

# Oral Communications

## Monday 14 July

### Cannabinoid pharmacology/cardiovascular pharmacology (14.30–15.30)

#### C001

##### **Ca<sup>2+</sup> dependent effect of CB<sub>1</sub> receptor antagonist AM251 on the mIPSCs frequency of cerebellum Purkinje cells**

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G-protein coupled cannabinoid CB<sub>1</sub> receptors play an important role in the modulation of the inhibitory GABAergic neurotransmission at interneurone-Purkinje (IN-PC) synapses in the mammalian cerebellum. We have recently reported that the cannabinoid receptor agonist WIN55,212 (WIN55) decreases mean miniature inhibitory postsynaptic current (mIPSC) frequency at IN-PC synapses, and that the effect of WIN55 was reversed by the CB<sub>1</sub> receptor antagonist AM251, which overall caused an increase in mIPSC frequency beyond control levels (Ma *et al.* 2008). These results were consistent with the presence of a basal level of CB<sub>1</sub> activation in the cerebellum. In order to investigate mechanisms underlying the additional action of AM251, we carried out further experiments designed to manipulate endocannabinoid release in acute cerebellar brain slices. Cerebellum brain slices were prepared from 3- to 5-week-old male TO mice. Slices were placed in a recording chamber and continuously perfused with artificial cerebrospinal fluid containing (aCSF) aerated with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Whole-cell patch clamp recordings were performed with 3–7 MΩ resistance electrodes filled with a CsCl-based intracellular solution. Inward bicuculline-sensitive mIPSCs were isolated in the presence of TTX, NBQX and CGP 55845. Changes in mIPSC frequency induced by drugs were normalised against control, and the data are presented as mean ± SEM. We found that the endocannabinoid uptake blocker, AM404 (1–10 μM) had no effect on mean mIPSC frequency (1.04 ± 0.03; n = 6, P > 0.05) or amplitude (1.04 ± 0.08; n = 6, P > 0.05). In these experiments, subsequent application of WIN55 was still able to reduce mIPSC frequency. The effect of orlistat, a diacylglycerol lipase inhibitor, was then examined: in the presence of orlistat (20 μM) the effect of AM251 (2 μM) was preserved (1.34 ± 0.168; n = 3, P < 0.05) as we reported earlier (Ma *et al.* 2008). We next examined the effects of the calcium chelator BAPTA-AM on AM251 action. BAPTA-AM (200 μM) alone had no overall effect on mIPSC frequency (0.92 ± 0.03; n = 5); in the continued presence of BAPTA-AM, AM251 (2 μM) now failed to increase the mIPSC frequency (0.95 ± 0.08; n = 5). The results with AM404 and AM251 suggest that: i) blockade of cannabinoid uptake is unable to increase local endocannabinoid levels to a sufficient extent to activate CB<sub>1</sub> receptors; ii) the inhibition of endocannabinoid syntheses does not affect the AM251 induced increase of mIPSC frequency. These results indicate that endocannabinoid tone does not play a significant role in basal level CB<sub>1</sub> activation at IN-PC synapses. The results with BAPTA-AM are consistent with AM251 acting via a calcium-dependent pathway to increase presynaptic GABA release. These results contrast with previous findings that BAPTA-AM (200 μM) had no effect on the GABA<sub>B</sub> receptor-mediated inhibition of GABA release at IN-PC synapses (Harvey & Stephens, 2004) and suggest differential modes of action of presynaptic G protein-coupled receptors at IN-PC synapses.

##### **References:**

Harvey VL and Stephens GJ. Eur J Neurosci. 2004; 20: 684–690.  
Ma YL *et al.* Br J Pharmacol. epub 2008.

