P053

Non-genomic effects of testosterone on contraction in the mouse *vas deferens*

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Several studies have demonstrated non-genomic effects of androgens on a range of tissue preparations, characterised by a short time lag and insensitivity to transcription and translation inhibitors (Falkenstein *et al.*, 2000). It is not known whether there are non-genomic effects of testosterone in the mouse vas deferens, an important model for studying junctional transmission. Vasa deferentia were excised from eight- to twelve-week-old male Balb/C mice (20–40 g) which had been humanely killed by an approved Schedule I method. The effects of testosterone on contraction were examined using electrical field stimulation and the exogenous application of the P2 purinoceptor agonist, α,β-methylene ATP; on membrane depolarisation using intracellular microelectrodes; and on whole cell Calcium (Ca^{2+}) transients using confocal microscopy and Oregon Green^{TM} BAPTA-1 as a fluorescent indicator of free Ca^{2+}, delivered to smooth muscle cells as a membrane-permeable AM form. Testosterone caused a rapid, concentration-dependent (3–300 μM) inhibition of neurogenic contraction (5 pulses, 10 Hz, 0.5 ms duration, supramaximal voltage) with an EC_{50} of 120 μM and 87 ± 8% inhibition at 300 μM (n = 6). Such contractions were resistant to the androgen receptor antagonist, flutamide, and the translation inhibitor, cycloheximide. This inhibitory effect was also observed with contractions elicited by the exogenous application of α,β-metATP. Combined with the observations from the electrophysiology experiments, this indicates a post-junctional site of action for testosterone. These observations might be due to effects on L-type Ca^{2+}channels as has been shown in vascular smooth muscle cells (Hall *et al.*, 2006). Although the concentrations of testosterone used in our studies are supraphysiological, raised levels of androgens are seen clinically in conditions such as androgen insensitivity syndrome and with abuse of anabolic steroids.

References:

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P054

Study on antioxidant effect of L-carnitine oral solution *in vivo* and *in vitro*

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L-carnitine has been reported to have antioxidant potential, but there are no clinical data for its role in balancing antioxidative systems in human body. The aim of the current study was to investigate the antioxidant effect of L-carnitine oral solution *in vivo* and *in vitro*. The productivity of superoxide anion and hydroxyl radical in L-carnitine oral solution and its dilute solution were measured. Twelve Chinese healthy volunteers were recruited (half male and half female, average age 27.7 years). After single oral administration of 2 g L-carnitine oral solution, plasma samples were collected at 0, 0.5, 1.0, 1.5, 2, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 12.0, 24.0 hours, and the urine were collected at 0, 0~2, 2~4, 4~8, 8~12, 12~24 hours. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-px), and the levels of nitric oxide (NO) and malondialdehyde (MDA) were measured. Results showed that the productivity of superoxide anion and hydroxyl radical in L-carnitine oral solution elevated significantly during being diluted ($P < 0.01$) and showed a linear correlation between them. After single oral administration of 2 g L-carnitine oral solution, the plasma level of MDA was reduced at 2.5 h ($P < 0.01$). The activities of SOD and GSH-px increased after 1 h ($P < 0.01$) and remained elevated at 24 h. CAT activity increased at 1 h ($P < 0.01$) but declined in half an hour. No significant change of NO was observed in plasma. L-carnitine administration resulted in an increase in SOD, GSH-px activities in urine at 2~4 h ($P < 0.01$). Also, MDA and NO production was reduced ($P < 0.01$). These changes in urine all recovered gradually after 8 hours. In conclusion, data obtained from this study indicate that L-carnitine supplementation could increase the endogenous antioxidant ability of human body and thereby protect human from oxidative stress. It may play an important role in the treatment of oxidation-induced disorders.

P055

Effects of an n-butanol extract from stems of *Tinospora crispa* on blood pressure and heart rate in anesthetized rats

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Tinospora crispa has been used in folkloric medicine for many purposes including control of blood pressure. However, this claim has not been confirmed by any scientific investigation. This study aims to investigate the effects and mechanisms of action, in anesthetized rats, of an n-butanol extract from stems of *Tinospora crispa* (TS-ext) on blood pressure and heart rate. TC-ext (1–100 mg/kg, i.v.) causes a decrease in mean arterial blood pressure (MAP) that was inhibited by propranolol, but not by atenolol, atropine or hexamethonium. With reserpine pretreated rats, the TC-ext had a dual effect: reducing hypotensive activity, followed by a small increase in blood pressure. In reserpinized rats, propranolol inhibited the hypotensive effect, but enhanced the hypertensive activity. When phentolamine was also given together with propranolol, the D-R curves were restored to the same level as that of the reserpinized control group. TS-ext (i.v.) had a dual effect on anesthetized rat heart rate: a small decrease, followed by an increase. The positive chronotropic effect was inhibited by propranolol and atenolol, but not by atropine or hexamethonium. Reserpine potentiated the positive chronotropic effect of the TS-ext, and a further potentiation was found when the animals were also pretreated with propranolol. This potentiation was restored to the same level as that of the reserpinized control group when phentolamine was given together with the propranolol. The negative chronotropic effect of the TS-ext was inhibited by atenolol, propranolol, but not by atropine or hexamethonium. Reserpine inhibited the negative chronotropic effect of TS-ext, but inhibition was not modified by propranolol and/or phentolamine. These results indicate that the TS-ext possesses at least three different active components that cause a hypotensive and both a negative and positive chronotropic effect in rats. The mechanisms responsible for these effects could be that these active components act directly via (1) β_2-adrenergic receptors causing a decrease in MAP and an increase in heart rate, (2) α-adrenergic receptors causing an increase in blood pressure and heart rate, and (3) other pathways that cause a decrease in MAP and heart rate. Further studies to isolate and identify the active substances responsible for these effects are in progress

P056

Effects of an n-butanol extract from stems of *Tinospora crispa* on rat atria and thoracic aorta and on a guinea pig trachea *in vitro*

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Our previous studies demonstrated that an n-butanol extract from stems of *Tinospora crispa* (TS-ext) in anesthetized rats caused a decrease in mean arterial pressure and a transient decrease, followed by an increase in heart rate. This study aimed to determine if active components react directly with the atria and blood vessels to cause these effects. Isolated right and left atria and thoracic aortic rings of the rat and the tracheal rings of the guinea pig (predominant β_2- adrenergic receptors) were tested *in vitro*. TS-ext (.01–3 mg/ml) caused a transient decrease, followed by an increase in the rate of a spontaneously beating right atrium. The positive chronotropic effect was inhibited by propranolol (10 nM) or atenolol (100 nM). However, when the concentrations of propranolol or atenolol were increased up to 100 nM or 1000 nM respectively, a negative chronotropic effect was found, and this was not modified by atropine. A similar result was found in the atria obtained from reserpinzied rats. The force of contraction of the electrical field stimulated left atrium was increased by TS-ext. This effect was inhibited by propranolol and atenolol. Atropine did not modify the D-R curve of the TS-ext induced increased force of the left atrial contraction, except at a high concentration of atropine (100 nM) and TS-ext, when the maximal contractile response was increased. This increased force of contraction of the left atrium induced by TS-ext was reduced in reserpenized rats and abolished by propranolol. High concentrations of TS-ext caused relaxation of the thoracic aortic ring preconstricted with phenylephrine. N-nitro-L-arginine or removal of the endothelium caused a rightward shift of the D-R curve. Propranolol caused a significant shift of the D-R curve to the left. TS-ext caused relaxation of isolated guinea pig trachea precontracted with carbamylcholine chloride. Propronolol caused a parallel rightward shift of the D-R curve of the TS-ext in a dose-dependent manner in a similar way to that of isoproterenol. These results indicate that the active components of the TS-ext act directly via the β_2-adrenergic receptors of the heart to cause an increase in both the rate and force of atrial contraction, and act via a different pathway to cause a decrease in heart rate, and on blood vessels to cause vasodilatation.

P057

Effects of novel silatran (substituted N(silatranylmethyl)acetamid) on cholinergic motility of the gastrointestinal tract

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New silicoorganic compound (Sil-1), which was synthesized by department of biochemistry at RSMU (Professor Baukov Yu), has acetylcholine-like structure. Aim of this study was to investigate effects of Sil-1 on intestinal propulsion in mice and on contractility of isolated ileum of guinea pig. *In vivo* assays were carried out using tissues from wild-type male mice. Sil–1(100–2000 mg/kg) was administered intraperitoneally. Direct effects of drug on intestinal contractions were determined *in vitro* using isolated segments of guinea pig ileum. Segments of ileum were dissected out and placed in organ bath containing Krebs solution (35°C). Isometric contractions were recorded through physiograph. Concentration–response curves for acetylcholine and *sil-1* were measured in a noncumulative manner. Sil-1 produced increase in intestinal transit that was blocked by atropine pretreatment. Toxicity of silatran was low – DL50 = 2000mg/kg. Acetylcholine and Sil-1 contracted the isolated ileum in a dose-dependent manner. Prior incubation of tissues with atropine abolished silatran and acetylcholine-induced contractions completely. The increase in intestinal transit produced by Sil–1 as well as contractions of ileum segments was blocked by atropine. Both the maximal response evoked by Sil-1 and the EC_{50} were significantly lower, than equal measures of ACh in the same experiments (Table1).

Table 1. Values are given as means ± S.E.M.

	Emax(g)	EC_{50} (M)	pD_2(-log pEC_{50})
Acetylcholine	1.7 ± 0.15	4.97×10^{-7}M	6.31 ± 0.04
Sil – 1	0.8 ± 0.11	3.85×10^{-7}M	3.41 ± 0.08

Thus, present results demonstrate that effects of silatran on intestinal transit and on contractility of isolated ileum are attributed to an interaction with muscarinic receptors and Sil-1 is a partial agonist of these receptors.

P058

Vasodilatory effects of endogenous cannabinoid virodhamine in the human pulmonary artery

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The endocannabinoid virodhamine is a partial agonist at the CB_1 and a full agonist at the CB_2 receptor, it has high efficacy at GPR55 receptor and relaxes the rat mesenteric artery via endothelial cannabinoid receptors. We demonstrated recently that abnormal cannabidiol causes a full relaxation of the human pulmonary artery via the endothelial cannabinoid receptor (Kozlowska *et al.*, 2007). The aim of our present study was to examine the influence of virodhamine on the human pulmonary artery. Vasodilatory effect of virodhamine was examined on endothelium-intact human pulmonary arteries preconstricted with serotonin (5-HT, 1 μM) or with potassium chloride (KCl; 60 mM). Virodhamine, but not WIN 55,212-2 (both 0.1–100 μM), relaxed 5-HT-preconstricted vessels concentration-dependently (pD_2 = 5.08 ± 0.07; E_{max}=94.3 ± 2.8; n = 30). The effect of virodhamine was reduced by endothelium denudation, two antagonists of cannabinoid endothelial receptors - cannabidiol and O-1918 and by a high concentration of the two antagonists of CB_1 receptors - rimonabant and AM251. Their respective pA_2 values were: 6.6; 6.3, 5.9 and 6.8. It was not modified by the vanilloid receptor antagonist capsazepine or the nitric oxide synthase inhibitor L-NAME. The inhibitors of cyclooxygenase - indomethacin and of fatty acid amide hydrolase - URB597 as well as a combination of selective blockers of small (apamin), intermediate and large (charybdotoxin) conductance Ca^{2+}-activated K^+ channels attenuated the virodhamine-induced relaxation. The potency of virodhamine to relax vessels was lower in KCl- than in 5-HT-preconstricted preparations. In conclusion, virodhamine relaxes the human pulmonary artery via the putative endothelial cannabinoid receptor and/or GPR55 receptor and its cyclooxygenase-derived vasodilatory prostanoid. One or both of these mechanisms may stimulate vasodilatory Ca^{2+}-activated K^+ channels.

Reference:

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P059

Evidence that JWH015 (CB_2 agonist) reduces MDMA-induced neuroinflammation but fails to provide protection

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3,4-Methylenedioxymethamphetamine (MDMA) produces microglial activation and increases interleukin-1β (IL-1β) release in rat brain (Orio *et al.*, 2004). Activation of the cannabinoid 2 (CB2) receptor inhibits microglial activation (Ehrhart *et al.*, 2005) and reduces pro-inflammatory cytokine release (Klegeris *et al.*, 2003) in neuronal degeneration models (Arevalo-Martin *et al.*, 2003). Since MDMA produces a persistent loss of serotonin (5-HT) axon terminals, we evaluated the effects of a CB_2 selective agonist on the neuroinflammation and neurotoxicity of the drug. Male Dark Agouti rats (175–200 g) were given JWH015 (2.4 mg/kg i.p.) 48, 24, 0.5 h before and 6 h after MDMA (12.5 mg/kg i.p.). Animals were killed at different times to analyze various parameters in hypothalamus and cortex: 3 h for the quantification of IL-1β (ELISA); 24 h for the immunohistochemical determination of microglial activation (labelled with OX-42) and 7 days to evaluate the density of [^{3}H]-paroxetine labelled 5-HT transporter (5-HTT) and cortical concentration of 5-HT (HPLC). To confirm that the effects were mediated by CB_2 receptor activation, a group of animals were given a CB2 selective antagonist (AM630) 15 min before the agonist. JWH015 reduced the MDMA-induced increase in IL-1β levels and microglial activation at 3 h and 24 h, respectively, both in cortex and hypothalamus. The hyperthermic response of MDMA was not modified by JWH015. AM630 attenuated JWH015-mediated inhibition of microglial activation. However, the CB_2 agonist did not modify the MDMA-induced reduction in cortical 5-HT content or 5-HTT density 7 days later. In conclusion, JWH015, by activating CB_2 receptors, attenuates MDMA-induced microglial activation and IL-1β release. However, the reduction of these markers alone is not enough to protect against the neurotoxic effect of MDMA.

