictors is a hallmark of experimental sepsis. Contrarily, alpha adrenoceptor-induced contractions of mesenteric arteries from LPS-treated mice were reported to be enhanced via the activation of Rho-kinase pathway which involves also the endothelin peptides (Buyukaslar et al., 2004). Thus, we investigated the effect of LPS on the contractile responsiveness of rat prostatic vas deferens with special emphasis on the role of endothelin peptides. Wistar albino rats (250–350 g) pretreated with LPS (4 mg/kg, ip) were then given bosentan (30 mg/kg, ip twice at 2nd and 12th hour after LPS). Parallel controls received saline (0.9% NaCl, ip). At 24th hour, prostatic sections of vas deferens were isolated into organ baths containing Krebs-Henseleit solution at 37°C and contracted by electrical field stimulation (EFS, 0.1–100 Hz, supramaximal voltage, 2 min duration for 10 s) or by cumulatively added phenylephrine (0.1 μM–0.1 mM). All data were expressed as means ± SEM of number (n) of observations. Ordinary one-way ANOVA or two-way ANOVA for repeated measures were used where appropriate and statistical significance was accepted when P < 0.05. LPS significantly augmented the contractile responses to EFS (e.g. mg contraction to 1 Hz control: 3.6 ± 0.4; LPS: 5.1 ± 0.4, P = 0.0076, n = 15) and to phenylephrine (i.e. two-way ANOVA for repeated measures applied to curves obtained from control versus LPS-treated animals revealed P = 0.0486). However, bosentan had no significant effect on neither EFS nor phenylephrine-induced contractions. Therefore, we conclude that LPS augments the EFS- or alpha adrenoceptor-mediated contractions of rat prostatic vas deferens via mechanisms that do not involve the endothelin receptors.

Reference:
Buyukaslar et al., Eur J Pharmacol. 2004; 498: 211–217.

C046
A potential inhibitory role of hydrogen sulphide on carrageenan-induced acute arthritis in rats
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Hydrogen sulphide (H2S), a known health-promoting endogenous metabolite synthesized by the activity of cystathionine-β-synthase (CBS), has been shown to possess anti-inflammatory properties in experimental arthritis tissues. However, it is not known how H2S acts in vivo. Histamine is a key mediator in inflammation, and our previous studies have shown that it can produce biphasic contractions of prostatic smooth muscle, with a peak response at 5 min following histamine administration. The aim of this study was to determine whether histamine can enhance the contractile responses to a histamine receptor agonist, thereby potentiating prostatic vas deferens responses. Wistar rats (250–300 g) pretreated with LPS (4 mg/kg, ip) was then given bosentan (30 mg/kg, ip twice at 2nd and 12th hour after LPS). Parallel controls received saline (0.9% NaCl, ip). At 24th hour, prostatic sections of vas deferens were isolated into organ baths containing Krebs-Henseleit solution at 37°C and contracted by electrical field stimulation (EFS, 0.1–100 Hz, supramaximal voltage, 2 min duration for 10 s) or by cumulatively added phenylephrine (0.1 μM–0.1 mM). All data were expressed as means ± SEM of number (n) of observations. Ordinary one-way ANOVA or two-way ANOVA for repeated measures were used where appropriate and statistical significance was accepted when P < 0.05. LPS significantly augmented the contractile responses to EFS (e.g. mg contraction to 1 Hz control: 3.6 ± 0.4; LPS: 5.1 ± 0.4, P = 0.0076, n = 15) and to phenylephrine (i.e. two-way ANOVA for repeated measures applied to curves obtained from control versus LPS-treated animals revealed P = 0.0486). However, bosentan had no significant effect on neither EFS nor phenylephrine-induced contractions. Therefore, we conclude that LPS augments the EFS- or alpha adrenoceptor-mediated contractions of rat prostatic vas deferens via mechanisms that do not involve the endothelin receptors.