#### C002

##### **The calcium chelator BAPTA blocks AM251-mediated increases in inhibitory neurotransmission at interneuron-Purkinje cell synapses in the mouse cerebellum**

YL Ma, G Stephens School of Pharmacy, University of Reading, Reading, Berks, UK  
Cannabinoid CB<sub>1</sub> receptors play an important role in the modulation of the inhibitory GABAergic neurotransmission at interneurone-Purkinje cell (IN-PC) synapses in the mammalian cerebellum. We have recently reported that the cannabinoid receptor agonist WIN55,212 (WIN55) decreases miniature inhibitory postsynaptic current (mIPSC) frequency at IN-PC synapses, and that the effect of WIN55 was reversed by the CB<sub>1</sub> receptor antagonist AM251, which overall caused an increase in mIPSC frequency to 143% of control levels (Ma *et al.* 2008). Here, cerebellar brain slices were prepared from 3 to 5-week-old male TO mice; mIPSCs (isolated in the presence of TTX, NBQX and CGP 55845) were recorded from PCs using the whole-cell patch clamp technique. AM404 (1–10 μM), a blocker of endocannabinoid uptake, had no effect on mean mIPSC frequency (1.04 ± 0.10; n = 6) or amplitude (1.04 ± 0.08; n = 6). Subsequent application of WIN55 was still able to reduce mIPSC frequency in all cells tested. We next examined the effects of the calcium chelator BAPTA-AM on AM251 action. BAPTA-AM (200 μM) alone had no overall effect on mIPSC frequency (0.92 ± 0.03; n = 5; P < 0.05); in the continued presence of BAPTA-AM, AM251 (2 mM) now failed to increase the mIPSC frequency beyond control level (0.95 ± 0.08; n = 5). These data are consistent with AM251 acting via a calcium-dependent pathway to increase presynaptic GABA release. The results contrast with our previous finding that BAPTA-AM (200 μM) had no effect on the GABA<sub>B</sub> receptor-mediated inhibition of GABA release at IN-PC synapses (Harvey & Stephens, 2004) and suggest differential modes of action of presynaptic G protein-coupled receptors at IN-PC synapses.

##### **References:**

Ma YL *et al.* Br J Pharmacol. epub 3 March 2008.  
Harvey VL and Stephens GJ. Eur J Neurosci. 2004; 20: 684–690.

#### C003

##### **Evaluation of cardioprotection produced by BRL37344 on Langendorff-perfused rat heart**

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β-3 adrenoceptors (ARs) have extensively been characterized in the heart. However, their effectiveness in cardiac contractility is still controversial. In addition, insufficient data is available in relation to their function in ischemia-reperfusion (I/R) injury. Here, we investigated whether activation of β-3 ARs before ischemia could modify the deteriorated cardiac function in I/R injury. We therefore studied the effects of the specific β-3 AR agonist, BRL37344 (10<sup>-9</sup>–10<sup>-6</sup>M), as well as its possible signalling pathways by using specific β-3 AR antagonist SR59230A (10<sup>-5</sup>M) and β-1/2 AR antagonist nadolol (10<sup>-5</sup>M). For this purpose, hearts isolated from 300–350 g male Wistar rats were subjected to 30 min global no-flow ischemia followed by 1 h reperfusion. Drugs were applied for 10 min prior to ischemia in which cardiac parameters (left ventricle developed pressure, ±dp/dt, heart rate) and coronary hemodynamics (coronary flow, coronary vascular resistance) were assessed simultaneously. Data are expressed as % of baseline values and statistical analyses were determined by Student's *t*-test. In order to estimate myocardial damage, coronary effluents were collected throughout the whole protocol at 10 min. intervals for creatine kinase and troponin-I measurements and infarct size were determined by TTC staining. BRL37344 produced positive inotropic and chronotropic effects as well as increased coronary flow and enhanced post-ischemic cardiac function at 10<sup>-7</sup> and 10<sup>-6</sup>M concentrations. In contrast, acute effects in contractility and improvement in recovery after ischemia were not observed at much lower (10<sup>-9</sup>–10<sup>-8</sup>M) concentrations. SR59230A administration attenuated both acute and post-ischemic effects of BRL37344 (10<sup>-6</sup>M), whereas the combination of SR59230A with nadolol produced further inhibition of post-ischemic values. In parallel to functional data, BRL37344 (10<sup>-7</sup> and 10<sup>-6</sup>M) displayed similar favourable changes in the infarct size and biochemical markers of myocardial damage which is reversed in the presence of SR59230A and nadolol. Hence, our results suggested that higher concentrations of BRL37344 have positive inotropic effects mediated by β-3 ARs and protect myocardium from I/R injury via activation of both β-3 and β-1/2 ARs. The present work was supported by the Research Fund of Istanbul University. Project no:T-817/27122005.