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P060

Neurotoxic doses of METH increase ethanol consumption and preference: Involvement of endocannabinoid system

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Mice treated with MDMA exhibited a higher consumption of and preference for ethanol (EtOH) compared with saline-treated animals (Izco *et al.* 2007), an effect related to the dopamine (DA) depletion induced by MDMA. Methamphetamine (METH) also produced selective loss of DA content and DA transporter (DAT) in mice striatum (Sanchez *et al.* 2003). We have now examined EtOH consumption and preference in METH-lesioned mice and determined whether changes in these parameters are related to alterations in the cannabinoid system mediated by CB1 receptors. Adult male C57BL/6J mice (25–30 g) were injected with saline (controls) or METH (8 mg/kg, i.p. ×3, 3 h intervals). EtOH preference and consumption were examined using a 2-bottle choice paradigm with increasing EtOH concentrations (3, 6, 10, 20% v/v). Mice injected with METH or saline were exposed to EtOH (10% v/v) and given the CB1 antagonist AM251 (1.25 mg/kg) 1 h before the onset of dark cycle. One and 4 weeks after METH or saline administration, density of the CB1 receptor and agonist-stimulated [^{35}S]GTPγS binding were determined in limbic brain and DA concentration and DAT density (labelled with [^{3}H]WIN-35.428) were quantified in striatum and prefrontal cortex. METH pretreated mice showed increased consumption of EtOH solutions and greater preference ratios compared with controls [$F(1.16)=16$, $P < 0.001$] [$F(1.16)=10.5$, $P < 0.001$]. The CB1 receptor antagonist, AM251, completely prevented the enhanced EtOH intake and preference in METH-treated mice. AM251 did not modify these parameters in saline-injected animals. There was no difference in the density of CB1 receptors or in the CB1 receptor-stimulated [^{35}S]GTPγS binding in limbic brain. These findings indicate that administration of neurotoxic doses of METH predisposes the mice to high voluntary consumption of EtOH and suggest that the long-lasting DA loss induced by METH is accompanied by a rise in endocannabinoid levels.

Financial support: PR75/06-15077 (MSC), 910258 (UCM-CAM), RD06/0001 (MSC).

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P061

Autoradiographic study of G-protein coupling to cannabinoid CB_1-receptors in the brain of rats with peripheral mononeuropathy

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Alterations in signal transduction of different types of neurotransmitter receptors involved in pain transmission and processing may occur during neuropathic pain. Several basic lines and clinical evidences have demonstrated the role of cannabinoid neurotransmission in the control and treatment of pain. In this autoradiographic study, we have evaluated the coupling efficacy of G-proteins to brain CB_1-receptors in a model of neuropathic pain. Peripheral mononeuropathy in male Sprague-Dawley rats, 2 months old (neuropathic animals, n = 8; sham-operated, n = 7), was performed following the 'spared nerve injury' model (Decosterd and Woolf, 2000). Abnormal pain behaviour -decreased hind-paw withdrawal thresholds to tactile and noxious mechanical stimulation- was at a stable maximum following 14 days of surgery. The functionality of cannabionid CB_1 receptors was assessed in brain and spinal cord sections by measuring the stimulation of [^{35}S]GTPγS binding induced by the cannabinoid agonist WIN55.212 (10 μM), The specificity of the agonist-induced response was confirmed using the selective CB_1 antagonist SR141716A (10 μM). In rats with mononeuropathy, agonist-induced specific stimulation of [^{35}S]GTPγS binding was significantly enhanced in cingulate cortex, septal nuclei and susbtantia nigra (+16%, +31% and +43% over basal binding, respectively) whereas it was reduced in other areas such as hippocampal dentate gyrus, entorhinal cortex and raphe magnus nuclei (−18%, −15%, −26% above basal binding, respectively). These results demonstrate a differential regulation, regionally-dependent, of the functionality of brain CB_1 receptors during neuropathic pain. These data may help to understand the role of cannabinoid neurotransmission in the pathogenesis of neuropathic pain as well as to develop potential therapeutical targets.

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Reference:

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P062

Identification and characterisation of putative modulator ligands at the orphan receptor GPR55

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Cannabinoids exert their effects on the central and peripheral nervous system via two G-protein coupled receptors, cannabinoid receptor type 1 (CB_1) and type 2 (CB_2). Activation of both CB_1 and CB_2 receptors contribute to the anti-nociceptive and immunomodulatory effects of cannabinoid ligands, with both CB_2-selective and non-selective ligands currently in the clinic for pain. However, recent studies with $CB_1^{-/-}$ and $CB_2^{-/-}$ mice have indicated that some effects are not mediated by CB_1 or CB_2, suggesting the possibility of a third cannabinoid receptor, with one such candidate being the orphan receptor GPR55. We have developed a functional antagonist aequorin assay to measure intracellular [Ca^{2+}] changes, using HEK293 cells transduced with human GPR55 (hGPR55). This assay technology utilizes the photoprotein aequorin (isolated from jellyfish *Aequoria victoria*), which upon the addition of coelenterazine and binding to intracellular Ca^{2+}results in the production of CO_2 and the emission of luminescence. Using this assay to support an SAR campaign to progress GPR55 antagonists, we found that several compounds screened using this method demonstrated an increase in response to EC_{90} concentration of agonist, indicative of positive modulation of the agonist response. Thus the present study will describe the detailed characterization of these compounds and determine if they are, in fact, positive modulator compounds of the GPR55 agonist response.

P064

The role of Ca^{2+}sensitisation in P2X receptor-induced contractions of rat small pulmonary artery

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P2X receptors are Ca^{2+}-permeable, ligand-gated cation channels, which are activated by ATP. In arteries, such as the rat small pulmonary artery (SPA), they elicit vasoconstriction (Chootip *et al.*, 2002), which is dependent upon extracellular Ca^{2+}. An increase in the sensitivity of the contractile proteins to cytoplasmic Ca^{2+}via Rho kinase (RhoK) and protein kinase C (PKC) can also play a role in vasoconstriction. Thus, the aim of this study was to determine the involvement of Ca^{2+}sensitisation in P2X receptor-mediated contractions of rat SPA, using the RhoK inhibitor Y27632 and PKC inhibitor GF109203X. 5 mm rings of rat SPA were mounted under isometric conditions in 1 mL organ baths at 37°C and a resting tension of 0.5 g. Contractions were elicited by addition of the P2X receptor agonist α,β-meATP (10 μM) or KCl (40 mM) to the bath. Data are expressed as mean ± SEM and were compared by Student's t-test or one-way ANOVA as appropriate. α,β-meATP-induced contractions were significantly decreased by preincubation with Y27632 (10 μM, 70.5 ± 2.4% of control, $n = 4$) or GF109203X (10 μM, 62.8 ± 3.4% of control, $n = 4$) and were further depressed when the inhibitors were co-applied (37.8 ± 6.6% of control, $n = 5$, $P < 0.01$). KCl-induced contractions were also significantly decreased by Y27632 (10 μM) (77.4 ± 3.4% of control, $n = 5$, $P < 0.05$) and GF109203X (10 μM) (44.8 ± 4.7% of control, $n = 4$, $P < 0.001$). Co-application of the two inhibitors (10 μM each) had no greater effect than GF109203X alone (48.7 ± 7.0% of control, $n = 10$, $P < 0.001$). These data show that RhoK and PKC play a role in P2X-evoked contraction of SPA. This is surprising, as the activation of these enzymes is thought to be dependent upon activation of G proteins.

Reference:

Chootip K *et al.* Br J Pharmacol. 2002; 137: 637–646.

P065

Effect of chronic administration of cyclooxygenase-2 inhibitors on the cardiovascular system in L-NAME induced hypertensive rats

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In this study, we examined the effect of chronic administration of COX-2 inhibitors on blood pressure, renal blood flow (RBF) and endothelial function in N-nitro-L-arginine methyl ester (L-NAME) induced hypertensive rats. Three groups of SD rats (300 – 350 g, $n = 6$) were used. The first group served as normotensive control, the second group received oral L-NAME (140 mg/kg/day) for 3 weeks and the third group received both L-NAME (140 mg/kg/day) and the selective COX-2 inhibitor, nimesulide (5 mg/kg/day), orally by gavage for 3 weeks. At the end of the treatment period, the rats were anesthetized with pentobarbital (65 mg/kg) and MAP, HR and RBF were continuously monitored and displayed on a data acquisition system. This was followed by the injection of acetylcholine (ACh, endothelium-dependent vasodilator, 0.1–0.4 μg/kg) and sodium nitroprusside (SNP, endothelium-independent vasodilator 1–4 μg/kg). MAP was increased and RBF was decreased in the L-NAME hypertensive rats. Nimesulide treatment did not affect MAP but significantly attenuated the decrease in RBF induced by L-NAME. ACh and SNP induced dose-dependent decreases in MAP. There were no significant differences in the vasodepressor effect of ACh between normotensive and hypertensive controls but the vasodepressor effect to SNP was increased. Nimesulide did not affect the vasodepressor effect of ACh or SNP. In conclusion, chronic administration of the selective COX-2 inhibitor, nimesulide increased RBF but did not affect MAP in L-NAME hypertensive rats. This suggests that during L-NAME treatment vasoconstrictor prostaglandins may be increased.

P066

***Aspidosperma ulei* inhibits $45Ca^{2+}$ influxes into rat brain synaptosomes and aorta**

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Plants that belong to the genus *Aspidosperma* (Apocyanaceae) are known to be very rich in indole alkaloids and have an ethnomedical history of use as traditional remedies for erectile dysfunction. We described earlier that an alkaloid-rich fraction (SF_{3-5}) from *Aspidosperma ulei* (Markgr) induces penile erection-like behavioural responses in mice possibly through the activation of central dopamine and blockade of presynaptic alpha-2 adrenoceptors with a subsequent enhancement in nitric oxide release from the penile nerves and arteries (Campos *et al.*, 2006) and that SF_{3-5} relaxes isolated rabbit corpus cavernosum strips smooth muscle, at least in part, through a blockade of calcium influx or its function (Campos *et al.*, 2007). In nervous tissue, the calcium (Ca^{2+}) release induces neurotransmitter exocytosis and synaptic plasticity in neurons and is essential for Ca^{2+} waves and oscillations in astrocytes. Coronary and other diseases in cardiac or brain blood vessels are considered to be due to the excessive influx of Ca^{2+} into cytoplasm. If Ca^{2+} channels in cell membrane are blocked by medicines or other substances with considerable calcium antagonistic effects, these diseases might be cured or controlled. In this work, we have investigated the effect of an alkaloidical rich fraction from *Aspidosperma ulei* root bark SF_{3-5} on calcium influx in synaptosomal and aorta preparations under basal and depolarizing conditions. F3-5 caused a decrease on basal and stimulated $^{45}Ca^{2+}$ influx into cortical slices of brain in both tested concentrations (50 and 500 μg/mL). However, the effects of both concentrations seem similar. It can be noted that Ca^{2+} uptake in isolated rat aorta rings in normal physiological status and the Ca^{2+} influxes induced by KCl was markedly inhibited by SF_{3-5}. The results show that extracellular Ca^{2+} influx through receptor-operated Ca^{2+} channels and potential-dependent Ca^{2+} channels can be blocked by SF_{3-5}. This implies that this Brazilian herbal medicine has calcium antagonistic effects.

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P067

F16618, a novel PAR1 antagonist that prevents vasoconstriction and restenosis

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F 16618, a new PAR1 antagonist, prevents human platelet aggregation *in vitro*. Besides this effect on platelet function, antagonism of PAR1 may reduce vasoconstriction and prevents restenosis during a chronic reperfusion of coronary atherosclerotic arteries. Male rats (OFA-SPF, 250–270 g) or male pigs (Landrace 20 kg) were used. The anti-vasoconstrictor effect of F 16618 was evaluated on denuded rat carotid arteries and pig coronary arteries using isometric tension measurements (Emka, France). Anti-proliferative properties of F 16618 were evaluated *in vitro* using cell proliferation assay (WST-1) on human aortic smooth muscle cells or *in vivo* in a rat model of vascular restenosis after balloon angioplasty on the left carotid. Neointimal thickening was evaluated histologically and expressed as neointima/media area ratio (N/M). The PAR1 agonist peptide, SFLLR, induced a concentration-dependent constriction with an EC_{50} value of 8.4 μM and 4.9 μM in rat carotid and pig coronary artery, respectively. F 16618 antagonized the SFLLR-induced contraction with a pA_2 value of 7.7 and 6.2 for carotid and coronary, respectively. F 16618 was also able to inhibit the serum induced human aortic smooth muscle cells proliferation in a concentration-dependant manner (100 ± 2.4% for vehicle group vs. 62.9 ± 2% ($P < 0.05$), 85.2 ± 2% ($P < 0.05$), 105.4 ± 3.9% (ns) for F 16618 32 μM, 10 μM and 3.2 μM respectively, $n = 60$ to 108). Finally, daily *per os* administration of F 16618 following angioplasty reduced the N/M ratio (1.37 ± 0.07 for vehicle group vs., 0.97 ± 0.12 ($P < 0.05$), 1.04 ± 0.11 ($P < 0.01$) for F 16618 at 20 and 40 mg/kg respectively, $n = 9$–15). In conclusion, F 16618 could also be of therapeutic interest for treating vasoconstriction and restenosis attendant to arterial injury besides its anti-aggregant properties.