Reference:
Buyukaslar et al., Eur J Pharmacol. 2004; 498: 211–217.

C047
Up-regulation of histamine H1R receptor and TNF-α gene expression by exogenous administration of histamine in the rat paw
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In addition to provoking acute symptoms of inflammation, histamine may be involved in late phase allergic responses, as implied by reports of H1R up-regulation in the nasal mucosa of patients with allergic rhinitis, as well as in animal models thereof (Dinh et al., 2005; Murata et al., 2004). In following recent reports indicating that histamine can induce H1R expression in various types of cells in culture (Das et al., 2007), we recorded the time course of H1R gene expression, as well as that of TNF-α, following intradermal administration of histamine in the rat paw. Male Wistar rats (250–300 g) were used in this study. Acute reaction to local subcutaneous histamine administration (100 μl, 5 mM) was followed by measuring paw oedema formation, with the use of a plethysmometer. H1R and TNF-α gene expression at various time-points following histamine administration was determined semi-quantitatively by conventional, end-point, RT-PCR on RNA isolated from subcutaneous tissue excised from rat paws. Histamine induced a significant increase in steady state mRNA levels of both H1R and TNF-α in the rat paw. The time course of this up-regulation was specific for the two transcripts. H1R expression was relatively low during the first 3 h, peaked at hours four to five, and then fell again at hour six, post-histamine. TNF-α gene expression responded in biphasic manner, with a steady increase during the first 3 h, followed by a reduction at hour four and a new increase, but at a lower rate, over hours five and six following the injection of histamine into the paw. This complex pattern of H1R and TNF-α gene expression is suggestive of histamine’s ability to elicit late phase allergic responses.

References:
Das AK et al., J Pharmacol Sci. 2007; 103: 374–382.
Dinh QT et al., Clin Exp Allergy. 2005; 35: 1443–1448.
Murata Y et al., Inflamm Res. 2004; 53 (Suppl 1): S11–12.
Obesity
(16.00–16.30)

C048
Age-related changes in cardiovascular risk factors among type 2 diabetic patients compared to age-matched healthy control subjects
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Type 2 diabetes mellitus (T2DM) accounts for about 95% of diabetic patients (over 190 million globally). In the UK it costs the NHS system £5.2 billion annually to diagnose, treat and care for DM patients with long-term complications. Cardiovascular dysfunctions are a major cause of morbidity and mortality in DM patients. This study attempted to identify biochemical risk factors in the plasma of type II diabetic patients compared to healthy male and female subjects employing different age groups (15–25 years, 26–40 years, 41–60 years and 61–80 years). Levels of insulin, glucose, triglycerides (TG), total cholesterol, High density lipoprotein (HDL), low density lipoprotein (LDL), C-reactive protein (CRP), homocysteine (HCys), tumour necrosis factor (TNF-α) and interleukin-6 (IL-6) were measured using established commercial assay procedures. The results show no significant differences in the age groups of T2 diabetes compared to controls. T2 diabetics have slightly elevated plasma insulin and body mass index compared to healthy controls. There were significant (P ≤ 0.05) increases in plasma glucose levels in T2 diabetics compared to healthy controls in all four age groups. Both TG and total cholesterol were significantly (P ≤ 0.05) elevated in diabetics compared to healthy subjects in all four age groups. Diabetics have elevated LDL and decreased HDL compared to controls. The levels of CRP and HCys increased significantly (P < 0.05) in all diabetic subjects compared to controls. In contrast, TNF-α remained more or less constant in control and diabetic subjects in all age groups. In contrast, IL-6 increased gradually in diabetic subjects compared to controls but this was only significant at age groups, 41–60 years and 61–80 years. The results clearly indicate that all diabetic subjects display signs of obesity, hyperglycemia, dyslipidaemia and low grade inflammation compared to healthy controls. But inflammatory cardiovascular risk factors seem to be more age-dependent.