#### C004

##### **Myofibroblast characteristics can be modulated by over-expression of components of the Wnt/Frizzled pathway**

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Myocardial infarction (MI) is a frequent cardiovascular event. The number of patients that survive the acute phase of MI increases, resulting in more patients entering the phase of wound healing. When wound healing is inadequate, it can lead to dilatation of the heart and result in heart failure. After MI, specialized fibroblastic cells called myofibroblasts appear in the infarcted area which may stabilize the infarct by actively contracting the infarct area and by deposition of the extracellular matrix proteins. Our laboratory has shown that the Wnt/Frizzled (Wnt/Fz) signal transduction cascade is upregulated during the wound healing, particularly in the myofibroblasts. This study was performed to further define the role of Wnt/Fz signalling in myofibroblast proliferation, differentiation and migration. To this end, we developed a rat cardiac fibroblast cell line which was immortalized by stable transfection with human telomerase. These cells were transiently transfected with expression plasmids encoding Fz1, Fz2 or β-catenin (the mediator of the canonical Wnt/Fz pathway), and treated with conditioned medium containing their endogenous ligands Wnt-3a or 5a. A Ca<sup>2+</sup> agonist and antagonist were applied to mimic non-canonical Wnt/Fz signaling. Proliferation rates were not significantly affected by over-expression of Wnt/Fz components. Next, we studied the differentiation of the fibroblast into the myofibroblasts. The levels of several differentiation markers, including collagen 1α2, alpha smooth muscle actin (αSMA) and fibronectin, were quantified by qPCR. Overexpression of Fz2+Wnt3a increased collagen 1α2 expression, whereas β-catenin over-expression was ineffective. Total fibronectin expression also was significantly increased by over-expression of Wnt/Fz components. αSMA expression levels were studied at mRNA and protein level. Treatment with Wnt5a, with or without Fz2, increased αSMA levels. Wnt3a addition, on the other hand, resulted in a decrease of αSMA levels. These results were confirmed by immunohistochemical staining. The effect of Wnt5a could be mimicked by treatment with a Ca<sup>2+</sup> agonist. These combined results indicate that Wnt/Fz signaling leads to fibrosis and can modulate fibroblast differentiation. Migration of the fibroblasts was determined using an *in vitro* wound assay, in which the time needed by the cells to migrate into a scratch in a confluent cell layer was determined. Addition of Wnt3a or Wnt5a conditioned medium or over-expression of Fz1 or Fz2 attenuated the migration and combinations inhibited the migration even further, as seen with a calcium inhibitor. Addition of siRNAs for Fz1 and two completely abolished this anti-migratory effect. The results support a functional role for the Wnt/Fz pathway in the control of migration of cardiac fibroblasts during wound healing, presumably mediated through the non-canonical pathway.

# Cardiovascular pharmacology (16.00–16.30)

## C005

### The neurotrophin nerve growth factor contributes to reparative neovascularization and cardiomyocyte survival after myocardial infarction