P068

Oral and intravenous anti-thrombotic activities of F 16618, a new selective PAR1 antagonist with minor risk of bleeding

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F 16618 is a new compound which competitively antagonizes PAR1 giving prevention *in vitro* against human platelet aggregation. The main goal of the study was to investigate the anti-thrombotic effects of F 16618 in an arteriovenous shunt and a photochemical-induced carotid artery thrombosis. The shunt was placed between the jugular vein and the carotid artery in anesthetized males rats (OFA-SPF, 280–300 g). The kinetics of thrombus formation were assessed by a thermic probe fixed on the shunt. F 16618 (10–80 mg/kg) was administered by gavage 15 min before anesthesia. In the second model, F 16618 (40–630 μg/kg) was administered in males guinea pigs (Hartley, 300–400 g) as a bolus, followed by a constant infusion over 15 min immediately after the source of light was turned on, and the kinetics of thrombus formation were assessed by measuring carotid blood flow. F 16618 dose-dependently increased the occlusion time from 10 to 80 mg/kg, p.o, in the arteriovenous shunt model. The first effective dose was 20 mg/kg and the maximal response was found at 40 mg/kg (54 ± 14%, $n = 7$, $P < 0.001$ vs. vehicle). A high dose of F 16618 administered i.v. significantly delayed the occlusion time (29 ± 8%, $n = 10$, $P < 0.05$) without any significant effect on the tail bleeding time (358 ± 26 s, $n = 9$, in the presence of F 16618, vs. 397 ± 38 s, $n = 8$, in the presence of vehicle). The first effective dose of F 16618 in the photochemical-induced carotid artery thrombosis model was 0.16 mg/kg (65 ± 30%, $n = 7$, $P < 0.05$) and the maximal effect was obtained with 0.32 mg/kg (bolus and infusion, 107 ± 35%, $n = 8$, $P < 0.001$ vs. vehicle). Collectively, these results demonstrate that F 16618, a new PAR1 antagonist, dose-dependently prevents platelet-induced arterial thrombosis with minor risk of bleeding by i.v. or oral administration.

P069

Detection of MIP-1β, MIP-1α and RANTES, endogenous ligands of chemokine receptor CCR5, in normal and atherosclerotic human vasculature

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Macrophage Inflammatory Protein (MIP)-1β, MIP-1α and Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES) are high affinity ligands of CCR5. This receptor, with its ligands, is suggested to have a role in atherosclerosis (Krzysiek *et al.*, 1999) and has also been detected in human vascular smooth muscle cells and localised to cells of human atherosclerotic plaque (Schecter *et al.*, 2000). Therefore our aim was to investigate the distribution of CCR5 ligands MIP-1β, MIP-1α and RANTES in the human cardiovascular system. RNA extracted from

the medial layer of human artery and vein including aorta, coronary artery, and saphenous vein, was reverse transcribed and PCR carried out using ligand specific primers (Yang *et al.*, 1997; Quinones *et al.*, 2007). Immunohistochemistry (IHC) was carried out on normal and diseased human cardiovascular tissues using ligand specific antibodies (Abcam, UK), with detection by an avidin/biotinylated enzyme complex. Dual labelling fluorescent IHC used antibodies against von Willebrand Factor (vWF, endothelial cell marker), smooth muscle α-actin (SMαA) and CD3 (T-cell marker). PCR products of the expected size for MIP-1β, MIP-1α and RANTES were detected in the smooth muscle layer of artery and vein. IHC showed localisation of MIP-1β-, MIP-1α- and RANTES-like immunoreactivity (LI) to the medial and endothelial layer of both artery and vein and to intramyocardial blood vessels, this was confirmed using dual labelling IHC. MIP-1β-, MIP-1α- and RANTES-LI was also localised to the medial layer and cells of atherosclerotic plaque in sections of human atherosclerotic coronary artery. Within the plaque, co-localisation was also detected with CD3-LI. Taken together with previous studies investigating CCR5 in the vasculature and atherosclerotic disease, these results suggest widespread distribution of CCR5 ligands in the vasculature and further support a role for CCR5 and its ligands in human atherosclerosis.

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P070

Localisation, binding and functional characterisation of β_2 adrenoceptors in canine isolated airways

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Guinea pigs have been used to assess β_2 adrenoceptor agonists (β_2A agonists) for asthma and COPD (Nials *et al.*, 1993) and dogs have been used for cardiovascular safety and pulmonary pharmacodynamic studies (Dürmüller et al, 2007). There is good translation between guinea pig and human β_2 pharmacology but less is known about the translation from dog to human. This work compared dog, human, guinea pig β_2 adrenoceptors. With local ethical approval lung tissue was obtained from humans and euthanized beagle dogs and guinea pigs. RT-PCR, radioligand homogenate binding experiments, autoradiography and isolated bronchus functional studies were used to characterise the effects of β_2A agonists. Gene expression for β_2 adrenoceptors was confirmed by RT-PCR. In autoradiographic studies specific binding (80–90%) of [^{125}I] iodocyanopindolol to sections of canine trachea, 1, 2 and 3 bronchi and parenchyma was inhibited by 1 μM propranolol. Studies with subtype selective β-adrenoceptor antagonists practolol (β_1), ICI 118551 (β_2) and CL 316243 (β_3) suggested that 35% of adrenoceptors were β_1 subtype and 65% β_2 subtype, with no evidence for β_3 expression based on the compounds used. This ratio was consistent throughout the bronchial tree. The binding affinities of standard β-adrenoceptor ligands for canine recombinant and native β_2 had a rank order of: ICI118551 = propranolol = salmeterol = formoterol > isoprenaline = salbutamol > practolol. There was a good correlation between canine β_2 and canine native tissue lung homogenate binding data ($r = 0.98$). In functional studies, isoprenaline, formoterol and salbutamol elicited concentration dependent reductions in the magnitude of electrical field stimulation response with potencies of isoprenaline and formoterol in good agreement across guinea pig, human and dog. Salbutamol was 5–10 fold more potent in dog than human or guinea pig. This study has demonstrated β_2 receptor protein expression in canine trachea, bronchus and lung tissue and functional responses to standard β adrenoceptor ligands in canine isolated bronchus. There is good translation across guinea pig, human and dog β_2 pharmacology for majority of the compounds tested.

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P071

Pharmacological profile of the adenosine antagonist, BG9928 at adenosine A_{2B} receptors from different species

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BG9928 has high and moderate affinity for the human adenosine (A) A_1 and A_{2B} receptors (R) respectively (Kiesman *et al.*, 2006), reduces myocardial ischemia in dogs (Auchampach *et al.*, 2004) and is in clinical trials for heart failure (Greenberg *et al.*, 2007). A_{2B} receptors are implicated in regulation of vascular smooth muscle tone, cell growth, hepatic glucose balance and mast cell degranulation. Inhibition of the A_{2B}R with BG9928 may contribute to beneficial effects observed to include improvement of diabetes and attenuation of cardiac and renal damage in rats with metabolic syndrome (Tofovic *et al.*, 2003). To better understand the role of the A_{2B}R in different animal models among different species, we performed pharmacological profiling of BG9928 together with several other widely used AR antagonists on the recombinant A_{2B}AR's cloned and stably expressed from human, dog mouse and rat. cDNAs for the mouse, rat, dog and human A_{2B} AR's were obtained by PCR-based methodology, subcloned into expression vectors and introduced into HEK 293 cells by lipofectamine. HEK-293 cells stably expressing A_{2B}R's were grown to 80–90% confluence in 96-well plates and Ca++ flux measured using FLIPR. Concentration-effect curves were constructed to the non-selective agonist, NECA in the absence and presence of BG9928 and a variety of known AR antagonists. At the amino acid level, human and rodent A_{2B}R's were about 89% homologous, while the rat and mouse A_{2B}R's are about 96.7% similar. The dog A_{2B}R is more similar to the human A_{2B}R (94.6% homology) than to either rat (90.96% homology) or mouse A_{2B}R's (90.06% homology). Although the rank order of potency for each antagonist was approximately the same against the A_{2B}R's from all four species, the antagonist potencies differed, especially with BG9928, with the lowest potency on the rat A_{2B}R, and the highest potency on the human A_{2B}R (see Table 1).

Table 1. Dissociation constants for various adenosine receptor antagonists against human, dog, mouse and rat A_{2B} receptors

K_B (nM)	Human	Dog	Mouse	Rat
BG9928	3.53 ± 1.17 (*n* = 3)	47.5 ± 10.2 (*n* = 6)	84.9 ± 20.2 (*n* = 4)	403 ± 146 (*n* = 2)
DPCPX	25.5 ± 6.5 (*n* = 3)	59.8 ± 18.1 (*n* = 6)	51.0 ± 18.6 (*n* = 6)	513.0 ± 0.0 (*n* = 2)
Enprofylline	1696 ± 661 (*n* = 3)	2932 ± 1383 (*n* = 2)	3699 ± 847 (*n* = 4)	5100 ± 4020(*n* = 2)
ZM241385	1.69 ± 1.13 (*n* = 2)	2.52 ± 2.06 (*n* = 2)	24.1 ± 13.4 (*n* = 4)	12.6 ± 2.5 (*n* = 2)
NECA*	870 ± 50 (*n* = 6)	409 ± 45 (*n* = 6)	1196 ± 214 (*n* = 5)	927 ± 58(*n* = 5)

*Agonist EC_{50} values derived from dose-response curves (*n*).

The present study shows inter-species differences in terms of compound potency among the A_{2B} R's. Inhibition of A_{2B} R's in animal models may contribute to some of the beneficial effects observed with BG9928 and may have relevance to potential clinical benefit in humans.

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P072

Signalling pathways activated by serotonin in vascular smooth muscle from human isolated pulmonary arteries

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Serotonin (5-HT) is a potent pulmonary vasoconstrictor and mitogenic agent whose plasma level is increased in patients suffering from pulmonary hypertension. However, the signalling pathways involved in the pharmaco-mechanical coupling in response to 5-HT in human pulmonary arteries are not yet fully elucidated. Thus, we investigated the cellular mechanisms involved in the contractile response induced by 5-HT in human isolated pulmonary arteries (HPA). Rings of HPA (inner diameter 0.5–0.8 mm) were dissected and mounted in an organ bath system to record isometric tension. In some experiments, rings were permeabilized with beta-escin (50 μM, 30 min), for studying the calcium sensitivity of the contractile apparatus. Cultured smooth muscle cells from HPA explants were used to determine the effect of serotonin on intracellular calcium concentration with microspectrofluorimetry (indo-1 as a calcium fluorescent probe). A 5-HT1 receptor agonist, 5-carboxamidotryptamine and a 5-HT2 receptor agonist, R-2,5-dimethoxy-4-iodoamphetamine hydrochloride, both induced a smaller contraction compared to 5-HT. Bath application of Ca^{2+}-free solution or voltage-operated Ca^{2+}channel blockers (nitrendipine or nifedipine) partially reduced the contraction to 5-HT. Thapsigargin and cyclopiazonic acid (CPA), two Ca^{2+}-Mg-ATPase inhibitors, known to deplete sarcoplasmic reticulum-Ca^{2+}stores, partially inhibited the contraction while removal of extracellular Ca^{2+} in those conditions further inhibited the contraction. Contractions were observed, in the presence of nitrendipine and CPA when changing from Ca^{2+}-free to Ca^{2+}containing solution, a protocol known to stimulate store-operated Ca^{2+}channels. Indeed, such contractions were completely blocked by nickel. Nickel or gadolinium, two blockers of voltage-independent Ca^{2+}channels also reduced the contraction to 5-HT. 10 μM 5-HT induced intracellular Ca^{2+}responses with various profiles in cultured HPA smooth muscle cells. Finally, Ca^{2+}-sensitization of HPA contractile apparatus was not altered by 5-HT. Our data show that in human pulmonary arteries, 5-HT1 and 5-HT2 receptors are functional. Voltage-operated and voltage-independent Ca^{2+}channels, as well as Ca^{2+}release participate to 5-HT-induced contractions.