C049
The effect of montelukast in metabolic syndrome in rats
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Metabolic syndrome is associated with systemic inflammatory status and increased coronary heart pathology. Leukotrienes (LTs), lipid mediators, are involved in atherosclerosis and other inflammatory cardiovascular diseases. This study investigated the effect of montelukast (MK, cysteinyl-LT1 R antagonist) in metabolic syndrome in rats. We worked on 4 groups of 8 male Wistar rats each (4 weeks of age) weighing between 40–45 g which received as follows: group I (control) - saline, group II - MK, 10 mg/kg/day for 15 weeks, group III - high fat diet (HF, 58% saturated fatty acids) for 15 weeks, group IV - MK (the same dose as group II) and HF diet for 15 weeks. After 15 weeks, we determined body weight, glycemia, blood pressure, insulin, glucose and insulin resistance, lipid profile, uric acid, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) in blood and in liver homogenate. A histopathological exam of liver, kidney and aorta was performed. For statistical analysis we used analysis of variance (ANOVA one way) followed by Tukey’s multiple comparison tests. Compared to control group, group III exhibited statistical significant increased levels of uric acid 12.15 ± 0.84 vs. 17.1 ± 0.85 mg/dL (P = 0.017), total cholesterol, low density lipoprotein cholesterol, triglycerides and oxidative stress parameters. The group IV presented statistical significant reduced levels of triglycerides (161.13 ± 46.51 vs. 215.83 ± 53.84 mg/dL; P = 0.002), MDA (2.85 ± 0.17 vs. 4.32 ± 0.41 nmol/mL, P = 0.001), increased levels of GSH (49.72 ± 15.02 vs. 27.38 ± 11.52 micromg/mL, P = 0.048) and SOD (46.88 ± 2.85 vs. 35.2 ± 4.43% inhibition, P = 0.0007) in liver homogenate.

C050
PAX4 enhances differentiation of human embryonic stem cells to insulin-secreting cells
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Guiding human embryonic stem cells (hESC) to differentiate into functional insulin-secreting β-cells in vitro can serve as an unlimited renewable source for cell transplantation to treat diabetes. Since the transcription factor PAX4 initiates terminal beta-cell differentiation during murine pancreatic development, we investigated whether constitutive expression of PAX4 in hESCs could promote progression towards a functional beta-cell phenotype in vitro. H7 hESC stably-transfected with Pax4 (H7.Px4) and untransfected H7 controls (H7) was allowed to spontaneously differentiate over a 3 week period following embryoid body (EB) formation. Expression of genes important during hESC maintenance, pancreatic development and mature β-cell function were assessed using standard RT-PCR and quantitative PCR at each differentiation time-point. Mature EBs were enzymatically dissociated and subjected to fluorescence-activated cell-sorting (FACS) using Newport Green to isolate a Zn2+ positive population of cells. The Zn2+ positive cells were subsequently assayed for c-peptide secretion using an ELISA kit. OCT4, HAND1, AP2β, KRT19, SLC2A1, GCK, ABCN1 and KCNJ11 transcripts were expressed at all time points in both control and H7.Px4 EBs (n=3 for each group). Interestingly, qPCR revealed substantially higher levels of expression of PDX1 and INS mRNA in H7.Px4 EBs than control EBs (n = 3) at the mid- to late- stages of differentiation. Following FACS, the Zn2+ positive cells were found to be positive for INS expression by RT-PCR and qPCR, they also contained c-peptide protein (7.7 ± 2 pg/10⁴ cells; n = 3) and secreted c-peptide in response to stimulation with the insulin-secretagogue, tolbutamide (100 μM; basal 23 ± 5 pg/10⁴ cells/15 min vs. tolbutamide 68 ± 4 pg/10⁴ cells/15 min; n = 3; Student’s t-test P < 0.001). These studies describe for the first time the enhancement of beta-cell differentiation in hESC by constitutive expression of PAX4, and also a novel method to separate differentiating insulin-secreting cells from undifferentiated precursors.