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Nerve growth factor (NGF), which was initially considered only for its important actions on the nervous system, has been recently implicated by us in angiogenesis and cardiomyocyte survival. This study investigated whether: i) endogenous NGF mediates the spontaneous angiogenesis response to myocardial infarct (MI) in mice and ii) NGF gene transfer to the mouse peri-infarcted heart stimulates therapeutic neovascularization and promotes the survival of endothelial cells (EC) and cardiomyocytes. Anaesthetized male CD1 mice ( $30 \pm 3$  g) underwent MI by permanent occlusion of the left descending coronary artery. To investigate the effects of endogenous NGF after MI, mice ( $n = 10$  each group) were intraperitoneally injected with a goat-raised antibody able to neutralize NGF (Ab-NGF, 3 mg/kg BW, IP, every 5 days) or with diluted goat IgG. Sham-operated mice were used as control. To understand the possible therapeutic role of NGF gene therapy, an adenovirus carrying the human NGF gene (*Ad.NGF*,  $10^8$  p.f.u.) or an empty vector (*Ad. Null*, control) was delivered to the MI border zone ( $n = 8$  mice per group). For aim 1, at 14 days post-MI, cardiac function was determined in terminally anaesthetized mice by using a Millar catheter advanced in the left ventricle (LV) chamber. Peak systolic LV pressure (LVP, mmHg) and maximal rate of LVP rise ( $dP/dt_{max}$ , mmHg/s) and fall ( $dP/dt_{min}$ , mmHg/s) were digitally recorded. Then, hearts were arrested in diastole by cadmium chloride injection and perfusion/ fixed. For aim 2, mice were killed at 14 days. Following staining of LV sections with silver stain, capillary density was counted in the peri-infarct myocardium. Apoptosis of EC and cardiomyocytes was determined by double staining for TUNEL plus lectin or alfa-sarcomeric actin, respectively. Analyses were performed in blind. Ab-NGF-injected mice showed a deterioration of the cardiac function ( $P < 0.05$  vs. control IgG for all comparisons). Moreover, Ab-NGF abrogated the spontaneous capillary growth after MI ( $P < 0.05$  vs. control IgG,  $P = N.S.$  vs. sham-operation) and increased the number of apoptotic EC and cardiomyocytes ( $P < 0.05$  for both comparisons vs. control IgG). Conversely, *Ad. NGF* improved angiogenesis and reduced the numbers of TUNEL-positive apoptotic EC and cardiomyocytes ( $P < 0.05$  for all comparisons vs. *Ad. Null*). Taken together, our results suggest that endogenous NGF is involved in spontaneous reparative neovascularization and survival of EC and cardiomyocytes after MI and that NGF over-expression may be used therapeutically to improve reparative neovascularization by promoting angiogenesis and to inhibit apoptosis in the setting of myocardial infarction.

## C006

### A3 adenosine receptor activation via 2-Cl-IBMECA protects the myocardium via recruitment of PI3K-Akt-iNOS intracellular signalling pathway during reperfusion

P Karjian, A Hussain, HA Rajaibe, H Maddock Coventry University, Coventry, UK  
Recent studies have emphasized the important roles that nitric oxide (NO), Akt or A3 adenosine receptors ( $A_3AR$ ) play in protecting against myocardial ischemia reperfusion injury (Maddock *et al.*, 2002; Zhao and Kukreja, 2002; Hausenloy and Yellon, 2004); In this study, we assessed whether phosphatidylinositol 3 kinase (PI3K)-Akt-iNOS (inducible NO Synthase) pathway was involved in  $A_3AR$  mediated cardioprotection. Isolated perfused rat hearts or cardiomyocytes were subjected to ischemia or hypoxia followed by reperfusion (R) or reoxygenation (RX) respectively. Hearts underwent triphenyl tetrazolium staining for infarct size assessment, or frozen for Western blot analysis. Cardiomyocytes were analysed for apoptosis/necrosis (A/N) & caspase-3 activity. 2-Cl-IBMECA (MECA,  $A_3AR$  agonist, 100 nM) was administered throughout R or RX in the presence and absence of Wortmannin (Wort, PI3K inhibitor, 100 nM), N(G)-nitro-L-arginine methyl ester (NAME, NOS inhibitor, 100  $\mu$ M) or Aminoguanidine (AG, iNOS inhibitor, 100  $\mu$ M). Data was analysed using ANOVA followed by Tukey's test ( $n = 6-12$ ). MECA when administered during R significantly reduced infarct size when compared to control ( $25 \pm 8\%$  vs.  $65 \pm 12\%$ ,  $P < 0.05$ ). In isolated myocytes A/N were significantly reduced in the presence of MECA compared to controls ( $18 \pm 3\%$  vs.  $35 \pm 5\%$  and  $23 \pm 3\%$  vs.  $33 \pm 4\%$  respectively,  $P < 0.05$ ). MECA inhibited caspase-3 activity compared to control ( $110 \pm 11\%$  vs.  $195 \pm 29\%$ ,  $P < 0.05$ ). The protective effect of MECA was abrogated by administration of one of Wort, NAME or AG. Western blot analysis further demonstrated that  $A_3AR$  activation during R induced a significant increase in p-Akt(Ser473) and iNOS expression in the presence of MECA compared to control hearts. MECA dependent upregulation of Akt and iNOS was blocked by Wort, NAME & AG. This is the first study to show that  $A_3AR$ s activation can protect the ischemic reperfused myocardium via recruitment of the PI3K-Akt-iNOS intracellular signalling pathway.