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P074

Addition of ketamine to propofol reduces the incidence of hypotensive episodes during total intravenous anesthesia in enalapril treated hypertensive patients

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Episodes of hypotension occurring during general anesthesia, especially with angiotensin converting enzyme-inhibitors (ACE-Is) treated hypertensive patients, are a serious problem for anesthetists. There are data available showing, that fall of blood pressure in ACE-Is treated patients is deep and demands ephedrine administration. We have shown previously, that ketamine (KET), an anesthetic with hyperdynamic action, given with propofol (PRO) in total intravenous anesthesia (TIVA) can prevent the fall of blood pressure in normotensive patients. In the present study we investigated whether small doses of KET given during general anesthesia with PRO can prevent the fall of blood pressure in hypertensive ACE-Is treated patients. We compared four groups of 40–60-year-old patients scheduled for maxillofacial trauma surgery: two long-term treated hypertensive ones (20 mg/day) ENA+PRO and ENA+PRO+KET, with two normotensive ones (PRO and PRO+KET). The antihypertensive treatment was continued until the day of surgery, the whole induction procedure was provided in the same manner. PRO in a dose of 2 mg/kg was given prior to 0.5 mg/kg of KET. Anesthesia was maintained with standard doses of PRO in TIVA, and KET was added in doses 0.5 mg/kg every 15 minutes. To support analgesia, in groups ENA+PRO and PRO,

fentanyl 0,2-0,5 mg was given. Systolic and diastolic blood pressure (SBP, DBP) as well as heart rate (HR) were monitored continuously throughout the study. Blood pressure in hypertensive patients was well controlled and values on the day of surgery were not significantly different than in normotensive groups. SBP during anesthesia was significantly lower in group ENA+PRO than in PRO group ($P < 0.01$). KET reduced the fall of SBP in ENA+PRO+KET group compared with the ENA+PRO ($P < 0.05$), and prevented hypotensive episodes occurring in the ENA+PRO group (10%) and PRO group (3%). All episodes responded well to fluid therapy. Addition of KET allowed reduction of the doses of PRO in order to achieve satisfactory anesthesia in groups ENA+PRO+KET ($P < 0.01$) and PRO+KET ($P < 0.05$). DBP was significantly lower only in ENA+PRO group ($P < 0.01$). No changes in HR were observed. Our results suggest, that in enalapril treated hypertensive patients small doses of ketamine added to propofol in total intravenous anesthesia can assure satisfactory anesthesia and prevent the occurance of hypotensive episodes often seen after propofol administration.

P075

Marked antithrombotic effect of combined spironolactone and quinapril administration in renovascular hypertensive rats

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An increasing amount of data show that aldosterone may disturb haemostasis, leading to cardiovascular events. The RALES and EPHESUS studies indicate that co-administration of an aldosterone receptor blocker with standard therapy reduces mortality in patients with heart failure, which may be potentially related to the effect of drugs on haemostasis. Thus, the aim of the present study was to investigate the effect of combined spironolactone and quinapril administration on haemostatic parameters in hypertensive rats. Wistar rats with induced 2K-1C hypertension were used in this study (300–350 g, $n = 12–15$). Spironolactone (SPIRO, 20 mg/kg), quinapril (QUI, 3 mg/kg), or VEH (gummi arabici, 5% aq.sol.) were administered p.o. for 10 days. The venous thrombosis was induced by a 2-hour ligation of the vena cava on the 11th day. The formed thrombus was removed and its dry weight was measured after 24 h. Systolic blood pressure (SBP) was measured by the tail-cuff method. Tissue plasminogen activator (t-PA), plasminogen activator inhibitor type-1 (PAI-1), tissue factor (TF) and thrombin-activatable fibrinolysis inhibitor (TAFI) plasma levels were measured by enzyme immunoassays. The results are presented in a Table 1.

Table 1.

$n = 12–15$	Thrombus weight (mg)	Incidence of thrombosis (%)	SBP (mmHg)	t-PA (ng/mL)	PAI-1 (ng/mL)	TF (pg/mL)	TAFI (μg/mL)
VEH	1.9 ± 0.4	91	143 ± 2	6.8 ± 0.3	9.9 ± 0.1	39.6 ± 1.4	10.6 ± 0.1
SPIRO	1.6 ± 0.3	93	132 ± 5*	6.9 ± 0.1	8.0 ± 0.1***	16.6 ± 0.2***	8.8 ± 0.1***
QUI	1.8 ± 0.6	88	130 ± 3**	7.0 ± 0.1	5.9 ± 0.1**	9.8 ± 0.2**	6.8 ± 0.1**
SPIRO +QUI	0.7 ± 0.3*	64	118 ± 6***	7.8 ± 0.2 *### ¤¤	5.2 ± 0.1 ***### ¤¤	2.9 ± 0.2 ***### ¤¤	6.4 ± 0.1 ***###¤

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. VEH; ###$P < 0.001$ vs. SPIRO; ¤$P < 0.05$; ¤¤ $p < 0.01$ vs. QUI.

Spironolactone and quinapril administered separately did not influence experimental thrombosis in renovascular hypertensive rats, although significant activation of fibrinolysis and inhibition of coagulation were observed. The most pronounced changes in haemostatic parameters with a marked reduction in venous thrombus formation were obtained after combined spironolactone and quinapril administration.

P076

Haemodynamic and platelet responses to nitric oxide inhibition during pulmonary thromboembolism

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Pulmonary embolism (PE) is a fatal disease that is due to a blockage of the pulmonary artery in the lungs by thrombi originating from the large vessels of the legs. Occlusion of pulmonary vessels causes ventricular overload which leads to haemodynamic dysfunction and subsequent cardiac hypertrophy and heart failure. We induced experimental PE in anaesthetised male C57-BL6 mice by injecting collagen intravenously. Thromboembolism was measured as accumulation of radiolabelled platelets in the pulmonary region. Collagen induced dose-dependent thromboembolism and an associated dose-dependent deterioration in cardiac haemodynamic function measured as dP/dt_{max} by cannulating the left ventricle with a Millar 1.4Fr pressure catheter was observed. We determined the effect of endogenous and synthetic nitric oxide synthase (NOS) inhibitors on platelet thromboembolism and the haemodynamic changes accompanying collagen (50 μg/kg) -induced PE. Experimental data were compared to vehicle controls by one-way ANOVA and all values are means ± SEM. Collagen (25, 50 & 75 μg/kg, $n = 4–6$) caused a dose-related increase in platelets counts due to an accumulation of thrombi in the pulmonary vasculature, this was associated with a dose-related (25, 50 & 75 μg/kg, $n = 7–11$) decrease in BP and dp/dt_{max}. In the presence of the synthetic NOS inhibitor L-NAME (50 mg/kg) collagen's platelet ($n = 4$) and hemodynamic ($n = 7$) responses were potentiated, however D-NAME (50 mg/kg) had no effect. In the presence of endogenous NOS inhibitor L-NMMA (500 μM) again collagen's platelet ($n = 4$) and hemodynamic ($n = 10$) responses were potentiated. However endogenous NO synthase inhibitor ADMA (1 mM) had no effect on collagen's platelet ($n = 4$) and hemodynamic ($n = 4$) responses. L-NAME, L-NMMA and ADMA all caused a significant increase in BP and dp/dt_{max}. Phenylephrine (2 μg/kg, $n = 8$) caused a similar increase in BP compared with L-NAME; however, unlike L-NAME there was no potentiation of the collagen induced haemodynamic response. This study demonstrates that dose-related PE leads to a dose related decrease in cardiac function and we present an animal model for measuring these parameters. Inhibiting NOS potentates PE leading to a further deteriation in cardiac function and this is likely to be mediated through enhanced platelet responses rather than an increase in BP.

P077

The functional role of the plasma membrane calcium ATPase (PMCA) in platelets

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The plasma membrane calcium ATPase (PMCA) extrudes calcium from a variety of cells within the cardiovascular system. In addition PMCA4 has been shown to regulate calcium dependent signalling molecules such as nitric oxide synthases (NOS). PMCA4 is expressed in platelets; however its functional role in this cell type remains undefined. Our aims were to investigate the roles of PMCA in calcium homeostasis and platelet function and to determine whether there is any association between PMCA and NOS-3, the NOS isoform found in platelets. PMCA inhibitor carboxyeosin (CE) (10–40 μM) dose-dependently elevated $[Ca^{2+}]_i$ in resting platelets. In contrast, CE (10–40 μM) dose-dependently inhibited collagen (5 μg/ml) and thrombin (0.1U/ml) induced calcium release and platelet aggregation. CE also inhibited platelet adhesion but enhanced clot retraction. Association between PMCA and NOS-3 was demonstrated by immunoprecipitation of PMCA from platelet lysates and detection of NOS-3 by Western blotting. Soluble guanylyl cyclase inhibitor ODQ (10 μM) had no effect on the inhibition of aggregation by CE and CE did not increase VASP phosphorylation. In conclusion, PMCA regulates resting $[Ca^{2+}]_i$ in platelets and positively regulates platelet adhesion and aggregation. These effects are time-dependent since in later stages of platelet activation, such as clot retraction, PMCA plays a negative regulatory role. PMCA associates with NOS-3 in platelets but the functional observations following inhibition of PMCA with CE are not dependent on NO-cGMP signalling. PMCA therefore regulates platelet function through mechanisms that remain undefined.

P078

Investigation of the slow kinetics of the prostanoid EP_3 receptor antagonists L-798106 and L-826266 on guinea-pig aorta

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EP_3 receptor antagonists are a recent addition to the prostanoid ligand armoury. We have examined the profiles of two such agents, L-798106 (([(2E)-N-[(5-bromo-2-methoxyphenyl)-sulphonyl]-3-[2′-(2-naphthyl-methyl)phenyl]acrylamide) and its 5′-chloro analogue L-826266 (Belley *et al.*, 2006). Muscle tension in isolated preparations of thoracic aorta and vas deferens (electrical-field stimulation) from Dunkin–Hartley male guinea-pigs (600 - 800 g) was recorded. EP_3 agonism (17-phenyl PGE_2 or ONO-AE-248) on aorta was measured under priming with phenylephrine (500 - 1500 nM). Selectivity of prostanoid ligands was confirmed using human recombinant prostanoid receptor FLIPR assays (co-transfection with chimeric G-protein cDNAs to allow activation of Ca^{2+}flux / HEK-293 EBNA cells). L-798106 and L-826266 (50–1000 nM) slowly inhibited established contraction of the guinea-pig aorta to the primed EP_3 agonist (incomplete at 60 min) in contrast to faster block on vas deferens. Exposure of aorta to antagonist for 3 h resulted in much greater block and parallel displacement of the EP_3 agonist log concentration-response curve; pA_2 value = 7.58 and 7.96 respectively. Phentolamine (100 nM) rapidly inhibited phenylephrine / EP_3 agonist responses. The potent TP antagonist BMS-180291 (Ifetroban) also slowly blocked U-46619 contraction of the aorta at concentrations of 0.3–3 nM ($pA_2 = 9.76$), whereas the less potent antagonists EP-045 and EP-092 had faster onsets. Studies on histamine H_1 antagonists showed that doxepin ($pA_2 = 9.6$), terfenadine ($pA_2 = 7.9$) and astemizole (7.5) were slow to reach steady-state block, while (+)-chlorpheniramine (9.1) was faster and diphenhydramine (7.8) even faster. The slow kinetics of L-798106, L-826266, terfenadine and astemizole, which have modest affinities, may relate to their very high lipophilicity (clogP = 6.87, 7.39, 6.54 and 5.64 repectively; ChemAxon software). In contrast, the slow kinetics of BMS-180291 and doxepin (cLogP = 3.60 and 3.59) are probably a consequence of their high affinity for TP and H_1 receptors respectively. Care is needed in using highly lipophiic EP_3 antagonists to elucidate receptor involvement.

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P079

Investigating the immunomodulatory effects of testosterone on cell type-specific expression of CX3CL1 in human aortic vascular cells

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Atherosclerosis is a chronic inflammatory disease and it has been documented that men with angina and heart failure often have lower levels of circulating testosterone. CX3CL1 (fractalkine), a novel member of the chemokine superfamily, is expressed on the surface of vascular cells and is up-regulated by pro-inflammatory cytokines *in vitro*, suggesting a role in the onset of atherosclerosis. The specific role of CX3CL1 in atherosclerosis, however, is not completely understood. Primary human aortic endothelial (HAEC) and smooth muscle cells (HASMC) were used to investigate *in vitro* expression of CX3CL1 following

pro-inflammatory treatment with tumour necrosis factor and interferon-gamma (TNF+IFN-γ, 100 ng/ml) in the presence or absence of testosterone (T, 0–100 nM). Data obtained through ELISA showed the up-regulation of CX3CL1 under inflammatory conditions (Table 1), and this was confirmed by immunocytochemistry. Cleaved and cell-bound CX3CL1 expression was cell-type specific suggesting a dual function, possibly as a chemotactic agent in endothelial cells and as an adhesion molecule in smooth muscle cells. Testosterone did not prevent CX3CL1 expression in this *in vitro* system (Table 1).

Table 1. ELISA data to show expression of CX3CL1 in HAEC and HASMC

Treatment	HAEC		HASMC	
	Cleaved CX3CL1	Bound *CX3CL1*	Cleaved *CX3CL1*	Bound *CX3CL1*
Control	1.836 (±0.48)	1.624 (±0.17)	0.649 (±0.01)	0.664 (±0.01)
TNF + IFN-γ	13.63 (±0.23)***	3.276 (±0.22)*	11.92 (±0.43)**	25.59 (±0.41)***
TNF + IFN-γ + T	14.16 (±0.32)	3.78 (±0.11)	13.65 (±0.49)	25.43 (±0.75)

(Mean values expressed as ng CX3CL1/mg total protein ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ One way ANOVA. n = 2–4).

In conclusion, upregulation of CX3CL1 by inflammatory agents is not prevented by testosterone in this *in vitro* system. This requires further investigation, but may be due to high levels of cytokines. Uncovering the role of CX3CL1 in atherosclerosis may lead to novel therapeutic targets for anti-atherogenic treatments.

P080

Neuroprotection of cis-beta-carotene from *Dunaliella salina* on SH-SY5Y cells following oxidative challenge

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The antioxidant property of trans-beta-carotene has been well documented, however, little is known about cis-beta-carotene. The present study aim to investigate whether cis-beta-carotene can exert neuroprotective effect on human neuroblastoma SH-SY5Y cells following hydrogen peroxide (H_2O_2) exposure. MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay was employed to analyze cell viability, and apoptosis was monitored by sub-G1 DNA content. The cell cycle distribution was analyzed by flow cytometry. Intracellular calcium concentration, mitochondrial membrane potential and caspase-3 activity were measured by flow cytometry. Western blot was performed to detect Erk1/2 phosphorylation. Pretreatment with cis-beta-carotene markedly blocked SH-SY5Y cell apoptosis. Under oxidative stress, cis-beta-carotene efficiently could maintain intracellular calcium homeostasis and the mitochondrial membrane potential. The activity of caspase 3 was significantly reduced in cis-beta-carotene treated-cells. Furthermore, H_2O_2-induced phosphorylation of Erk1/2 was also inhibited by cis-beta-carotene. These results provided an experimental basis for the pro-survival effect of cis-beta-carotene on dopaminergic neurons under oxidative damage.

P081

Apelin peptides are upregulated in human atherosclerosis

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Apelin peptides are endogenous ligands for the previously designated orphan G-protein coupled receptor APJ. We have shown APJ localised to human cardiomyocyte, vascular endothelial and smooth muscle cells (Kleinz *et al.*, 2005) with apelin peptides restricted to the endothelium (Kleinz *et al.*, 2004). APJ deficiency is protective against oxidative stress-linked atherosclerosis in mice (Hashimoto *et al.*, 2007). Our aim was to investigate whether the apelin-APJ system is altered in atherosclerosis in man. Human coronary artery (CA) samples (obtained with ethical approval) were extracted using SepPac C_{18} cartridges. Apelin peptide levels were determined by radioimmunoassay (RIA) using anti-apelin-36 serum and [^{125}I](Pyr^1)apelin-13. Levels were expressed as mean pg/g wet mass ± SE mean. n-Values are the number of patients from whom tissues were obtained. Immunohistochemistry (IHC) was carried out on sections of human normal and diseased coronary artery using apelin- and APJ-specific antibodies (Phoenix Pharm., Burlingame, CA, USA) with detection by peroxidise/anti-peroxidase method. RIA revealed a significant increase in apelin peptide levels in human atherosclerotic CA (73.2 pg/g, $n = 6$) compared to histologically normal CA (24.1 pg/g, $n = 6$, $P < 0.01$, Student's t-test). IHC revealed apelin peptides restricted to the endothelium in normal CA, whereas intense staining of the atherosclerotic plaque was observed in diseased CA. The localisation of APJ was not altered in atherosclerosis. These data show that apelin peptide levels increase in atherosclerosis in man and that the peptides are localised to the atherosclerotic plaque. Since apelins are mediators of oxidative stress in vascular tissue *in vitro* (Hashimoto *et al.*, 2007), the increase in apelin levels may be detrimental in atherosclerosis.

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P082

Apelin peptides are functional antagonists of endothelin-1 vasoconstriction in the human vasculature

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We have shown that the novel G-protein coupled apelin receptor is present on human vascular smooth muscle cells, endothelium and cardiomyocytes. This receptor is activated by a family of apelin peptides to elicit cardiovascular effects in experimental animals, including hypotension. Our aim was to identify the role of apelin receptors in human vascular and cardiac tissues. We hypothesised that activation of endothelial receptors by the three predominant endogenous isoforms [Pyr^1] apelin-13, apelin-13 and apelin-36 is linked to vasodilatation, that might oppose ET-1 constriction in normal blood vessels. Additionally we investigated the consequence of endothelium removal on apelin responses, to mimic conditions of endothelial dysfunction in disease, and used human saphenous vein and paced atria to discover the role of apelin receptors on vascular smooth muscle and cardiomyocytes. In human endothelium-intact mammary artery all three apelins induced concentration dependent reversal of ET-1(10 nM) constriction with comparable potency and efficacy: [Pyr^1]apelin-13, pD_2 8.8 ± 0.1, E_{max} 39 ± 3% reversal; apelin-13, pD_2 9.2 ± 0.2, E_{max} 51 ± 7%; apelin-36: pD_2 9.1 ± 0.2, E_{max} 43 ± 6%. This vasodilatation was abolished by endothelial removal or preincubation of endothelium-intact mammary artery with indomethacin, but unaffected by preincubation with L-NAME. Apelins were potent constrictors of endothelium-denuded saphenous vein: [Pyr^1]apelin-13, pD_2 8.8 ± 0.5, E_{max}(%KCl) 30 ± 5; apelin-13, pD_2 9.1 ± 0.2, E_{max} 19 ± 5; apelin-36, pD_2 9.2 ± 0.5, E_{max} 17 ± 6. In human paced atrial strips, all three peptides increased force of contraction with subnanomolar potencies: [Pyr^1]apelin- 13, pD_2 9.9 ± 0.2, E_{max} 49 ± 12% CaCl2; apelin-13: pD_2 10.1 ± 0.3, E_{max} 64 ± 16; apelin-36: pD_2 10.4 ± 0.2, E_{max}39 ± 14. These data demonstrate for the first time that the three principal endogenous forms of apelin have comparable potency and efficacy in human isolated vascular tissues. In normal vasculature we hypothesise that apelin peptides will oppose ET-1 induced tone whereas in conditions of endothelial dysfunction these peptides will contribute to unwanted vasoconstriction. Apelins are the most potent inotropic agents yet discovered.

P083

***In vitro* and *in vivo* investigation of NS11021, a novel opener of large-conductance Ca^{2+}-activated K^+ channels on erectile tissue in rats**

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Large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}), located on the arterial and corporal smooth muscle, are attractive targets for treatment of erectile dysfunction. This study examines the effect of NS11021, a new opener of BK_{Ca} channels and sildenafil, a known inhibitor of phosphodiesterase type-5, in rat and human erectile tissue *in vitro* and in an anesthetized rat model *in vivo*. Human erectile tissue was obtained from patients undergoing penile surgery. Isometric tension of human and rat penile arteries and corpus cavernosum strips were recorded. Whole cell patch clamp was used to measure BK_{Ca} currents. In anesthetized male Wistar rats intracavernosal pressure was measured before and after infusion of NS11021, sildenafil, and vehicle. In patch clamp recordings, NS11021 concentration-dependently increased BK_{Ca} currents, an effect which was inhibited by iberiotoxin (IbTX), a selective BK_{Ca} channel blocker. In isolated organ bath experiments, NS11021 (100 nM–0.1 mM) and sildenafil (0.1 nM–30 μM) induced relaxations in both intracavernous artery and corpus cavernosum isolated from rat. NS11021 relaxations were inhibited by high extracellular K^+ solution. IbTX decreased the vasorelaxation induced by NS11021 and sildenafil in human erectile tissue. NS11021 and sildenafil, but not vehicle increased erectile response in anesthetized Wistar rat. The effect of NS11021 on erectile function was abolished in the presence of tetraethylammonium, an inhibitor of Ca^{2+}-activated K^+ channels. In summary, NS11021 through opening of BK_{Ca} channels relaxes both intracavernous arteries and corpus cavernosum strips from rat and man and facilitates erectile responses to cavernous nerve stimulation in anaesthetized rats *in vivo*.

P084

Lipopolysaccharide-induced vascular hypocontractility to endothelin-1 in rats is dependent on non-voltage-gated calcium channels but not on calcium sensitization

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Sepsis is characterized by generalized vasodilation and hyporesponsiveness to vasoconstrictors. The present study aimed to identify the intracellular mechanisms responsible for hypocontractility of vascular smooth muscle following exposure to lipopolysaccharide (LPS). Pulmonary and aortic rings from male Wistar rats (250–300 g) were isolated and incubated for 20 h in culture medium (DMEM+10% FBS) or with LPS (*E. coli* O55:B5, 10 μg/ml). Tissue contraction to endothelin-1 (ET-1: 0.3–100 nM), phenylephrine (PE: 1 nM–10 μM) or 80 mM KCl, and relaxation to nifedipine (0.1–30 μM) were measured. Four-parameter non-linear regression analysis was carried out using Graphpad Prism and significance between groups was determined with Student's paired t-test. Expression levels of protein kinase C (PKC) and phosphorylation of Rho kinase (ROCK), CPI-17 and MYPT1 were assessed by immunoblotting. LPS significantly (**$P < 0.01$) decreased maximal contraction induced by ET-1 (26 ± 4%, $n = 9$), PE (78 ± 2%, $n = 5$) or KCl (20 ± 2%, $n = 64$) in the aorta, whereas pulmonary artery responses were unchanged. In the absence of external calcium, contractile responses to ET-1 were similar between control and LPS-treated aortic tissue. Nevertheless, contraction of the aorta to subsequent increases in extracellular Ca^{2+}(0.01–10 mM) (measured in the presence of 10 μM nifedipine) remained reduced by 57 ± 8%, $n = 5$, compared with control vessels. Vascular relaxation to nifedipine was not affected by LPS and there were no significant changes in the protein expression levels of PKC or phosphorylation levels of ROCK, CPI-17 and MYPT1. In conclusion, LPS induces vascular hyporeactivity to vasoconstrictors in the rat aorta but not in the pulmonary artery. For ET-1, the mechanism of this LPS-induced aortic hypocontractility does not involve decreased calcium sensitivity, but involves changes in external calcium influx through channels other than the voltage-gated calcium channels.

P085

Relationship between Factors II and X on INR in patients with chronic warfarin therapy

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Anticoagulation remains an important therapeutic basis for prophylaxis and management of cardiovascular diseases. Warfarin is the most widely used oral anticoagulant compound, in spite of its complex kinetic and dynamic profiles, making a rational dosing regimen difficult. The aim of the present work was to clarify the existing relationship between several warfarin response markers (Factors II, X and INR) obtained from patients undergoing chronic warfarin therapy and throughout a wide INR range. Data were collected from 80 adult outpatients (the study was approved by the Regional Healthcare Authority). All patients received warfarin in a monotherapy regimen and had been on a stable daily dose for at least two weeks before blood sampling. Prothrombin times (expressed as INR) and factors II and X activities were simultaneously determined using a Behring Fibriminter II and Behring coagulation reagents (calibration according to manufacturer's guidelines and samples determined in duplicate). Figure 1 summarise the obtained results. For INR commonly adopted therapeutic window values (2.0–3.5), moderate correlations were observed regarding factors II and X ($r = -0.35$ for factor II; $r = -0.36$ for factor X). However, for INR values greater than 3.5, factors II and X activities appear to be almost unchanged. In the present work, a nonlinear shape between INR and factors II and X activities was observed. In fact, lower values of both clotting factors are closely related to non-proportional increase on INR value, which means that in this circumstances the predictive capacity and the interest of factors II and X as warfarin response markers can be considered minimal.

P086

Kisspeptins as inotropic agents in human and mouse heart

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Kisspeptin, a 54-amino acid peptide (KP-54), has recently been found to act as a regulator of the gonadotropic axis (Seminara *et al.*, 2003) in addition to having roles as a metastasis suppressor and in trophoblast invasion. KP-54 and the C terminal fragments KP-13 and KP-10 bind to the recently de-orphanised KISS1 receptor, previously known as GPR54. Kisspeptin inhibits matrix metalloproteases-2 and -9, both of which are produced by cardiomyocytes. Additionally, we have reported vasoconstrictor activity of the kisspeptins in human umbilical vein and atherosclerosis-prone vessels (Mead *et al.*, 2007) and have detected kisspeptin and KISS1 immunoreactivity in endocardial endothelial cells and cardiomyocytes of the human heart (Mead E, unpublished data). We hypothesise that kisspeptin is an inotropic agent in both human and mouse heart, acting through the KISS1 receptor. 4mm human atrial appendage strips and atria from male 129S6/SvEv mice (20–30g; euthanised by CO_2 inhalation) were set up in organ baths and paced using field stimulation (<4 V). Tension on the tissue was adjusted to 50% of optimum resting tension before construction of cumulative concentration response curves to KP-54, -13 and -10 and termination by $CaCl_2$ addition (6.7 mM). Agonist responses were expressed as $\%CaCl_2$ and data are mean ± SEM. In human paced atrial appendage, KP-10, KP-13 and KP-54 induced positive inotropic effects with comparable potency (pD_2: 10.60 ± 0.73, 10.38 ± 0.57, 10.10 ± 0.44) and maximal response (E_{max}: 75.7 ± 8.0, 85.2 ± 14.0, 89.8 ± 13.9) respectively ($n = 5$; $P > 0.05$, ANOVA). In mouse atria, KP-54 also induced positive inotropic effects (pD_2: 10.19 ± 0.51, E_{max}; 27.3 ± 5.8; $n = 4$). In conclusion, kisspeptins appear to act as inotropic agents in both the human and mouse heart. All three peptides had comparable potency, indicating that the activity is retained in the final 10 amino acids.