#### References:

- Maddock HL *et al.* Am. J. Physiol. Heart Circ Physiol. 2002; 283: H1307-H1313.  
Hausenloy DJ and Yellon DM. Cardiovasc Res. 2004; 61: 448-460.  
Zhao TC and Kukreja RC. J. Mol Cell Cardiol. 2002; 34: 263-277.

# Nitric oxide (14.30–15.30)

## C007

### The excitatory role of nitric oxide on 2-deoxy-D-glucose-induced gastric motility in central nervous system

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2-deoxy-D-glucose (2-DG) increases the gastric acid secretion and the motility via the vagus nerve but the underlying mechanism remains to be established. Since nitric oxide (NO) is involved in food-induced gastric dilatation, investigating a possible interplay between 2-DG and NO appears to be reasonable. Thus, we investigated the effects of nitric oxide synthase (NOS) inhibitors L-NAME (10 mg/kg, i.v.) and L-NNA (10 mg/kg, i.v.) on 2-DG (200 mg/kg, i.v.)-induced gastric motility in Wistar albino rats (250–350 g) of both sexes. Animals were anaesthetized with urethane (1.5 g/kg, i.p.), cannulated for iv drug injections while the left vagus nerve was electrically stimulated (0.1–10 Hz, 0.5 ms duration, 12 V, for 60 s) and intragastric pressure and motility changes were monitored by using a latex gastric balloon connected to a computerized data recording system (Biopac, USA). Wilcoxon signed rank test or two-way ANOVA for repeated measures was utilized for data analysis and  $P < 0.05$  was taken as statistical significance. 2-DG increased the mean intragastric pressure (cmH<sub>2</sub>O, baseline:  $5.2 \pm 0.3$ ; after 2-DG:  $14.4 \pm 1.5$ ,  $n = 7$ ,  $P = 0.0156$ ) and significantly increased the motility index as expressed by integrating the total peristaltic activity during 10 min after the intervention while NOS inhibitors have significantly attenuated them (i.e. motility index, 2-DG:  $3634.6 \pm 1135.8$ ; L-NAME:  $1326 \pm 490.8$ ,  $n = 7$ ,  $P = 0.0156$ ). However, pretreatment with NOS inhibitors significantly augmented the gastric responses to peripheral electrical stimulation of the vagus nerve (i.e. cmH<sub>2</sub>O increase in response to 3 Hz, control:  $4.8 \pm 0.9$ ; L-NAME:  $9.3 \pm 1.3$ ;  $n = 7$ ,  $P = 0.0156$ ). Similar results were also obtained by using L-NNA as well. Therefore we conclude that NO acts as an excitatory mediator in gastric responsiveness to 2-DG that takes place in the central nervous system while its inhibition at the periphery by NOS inhibitors augment the responses to electrical stimulation of vagus nerve.

## C008

### The PARP inhibitor 3-aminobenzamide reduces the aggravating effect of rt-PA on NMDA-induced cerebral lesion in rats

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Thrombolytic therapy with recombinant tissue plasminogen activator (rt-PA) is the only treatment for clinical ischemic stroke. However, rt-PA exhibits vascular and neuronal side effects. Direct neuronal toxicity of rt-PA is in part mediated by potentiation of excitotoxicity via N-methyl-D-aspartate (NMDA) receptors. Furthermore, NMDA receptor activation is known to stimulate the nuclear enzyme poly(ADP-ribose)polymerase (PARP) that contributes to NMDA toxicity. In this context, the present study investigated whether PARP is implicated in the neurotoxicity of rt-PA due to NMDA receptor activation. Male Sprague-Dawley rats (270–310 g) were anaesthetized with chloral hydrate (400 mg/kg i.p.). A stereotactic administration of 3-aminobenzamide (3-AB, 54 µg/20 µl) or its vehicle (PBS) was performed into the left ventricle (1.5 mm lateral, 0.8 mm posterior, 4.5 mm deep from the bregma). Thirty minutes after this injection, NMDA (50 nmol) or NMDA (50 nmol) + rt-PA (3 µg) was injected in 2 µl PBS into the left striatum (3.5 mm lateral, 0 mm anteroposterior, 7 mm deep from the bregma). Sensorimotor functions were evaluated 24 h after intrastriatal infusion using a grading scale on nine points. The lower the score, the more severe the deficit. Rats were then killed with an overdose of pentobarbitone (200 mg/kg i.p.), their brains were removed and frozen. Cryostat-cut coronal sections were stained with cresyl violet for lesion determination. The intrastriatal injection of NMDA led to a  $30 \pm 1 \text{ mm}^3$  brain lesion which was associated with a neurological deficit ( $7.6 \pm 0.4$ ). Cojunction of rt-PA did not alter the neurological deficit ( $7.5 \pm 0.3$ ) but enhanced by 43% the brain lesion induced by NMDA ( $43 \pm 2 \text{ mm}^3$ ,  $P < 0.001$ ). The PARP inhibitor 3-AB reduced by 54% the aggravating effect of rt-PA ( $36 \pm 2 \text{ mm}^3$ ,  $P < 0.05$ ). In conclusion, our study points out that PARP activation contributes to the neurotoxicity of rt-PA and that its inhibition could be a strategy to improve the safety of rt-PA therapy in stroke.