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P087

Reversal of cardiac fibrosis by continuous low dose corticosterone infusions in spontaneously hypertensive heart failure (SHHF) rats

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Cardiac fibrosis plays a crucial role in heart failure related cardiac remodeling. Excessive collagen deposition results in stiffening of the heart and dispersed electrical conductance and hence contributes to heart function impairment and arrhythmias. Thus, reducing cardiac fibrosis seems to be an important therapeutic goal. This is supported by the fact that the therapeutic outcome of chronic heart failure patients is closely linked to the extent by which cardiac fibrosis is reduced. Glucocorticoids are well known for their anti-inflammatory and anti-fibrotic properties, but their effects on hypertension induced heart failure related cardiac fibrosis, so far have not been thoroughly explored. Therefore, 42 weeks old male lean spontaneously hypertensive heart failure (SHHF) rats ($n = 8$ per group), were treated with continuous intravenous infusions of corticosterone (at 6 or 60 μg/day). We have previously shown that this rat strain possesses marked cardiac fibrosis, which is stable between 38 and 52 weeks of age and therefore is an attractive model to study anti-fibrotic agents in the setting of hypertension and heart failure related cardiac remodeling. Following four weeks of treatment, left ventricular (LV) pressure recordings (under pentobarbitone anesthesia, using a catheter tip micromanometer) were conducted to monitor LV function. Sirius red histology was used to assess LV collagen levels. LV procollagen I and III gene expression was determined by quantitative RT-PCR. Mean arterial blood pressures and heart rates (pentobarbitone anesthesia) were similar between rat groups. Corticosterone infusions decreased LV collagen levels ($P < 0.05$) by 26 and 51% (at the 6 or 60 μg/day infusions). In parallel procollagen I and III mRNA were dose dependently decreased by the corticosterone infusions, but this only reached significance for the 60 μg/day corticosterone dose (67 and 65% decrease for procollagens I and III respectively, $P < 0.05$). In addition, LV end diastolic pressure was decreased (by 47%, $P < 0.05$) by the 60 μg/day corticosterone infusions, whereas basal or maximal (dobutamine induced) left ventricular contractilities and relaxation were not changed. Thus, continuously infused low dose corticosterone potently reverses pre-existent cardiac fibrosis. However, whether and under which conditions reversal of cardiac fibrosis by glucocorticoids is of potential therapeutic benefit still needs to be investigated.

P088

Evaluation of performance of a new algorithm for minipig ECG analysis

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Due to its physiological similarities with humans and its sensitivity to a wide variety of drugs, the minipigs have become very popular for pharmaceutical studies in Europe (Svendsen, 2006; Stubhan *et al.*, 2008). One of the main difficulties for minipig ECG analysis is the spontaneous changing in the T-wave polarity, and the

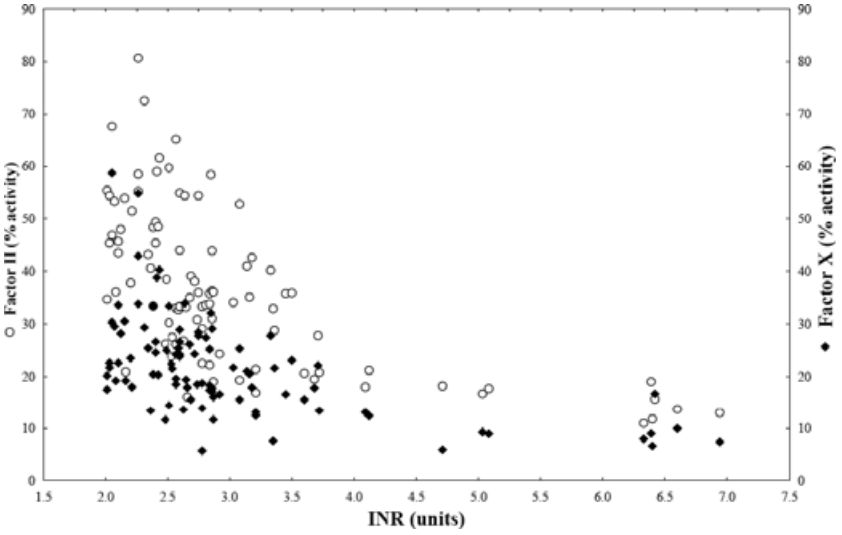

Figure 1 of P085. Relationship between both clotting factors (II and X) and INR.

diversity of T-wave morphologies that can occur. We have developed a new algorithm to cope with this issue. A key feature is its ability to track the different T-wave end candidates and discriminate between them by taking into account both the shape and the previous T-wave end positions. To evaluate the performance of the algorithm, we tested its ability to accurately position fiducial points and to track changes of interval duration on long files. Evaluation was carried on a database composed of 15 ECG signals files, recorded on freely moving pigs and minipigs, with durations varying from 15 min to 27 h. 150 zones were selected with 10–22 beats each, representative of the diversity of shapes and noises that can be found. Fiducial points were marked manually by an expert, who also set a tolerance, defining accepted distance between manual and automated positioning for each zone. Detection sensitivity (ability of the analyzer to detect every fiducial point) and positive predictivity (ability of the analyzer to detect only fiducial points) were above 94% for each fiducial point considered (for T-end : 94.4 ± 12.7% and 97 ± 8.2%, respectively). The mean parameter values for interval durations obtained by automated analysis are highly comparable to those obtained manually for each analysis zones, enabling representative tracking of change of QT duration.

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P089

The relaxant effect of hydrogen sulphide is enhanced in aorta but not in anococcygeus muscle isolated from streptozotocin-induced diabetic rats

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Hydrogen sulphide (H_2S) is considered as a gaseous transmitter which has potent relaxant effect on vascular and nonvascular smooth muscles (Hosoki *et al.*, 1997). We have investigated the relaxant effect of H_2S in thoracic aorta and anococcygeus muscle isolated from nondiabetic and streptozotocin (35 mg/kg)-induced diabetic (4 weeks) male Sprague–Dawley rats (200–300 g). The tissues were mounted in organ baths filled with Krebs–Henseleit solution (37°C) and isometric changes in tension were recorded. The relaxation response to H_2S donor sodium hydrogen sulphide (NaHS) was elicited in phenylephrine-precontracted aorta and anococcygeus muscle, and expressed as % papaverine (0.1 mM)-induced relaxation. Data are given as mean ± SEM (n = 5–7 in each group). Statistical analysis was done by using Student's t-test and $P < 0.05$ was considered significant. NaHS elicited concentration-dependent relaxation response in a narrow range of 0.1–1 mM and with a maximum of 58.21 ± 7.75% in aorta and 85.18 ± 3.36% in anococcygeus muscle. ATP-sensitive potassium channel (K_{ATP}) blocker glibenclamide inhibited the relaxation response in the aorta ($P < 0.05$) but not in the anococcygeus muscle. Incubation with the non-selective potassium channel blocker tetraethylammonium (1mM) and the inhibitors of guanylate cyclase (ODQ, 100 μM), adenylate cyclase (SQ-22536, 100 μM), protein kinase A (KT-5720, 1 μM), protein kinase C (H-7, 30 μM) did not affect NaHS-induced relaxation in the anococcygeus muscle. The maximum relaxation elicited by NaHS in aorta isolated from diabetic rats was increased to 95.18 ± 1.65% which was significantly different from nondiabetic rats ($P < 0.05$). However, the relaxation to NaHS in anococcygeus muscle from diabetic rats (77.54 ± 5.66%) was not altered. Our results show that, unlike vascular tissues i.e. aorta, NaHS-induced relaxation in the anococcygeus muscle does not include K_{ATP} channels. Also other potassium channels, guanylate cyclase, adenylate cyclase, protein kinase A and C are not involved in this response. Furthermore, enhanced relaxation to NaHS in aorta but not in the anococcygeus muscle in short term experimental diabetes, indicates another difference between vascular and nonvascular tissues in the relaxant effect of H_2S.

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P090

Red wine decreases postischemic myocardial injuries in isolated rat heart

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Mild-to-moderate red wine consumption is associated with a reduced incidence of mortality and morbidity from coronary heart disease, especially myocardial infarction. An important consequence of myocardial ischemia and reperfusion is the occurrence of arrhythmias, including a potentially lethal ventricular fibrillation. The possible antiarrhythmic effects of native red wine have not been investigated yet. The aim of the present study was to study the effects of native red wine (Cabernet Sauvignon) perfusion on the postischemic myocardial injury *in vitro*. The experiments were carried out on isolated rat hearts (Wistar rats, n = 30, weight 250–280 g) of both sexes according to Langendorff. Postischemic myocardial injuries during reperfusion were determined by changes in coronary flow rate, lactate dehydrogenase (LDH) release rate, electrocardiogram analysis, incidence and duration of arrhythmias. The effects of red wine were compared to the effects of clinically used antiarrhythmic drug propafenone. Statistical analysis was performed with one-way ANOVA, followed by Bonferroni post-test. Red wine was proved to be effective in the prevention of arrhythmias. The duration of arrhythmias decreased in red wine (3%) group to 1.9 ± 0.7% ($P < 0.001$), in red wine (1%) group to 2.6 ± 0.6% ($P < 0.001$) and in propafenone treated group to 8.2 ± 2.2% ($P < 0.01$), compared to the control group of hearts. Red wine at 1% concentration decreased LDH release rates by 81.8 ± 7.4% ($P < 0.001$) and propafenone by 76.2 ± 6.2% ($P < 0.001$) compared to the control group of hearts. Between propafenone and red wine treated groups no significant differences were observed. Red wine showed as potent antiischemic and antiarrhythmic effects in ischemia-reperfusion injury of isolated rat hears as propafenone.

P091

The influence of regular 6-weeks red wine intake on vascular reactivity in subjects with risk factors for atherosclerosis

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Endothelial dysfunction, caused by a number of risk factors, is an early phenomenon in the process of atherogenesis, which is clinically expressed later in life. Several studies suggest that short-term red wine intake induces favourable effects on vascular reactivity probably through improving endothelial performance. The aim of the present study was to evaluate the effects of moderate regular 6-weeks native red wine drinking on the brachial artery diameter and reactivity of subjects with risk factors for atherosclerosis. Twelve healthy adult subjects (6 males and 6 females) with slightly elevated blood pressure values, no history of diabetes mellitus, BMI between 20 and 26 kg/m^2, absence of liver and endocrine disease, and total plasma cholesterol levels between 6 and 7 mmol/l were recruited for the study. Subjects were randomly allocated to drink either 0.2 l (women) or 0.3 l (men) of native red wine daily for 6 weeks. Ten normal subjects were used as controls for vascular reactivity. The ultrasound measurements of brachial artery reactivity were repeated for three times at every week examination: the initial ultrasound measurements, after the cuff inflation (flow-mediated vasodilation) and 20 min after a glass of red wine (red wine-mediated vasodilation). In subjects involved in our study the mean baseline brachial artery diameter increased from the beginning to the end of the study (0.34 ± 0.06 mm vs. 0.40 ± 0.03 mm; $P < 0.05$). Red wine intake increased flow-mediated vasodilation compared to the controls (109.1 ± 8.7% vs. 116.0 ± 7.9%; $P < 0.05$). Red wine-mediated vasodilatation was significantly improved after 6 weeks of regular red wine intake compared to the controls (107.3 ± 6.6% vs. 112.8 ± 7.6%; $P < 0.01$). Basal tonus and endothelial-dependent vasodilation of brachial artery in subjects with risk factors for atherosclerosis was significantly improved after regular 6-weeks red wine intake.

P092

Effects of articaine on action potential configuration and ionic currents in isolated canine ventricular cardiomyocytes

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Articain is widely used in everyday clinical practice as a local anesthetic but little information is available on its cellular cardiac effects (Moller and Covino, 1993). Our aim was, therefore, to elucidate the dose-dependent effects of articaine on the action potential morphology and the underlying ionic currents in canine ventricular myocytes isolated from the hearts of adult male mongrel dogs weighing 10–20 kg. Action potentials were measured on enzymatically dispersed left ventricular mid-myocardial cells using sharp microelectrodes (Szabo *et al.*, 2005; Horvath *et al.*, 2006). Conventional patch clamp and action potential voltage clamp techniques were used to study the effects of articaine on the transmembrane ionic currents (Hamill *et al.*, 1981; Fischmeister *et al.*, 1984). Articaine produced dose-dependent changes in various action potential parameters. The drug shortened the action potentials, decreased their amplitude and maximum velocity of depolarization (V_{max}), suppressed the early repolarization and depressed the plateau phase. The EC_{50} value obtained for the V_{max} block was 162 ± 30 μM. The reduction of V_{max} was more prominent at shorter pacing cycle lengths indicating a rate dependent V_{max} block, having rapid offset kinetics (tau = 91 ± 20 ms). The effect on action potential duration was also frequency dependent. The reduction of action potential duration was more pronounced at lower stimulation rates. Under voltage clamp conditions a variety of ionic currents were blocked by articaine: I_{K1} (EC_{50} = 372 ± 46 μM), I_{Kr} (EC_{50} = 278 ± 79 μM), I_{Ks} (EC_{50} = 326 ± 65 μM), I_{to} (EC_{50} = 365 ± 62 μM) and I_{Ca} (EC_{50} = 471 ± 75 μM). The Hill coefficients were close to unity indicating a single binding site for articaine, except for I_{K1}. Articaine can modify cardiac action potentials and ionic currents at concentrations higher than the therapeutic range which can be achieved only by overdose. Since its suppressive effects on the inward and outward currents are relatively well balanced, the articaine-induced changes on action potential morphology can be moderate even in the case of intoxication.

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P093

Ca^{2+}-signalling pathways involved in P2X receptor-induced contractions of rat small pulmonary artery

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P2X receptors are Ca^{2+}-permeable, ligand-gated cation channels, which are activated by ATP. In arteries, including the rat small pulmonary artery (SPA), they elicit vasoconstriction (Chootip *et al.*, 2002), which is dependent upon extracellular Ca^{2+}. The aim of this study was to determine the relative contributions of Ca^{2+}influx via the P2X receptor pore and voltage-dependent CaV1.2 (L-type) Ca^{2+}channels, and the role of Ca^{2+}-induced Ca^{2+}release (CICR) in P2X receptor-mediated contractions of rat SPA. 5 mm rings of rat SPA were mounted under isometric conditions in 1ml organ baths at 37°C and a resting tension of 0.5 g. Contractions were elicited by addition of the P2X receptor agonist α,β-meATP (10 μM) or KCl (40 mM) to the bath. Data are expressed as mean ± SEM and were compared by Student's t-test or one-way ANOVA as appropriate. Contractions evoked by α,β-meATP were abolished when tissues were bathed in Ca^{2+}-free buffer (n = 5) and inhibited by 56 ± 6% by nifedipine (1 μM) (n = 5, $P < 0.05$), 47 ± 9% by $CdCl_2$,

(100 μM) ($n = 5$, $P < 0.05$) and 56 ± 9% by nifedipine (1 μM) plus $CdCl_2$, (100 μM) ($n = 6$, $P < 0.01$). These treatments each abolished contractions evoked by KCl ($n = 4$–5). To study the role of CICR, the sarcoplasmic Ca^{2+}stores were depleted by pretreatment with ryanodine (10 μM) and caffeine (10 mM). Under these conditions the contractions to α,β-meATP were unchanged (99.9 ± 7.5% of control, $n = 7$), whereas the response to KCl was significantly reduced (54.4 ± 3.6% of control, $n = 5$, $P < 0.05$). These data show that P2X-evoked contraction of SPA predominantly depends equally upon influx of extracellular Ca^{2+}through the P2X receptor pore and CaV1.2 voltage-operated Ca^{2+}channels. However, CICR from sarcoplasmic reticulum Ca^{2+}stores does not appear to play a role.

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P094

Effects of simvastatin, proanthocyanidin and silymarin on atherosclerosis developed in rabbits

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Aterosclerosis is a complex and slow developing illness of the cardiovascular system and it is known that there are many factors effecting its formation. In recent years, the most valid theory is oxidation. The aim of our study is to investigate effects of antioxidants and simvastatin on vessel activity and plate formation in rabbits fed with a high cholesterol diet. In our study, 35 male rabbits (New Zealand, 2200–2500 kg) were used. Experiment groups were fed with 2% cholesterol (Col) diet for 12 weeks. In addition to cholesterol diet, proanthocyanidin (Pro) 100 mg/kg, silymarin (Sily) 10 mg/kg and simvastatin (Sim) 10 mg/kg were given. Isolated organ bath responses were evaluated from the aspects of histologic and lipid profiles. In the conducted histological investigation, it was observed that there was a significant reduction in the numbers and dimensions of arteriosclerotic plates in the Pro+Col, the Sim+Col and the Sily+Col groups, respectively while lipid profiles got better. A concentration dependent relaxation is observed at control group during the process at isolated organ bath. In the cholesterol group a meaningful relaxation reduction compared with the control group was observed. In the Pro+Col and the Sily+Col groups a relatively less relaxation compared to Sim+Col and more compared to cholesterol group is observed which was found to be statistically meaningful ($P < 0.05$). However; statistically there was no significant difference between the Pro+Col and the Sily+Col groups ($P > 0.05$) It can be concluded that antioxidants cannot prevent the developed arteriosclerosis. However, they can keep it in its status and prevent its further progress. Besides, antioxidants have a regulating role on blood lipid profiles. We believe that antioxidants such as proanthocyanidin and silymarin can only be used as a support in addition to antihyperlipidemic drugs.

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P095

L-NAME augments β-adrenoceptor desensitization in isolated rat main pulmonary artery

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Beta-adrenoceptor (beta-AR) stimulates adenylyl cyclase activation followed by an elevation in intracellular cAMP level in smooth muscle. There are controversial studies suggesting the role of endothelium/nitric oxide in beta-AR-induced vasodilations. Furthermore, beta-AR-mediated vascular responses can undergo desensitization and the mechanism is not yet fully understood. Therefore, we studied the mechanisms of isoprenaline (activates all subtypes of beta-AR) -induced vasorelaxation in isolated rat (Sprague Dawley, male, 200–300 g) main pulmonary artery (PA). PA rings were mounted in organ baths filled with Krebs–Henseleit solution at 37°C (pH 7.4). Isometric tension changes were recorded and expressed as % of precontraction with submaximal U-46619 (TxA_2 agonist; 30 nM). Data are given as mean±SEM. Statistics was done by Student's *t*-test and $P < 0.05$ was accepted as significant. Cumulative isoprenaline relaxation responses were elicited (10^{-10} to 10^{-6} M, E_{max}=75.1 ± 3.3%, $n = 8$) in endothelium intact PA rings. These relaxations were not altered by nitric oxide synthase inhibitors N_ω-nitro-L-arginine methyl ester (L-NAME; E_{max}=68.9 ± 6.1%, $n = 6$), N(5)-(1-iminoethyl)-L-ornithine (L-NIO; E_{max}=76.3 ± 4.3%, $n = 4$) or endothelium removal (E_{max}=75.4 ± 2.2%, $n = 6$). On the other hand, 60 mM K^+ inhibited isoprenaline-induced vasorelaxation responses (E_{max}=18.8 ± 4.3%, $n = 4$). A slight desensitization (E_{max}=59.5 ± 5.9%, $n = 4$) was observed in the second isoprenaline relaxation curve which was elicited 1 hour after the initial curve. Removal of endothelium did not affect this desensitization pattern having a maximum relaxation of 61.3 ± 1.2% ($n = 4$). However, unexpectedly L-NAME enhanced desensitization in both endothelium intact (E_{max}=35.8 ± 4.7%, $n = 4$) and denuded (E_{max}=41.2 ± 4.0%, $n = 4$) rings. Our preliminary results suggest that vasodilator effects of isoprenaline were mainly due to activation of beta-AR on vascular smooth muscle rather than the release of endothelium-derived relaxing factors (such as nitric oxide). In addition, we may propose that a modest desensitization occurs in rat PA without the effect of aging factor and this desensitization is susceptible to non specific effects of L-NAME.

P096

The role of serotonin, 5-HT2B, 5-HT4 receptors and catecholamines in myocardium contraction regulation in patients with chronic heart failure

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The role of serotonin (5-HT) in myocardium regulation of patients with chronic heart failure (CHF) is not well identified. The contraction force of human atrial strips in response to application of 10.0 μM 5-HT, agonists of 5-HT2B, 5-HT4 receptors and adrenaline (A) was measured in isometric setup force. We determined the expression of 5- HT2B, 5-HT4 receptors in human right atria by immunohistochemistry. The patients with CHF were divided into three groups: patients with diastolic cardiac dysfunction (1 group), patients with diastolic cardiac dysfunction with hypertrophy of left ventricle (HLV) (2 group), and patients with diastolic and systolic cardiac dysfunction (3 group). The control group is people without cardiovascular diseases. The Tatarstan Ethical Committee has approved the study. 5-HT had positive inotropic effect on atria. 5-HT2B and 5-HT4 immunoreactivity were found in all groups, but not in all cases. The agonist 5-HT2B receptor had positive inotropic response only on atria of 2 group. The agonist 5-HT4 receptor had positive inotropic response on atria of all groups, but not in all cases. Patients with HLV had a maximal inotropic response to 10.0 μM 5-HT (149.39 ± 25.92%) and a minimal inotropic response to 10.0 μM A (69.13 ± 17.76%) in comparison with patients in the first (64.75 ± 23.46%, 204.26 ± 49.20%, respectively) and third (61.28 ± 15.43%, 148.19 ± 40.92%, respectively) groups. Thus, we established that HLV is characterized by the predominance of serotoninergic regulation under adrenergic regulation. Expression of the 5-HT2B and 5-HT4 receptors in failing human heart was demonstrated.

P097

The particularities of biological activity of a new cardiotonic with different ways of administration

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The new original compound - dipotassium salt N-succin-d, L-triptophane-suphane shows inotropic activity, reduces acute heart failure, possesses the antiangor and antioxidant properties. The aim of the study is to define experimentally the criteria of possible negative influence of sulphane on the organism with different ways of administration. The studies were carried out on Wistar rats ($n = 65$), the ages of acute toxicity are defined, the ability for dermal resorbtion (10-times per 4 hours, 20 mg/cm^2), the particularities of toxical dynamics (30-times per os, 500 mg/kg). On the model of adrenalin inducted heart failure sulphane normalizes the indexes of lipid metabolism, and decreases the activity of free-radical reactions. According to the criteria DL_{50} it is one of nontoxic compound. With dermal administration the changes have been seen in coagulogram and central nervous system function. The long term peroral administration ($5 \times ED_{50}$) showed positive reaction to the lipid blood spectrum, cardiotonic and antioxidant reaction, central nervous system stimulation. In the condition on one-time inhalant exposition (concentration 24,16 and 9 mg/m^3) we have seen the quick repolarization of atrium and ventricle of heart, the atrium beat contraction (24.16 mg/m^3), reducing of physical activity of rats and increase of their emotional reactivity (24,16 and 9 mg/m^3). In this way the original nonglycoside cardiotonic suphane is a non toxic compound which causes a positive effect on the lipid metabolism, shows the cardiotonic and antioxidant reaction on the doses level bigger than ED_{50}. The criteria of toxic genesis of suphane without reference to the administration way are the changes of central nervous system functional condition.

P098

5-HT4-elicited positive inotropic response is mediated by cAMP and regulated by PDE3 in failing rat and human ventricle

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The ventricle of the failing rat heart becomes sensitive to serotonin (5-HT) paralleled by the appearance of functional Gs-coupled 5-HT4 receptors. The objective of this study was to explore regulatory functions of phosphodiesterases in the ventricular 5-HT4-mediated functional effects induced in failing rat and human heart. Post-infarction heart failure was induced by coronary artery ligation in male Wistar rats. Contractility was measured in rat left ventricular papillary muscles 6 weeks after surgery and in ventricular trabeculae from explanted human hearts. cAMP was quantified by RIA. In papillary muscles from postinfarction rat hearts 5-HT4 stimulation exerted positive inotropic and lusitropic effects accompanied by increase of cAMP. The inotropic effect was increased by the non-selective PDE inhibitor IBMX (10 μM) and by the PDE3 inhibitor cilostamide (1 μM). The PDE2 inhibitor EHNA (10 μM) and the PDE4 inhibitor rolipram (10 μM) did not increase the inotropic response. Combined PDE3/4 inhibition enhanced the inotropic response beyond the effect of PDE3 inhibition alone and increased the sensitivity to serotonin. The lusitropic effect was increased only during combined PDE inhibition. In failing human ventricles, the 5-HT4-mediated positive inotropic response was regulated by PDEs in a similar manner as in post-infarction rat hearts. The 5-HT4-mediated positive inotropic response in failing rat ventricle is cAMP-dependent. PDE3 is the main regulator of this response and the involvement of PDE4 is demasked by inhibition of PDE3 in both post-infarction rat and failing human hearts.

P099

Comparison of the re-assertion profile of the β2 adrenoceptor agonists, carmoterol and indacaterol with salmeterol in the electrical field stimulated (EFS) guinea pig isolated trachea

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In the electrical field stimulated (EFS) guinea pig isolated trachea, salmeterol shows persistent receptor activation with a profile termed re-assertion (Nials *et al.*, 1994). The aim of this communication is to compare the re-assertion profiles of carmoterol and indacaterol (Battram *et al.*, 2006 and Cazzola *et al.*, 2005) with that of salmeterol in the EFS stimulated guinea pig isolated trachea. Tracheas were removed from euthanized male Dunkin-Hartley guinea pigs (475–525 g) and strips mounted in 5 mL organ baths in warmed (37°C), aerated ($95\%O_2/CO_2$) Krebs' solution (flow rate of 1 ml/min) containing indomethacin (3 μM) and guanethidine (10 μM) under 1 g wt. initial tension. Following equilibration for 60 min tissues were then stimulated by EFS (0.1 ms, 10 Hz for 10 s every 2 min) at sub-maximal voltage (10–30 V). Salmeterol and indacaterol were tested at approximately IC_{50} concentration, whereas carmoterol was tested at approximately 10 and 100 times the IC_{50}. Following plateau, a cumulative concentration response curve to sotalol (10–100 μM) was constructed until 100% reversal of the EFS response was obtained. Tissues were then infused with agonist Krebs' solution. If the inhibition of EFS re-asserted this was left to plateau and the sotalol/wash cycle was repeated for a maximum of 3 cycles. Data is summarised in table 1 and is mean with 95% confidence intervals, n = 4–18.

Table 1. Reassertion profiles of salmeterol, carmoterol and indacaterol

Compound	Initial % inhibition induced by compound	Sotalol/wash re-assertion cycle		
		1% inhibition	2% inhibition	3% inhibition
Salmeterol A (3 nM)	67.0 (60.4–73.6)	75.9† (69.8–82.0)	75.8† (70.2–81.3)	81.2† (74.2–88.1)
Carmoterol A (10 nM)	93.6 (91.4–95.8)	72.7* (57.6–87.7)	9.7* (–8.4–27.8)	n/d n/d
B (100 nM)	91.3 (88.4–94.2)	75.7* (66.6–84.7)	59.7* (43.0–76.3)	68.6* (54.1–83.1)
Indacaterol A (3 nM)	45.8 (33.6–57.9)	49.1† (37.7–60.5)	50.5† (40.5–60.5)	48.0† (36.8–59.2)

*significantly ($P < 0.05$) and †not significantly ($P > 0.05$) different from initial % inhibition, unpaired t-test.

Salmeterol and indacaterol exhibited multiple re-assertions. The degree of inhibition at the third re-assertion cycle is not significantly different from initial inhibition induced, at IC_{50} concentrations ($P > 0.05$). In contrast carmoterol only exhibited more than one re-assertion profile with concentrations of 100x IC_{50}. Thus carmoterol, but not indacaterol exhibits a concentration dependent re-assertion profile.

References:

Battram *et al.* J Pharmacol Exp Ther. 2006; 317: 762–770.
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P100

***In vitro* and *in vivo* characterisation of anti-murine IL-13 antibodies recognising two distinct functional epitopes**

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IL-13 sequentially binds to IL-13Rα1 and IL-4Rα to form a high affinity signalling complex. The IL-13Rα1-IL-4Rα receptor complex, expressed on cells in the airways, signals through STAT6 and stimulates the production of chemokines, cytokines and mucus. We have generated antibodies, using the UCB selected lymphocyte antibody method (SLAM), that block either binding of murine IL-13 to IL-13Rα1, or block recruitment of IL-4Rα to the IL-13-IL-13Rα1 complex. Using surface plasmon resonance, mAb A was shown to bind to IL-13 with high affinity and prevent binding of IL-13 to IL-13Rα1. In contrast mAb B, bound IL-13 with matched affinity to mAb A, was shown to prevent recruitment of IL-4Rα to the IL-13-IL-13Rα1 complex. *In vitro*, mAbs A and B demonstrated equipotent neutralisation of IL-13-stimulated STAT6 phosphorylation (IC_{50} mAb A 37 ng/ml; IC_{50} mAb B 38 ng/ml), and TF-1 cell proliferation (IC_{50} mAb A 80 ng/ml; IC_{50} mAb B 90 ng/ml). *In vivo*, mAbs A and B demonstrated equipotent, dose-dependent inhibition of eotaxin generation in mice (male, Balb/c, 21 g) stimulated by intraperitoneal administration of murine recombinant IL-13 (ED_{75} mAb A 10.2 mg/kg i.v.; ED_{75} mAb B 6.81 mg/kg i.v., n = 6–8). In an allergic lung inflammation model in mice (male, Balb/c, 24 g), mAb A and B dose-dependently inhibited muc5ac mucin mRNA upregulation in lung tissue measured 48 h after intranasal allergen challenge (ED_{75} mAb A 0.62 mg/kg s.c.; ED_{75} mAb B 0.41 mg/kg s.c., n = 8). In summary, we have demonstrated high affinity neutralising antibodies recognising two distinct functional epitopes on IL-13 are equipotent in blocking IL-13 signalling events in cell-based assays. *In vivo*, mAbs A and B were equipotent in blocking eotaxin generation stimulated by recombinant IL-13, and mucus gene upregulation stimulated by endogenous IL-13. These data support the design of therapeutics for the treatment of allergic airways disease that inhibit assembly of the high affinity IL-13 receptor signalling complex, by blocking the binding of IL-13 to IL-13Rα1 or subsequent recruitment of IL-4Rα.

P101

Pharmacological and toxicological properties of ofloxacin and ambroxol, drugs used in respiratory diseases treatment

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Diseases of the respiratory system prove to be the most wide-spread in contemporary Ukraine, constituting 36% of the first registered. Their pharmacological correction is primarily based on application of the 3-cycle fluor-hynolon antibiotics (ofloxacin – *OF*) and mucolytic drugs (ambroxol – *AM*). The aim of our study was to assess peculiarities of OF and AM pharmaco- and toxicodynamics. Experiments were conducted in the adult male Wistar rats and white mice, and involved integral, physiological, biochemical, immunologic, allergologic and morphological methods. In therapeutical doses OF exhibits high antimicrobial activeness due to its ability to inactivate DNA-hyrase, impair the synthesis of DNA and the process of cell division in microorganisms. AM helps regain reological properties of sputum and eliminate cough due to both its secretory motor activity and stimulation of the low viscosity tracheo-bronchial secret formation and surfactant production. AM normalizes functions of serous and mucous glands of the bronchus mucous membrane, possesses anti-edematous, anti-inflammatory and immune-modulating activity, and protects the lung tissue under oxidative stress. AM exhibits a fast therapeutical effect, its maximum concentration in blood is reached in 2 h and $T_{1/2}$ in 9–10 h. Our experiments showed that OF belongs to the non-toxic and AM, to minor toxic compounds by the DL_{50} criterion (per os, rats and mice resp.). The drugs proved not to have cumulative and sensibilizing properties, and AM possessed immune toxic effect. Within the sub-chronic per os OF injection to rats ($1/10$ DL_{50}) its toxicodynamics was characterized by the depression in CNS, infringement in cardiovascular system functioning and lipid metabolism in liver, and within its inhalation (22, 4.2, 2.6 and 1.1 mg/m^3), by the advancement of deceleration phenomena in CNS with the absence of disbiotic action. The AM sub-chronic per os injection to rats ($1/20$ DL_{50}) proved to infringe the general trophic processes, protein and lipid metabolism, activate processes of lipoperoxidation, and the drug inhalation (28.5, 11.6 and 6.8 mg/m^3) advanced the processes of excitement in cerebral cortex and stimulated the phagocyte nexus of non-specific resistance. We concluded in statement that the excess of therapeutical doses of OF and AM leads to emergence of the significant toxic effects.

P102

Offset rates of tiotropium and ipratropium at human recombinant muscarinic M_1-M_5 receptors using a dilution-offset methodology

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Tiotropium is a once a day inhaled M_3 antagonist characterised by slow offset from the M_3 receptor. No previous study has reported receptor offset for tiotropium at all of the muscarinic receptors (M_1–M_5). In this report the affinity and offset rates of tiotropium, ipratropium and atropine at human recombinant muscarinic M_1–M_5 receptors has been investigated. Competition binding experiments were performed using membrane preparations from CHO cells expressing recombinant human muscarinic receptors M_1–M_5. Test compounds or solvent were incubated with ^{3}H-N-methyl scopolamine (^{3}H-NMS) for 24 h, before the reaction was stopped by rapid filtration. Ki for each compound was calculated using the Cheng–Prusoff equation. To measure receptor offset, test-compounds were incubated with membrane for 2 h at 100x Ki. At various time points (between 15–1440 mins) thereafter the mixture was diluted such that compounds were at 1 x Ki and ^{3}H-NMS added at 12.5x K_D. The off rate of the test-compound is inferred by the on-rate of ^{3}H-NMS, and is expressed as the time taken to reach 50% of total ^{3}H-NMS for solvent treated membranes. Offset values are expressed as arithmetic mean with 95% confidence intervals. When the time to 50% recovery is estimated outside of the time points measured (15–1440 mins), results are quoted as >1440 or <15 min. When this occurs means are obtained using 15 and 1440, data is then quoted as > or < of the mean given. Statistical comparisons were obtained using ANOVA with $P < 0.05$. Results are shown in Table 1. All data n ≥ 6 except for M_3 Atropine (n = 3)

Table 1:

Compound	Time to 50% recovery (mins). () = 95% confidence limits.				
	M_1	M_2	M_3	M_4	M_5
Ipratropium	<15 (14.7–16.1)	<15 (14.8–15.5)	<15 (15.1–15.6)	<15 (14.9–15.3)	<39 + (32.6–47.4)
Tiotropium	>1430 (1420–1440)	>781* (649–940)	>1350 (1280–1420)	>1440 (>1440)	>1440 (1430–1450)
Atropine	<15 (14.7–15.5)	ND	<15 (2.85–40)	<15 (<15)	54.7 + (34–88.1)

*Tiotropium offset is significantly faster from M_2 versus $M_{1,3,4,5}$*Ipratropium and Atropine offset is significantly slower at M_5 than at M_1-M_4; ND, not determined.

The data indicate that tiotropium has (i) much slower offset rates at M_1-M_5 receptors compared with atropine and ipratropium and, (ii) a faster offset at M_2 and similar off rates at $M_{1,3,4}$ and $_5$. Our data with tiotropium offset is in general agreement with the observations reported by Disse et al (1999) in that tiotropium has a faster off rate from M2 when compared to M1 and M3 but the reported values do differ. Disse et al (1999) reported that the $t_{1/2}$ of tiotropium is 34.7 h at M_3 and 3.6 h at M_2 whereas or data indicate >22.5 h (M3) and >13 h at M_2 (M2). Different methodologies may account for this as Disse et al (1999) assessed off rates directly using radiolabelled compound whereas our assay assessed off rates indirectly with a dilution-offset methodology using ^{3}H-NMS and unlabelled compounds. Dilution-offset allows the measure of relative off rates without the need to radiolabel all compounds being investigated.

Reference:

Disse B *et al.* Life Sciences. 1999; 64: 457–464.

P103

Assessment of the functional potency and duration of action of the muscarinic antagonists tiotropium, atropine and ipratropium in the human isolated bronchus using both IC_{50} and Schild analysis

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Functional antagonist affinity determination in human bronchial rings using pA_2 and IC_{50} methodologies was performed. For pA_2s antagonist was pre-incubated for 1, 2 or 4 h prior to concentration response curves (CRCs) to carbamyl choline (CCh) and pA_2 determination. For IC_{50} determination electrically filed stimulated tissues were each exposed to a single concentration of antagonist for 4h and the IC_{50} constructed across tissues from the same donor. Tissues were then washed overnight to determine duration of action, expressed as $T_{50\%}$ (time for 50% recovery of a just maximal concentration). In pA_2 experiments atropine and ipratropium produced concentration related rightward shifts in CCh CRCs, with no suppression of agonist maximum responses and slopes not significantly different to 1.0. Ipratropium pA_2 = 9.68, 9.39 and 9.33 following 1, 2 and 4 h incubation. Atropine pA_2 = 9.07 and 8.87 using 1 and 2 h incubations. Affinity was not significantly affected by drug incubation time. Tiotropium caused concentration dependent suppression of the CCh maximum response consistent with non-surmountable antagonism and no pA_2 could be determined. In IC_{50} experiments tiotropium (0.44 nM) was more potent than atropine (2.37 nM) and ipratropium (2.26 nM) and all concentrations had reached plateau by 4 h. At a just maximal concentration, atropine and ipratropium had rapid onset of action ($T_{½on}$ = 8.9 and 22.2 min) and short duration of action, $T_{50\%}$ of 4.13 and 7.37 h. In contrast tiotropium showed slower onset ($T_{½on}$ = 93.2 min) and prolonged duration of action ($T_{50\%}$ > 14.0 h). Therefore the IC_{50} protocol was more appropriate for determining potency of slower onset/offset compounds and also allows determination of onset and duration at each individual concentration.

P104

Lipoxygenase products involve in indomethacin-induced potentiation of the contractile response to antigen in ovalbumin-sensitized guinea pig tracheas

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Antigen exposure to epithelium-intact tracheas isolated from ovalbumin-sensitized guinea pigs causes a contractile response that is potentiated by the cyclooxygenase inhibitor, indomethacin. In the present study we investigated the mediator(s) involved in this potentiation. Male albino guinea-pigs (300–400 g) were sensitized with 10 μg ovalbumin +0.1 g aluminium hydroxide 21–30 days before isolation of their tracheas. Trachea open-ring preparations were mounted in organ baths filled with Krebs-Henseleit solution (37°C) and isometric changes in tension were recorded. Contractions are expressed as % of contraction to 1 mM carbachol and given as mean ± SEM (n = 5–7). Statistical analysis was done by ANOVA/Newman-Keuls and $P < 0.05$ was considered significant. Ovalbumin (10^{-7}–10^{-2} mg/ml) elicited a contractile response with a maximum of 68.11 ± 4.85% which was increased to 88.77 ± 1.26% in the presence of 3 μM indomethacin ($P < 0.05$). The lipoxygenase inhibitor AA-861 did not affect ovalbumin contraction but prevented its potentiation by indomethacin ($P < 0.05$). The leukotriene receptor antagonist cinalukast inhibited both the contractile response to ovalbumin and its potentiation ($P < 0.05$). The antagonists of platelet activating factor (BN-52021), adenosine (CGS-15943), and endothelins (ET_A; BQ-123 and ET_B; BQ-788) did not have any effect on ovalbumin contraction and its potentiation in the presence of indomethacin. Capsaicin and neuropeptide receptor antagonists neurokinin1 (NK1) (L-732128), NK2 (MEN-10376) and NK3 (SB-218795) also did not inhibit the contractile response. However, capsazepine, the antagonist of capsaicin at 'transient receptor potential vanilloid1' (TRPV1) receptors did not alter ovalbumin response but inhibited its potentiation by indomethacin ($P < 0.05$). In conclusion, the potentiation of antigen-induced contraction by cyclooxygenase inhibition is likely due to the shift of arachidonic acid towards lipoxygenase pathway. Since some lipoxygenase products are potent vanilloid agonists and are structurally similar to capsaicin (Hwang *et al.*, 2000), their increased synthesis may result in the potentiation response by stimulating the TRPV1 receptors besides 'CysLT' receptors.

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Reference:

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