## C009

### Real-time measurement of non-lethal platelet thromboembolic responses in the anaesthetised mice

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*In vitro* platelet aggregation is a poor predictor of platelet function *in vivo* where the vascular endothelium plays an important role in the platelet response. We therefore developed methodology for assessing *in vivo* platelet thromboembolism non-invasively and in real-time without damaging the vascular endothelium. Platelets were isolated from anaesthetised donor mice, radiolabelled with <sup>111</sup>Indium Oxine and re-infused into anaesthetised recipient mice. Circulating platelets were monitored with a miniaturised crystal scintillation probe placed over the pulmonary vascular region. Intravenous injection of ADP (0.4–400 µg/kg), thrombin (100–1000 IU/kg) and collagen (25–100 µg/kg) induced dose-dependent changes in platelet counts that consisted of a rapid increase in the pulmonary probe as platelets aggregated and became trapped in the pulmonary vasculature. Counts then returned to baseline in a time-frame that varied with the agonist used. We confirmed the presence of platelet aggregates histologically and demonstrated synergistic responses to combined administration of threshold doses of collagen and adrenaline. The nitric oxide synthase (NOS) inhibitor L-NAME (10 mg/kg) was infused to inhibit NO from platelets and the vascular endothelium and significantly enhanced platelet responses to thrombin ( $****P < 0.001$ ,  $n = 6$ ). The endogenous produced NOS inhibitor, L-NMMA was infused at a range of concentrations. L-NMMA had no effect at concentrations up to 1 mM but at higher concentrations (400 mM) significantly enhanced thrombin induced thromboembolism ( $**P < 0.05$ ,  $n = 6$ ). Responses to thrombin and collagen were not significantly different in NOS3<sup>-/-</sup> mice compared with wild-type controls ( $P > 1$ ,  $n = 6$ ). We therefore present a model for assessing platelet thromboembolic responses *in vivo*. We have shown that pharmacological inhibition of NOS powerfully potentiates platelet thromboembolic responses but that lower concentrations occurring physiologically and pathologically do not affect the platelet response. Although NOS-3 has important haemostatic roles in producing NO in the vascular endothelium and possibly platelets, compensatory mechanisms following NOS-3 ablation led to normal thromboembolic responses in these mice.

## C010

### Effects of acylation inhibitors on nitric oxide production, eNOS localization, endothelial cell proliferation and vascular reactivity

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Endothelial nitric oxide synthase (eNOS), the enzyme that catalyzes nitric oxide (NO) synthesis in endothelial cells, is N-myristoylated and S-palmitoylated at specific residues. N-myristoylation and S-palmitoylation mediate the localization of eNOS in Golgi complex and cholesterol rich microdomains of the plasma membranes, including caveolae and lipid rafts, where it must be present to function properly. In addition to eNOS, other important proteins like caveolin-1 (the major coat protein of caveolae) and Src expressed in endothelial cell types are also palmitoylated. In order to analyse the importance of global palmitoylation in vascular biology, we investigated the effects of protein fatty acylation inhibitor 2-bromopalmitate and long-chain acyl-CoA synthase inhibitor Triacsin C. Basal NO release was determined by NO-specific chemiluminescence analyzer. Western blotting was used to identify eNOS protein expression levels. Our results show that, Triacsin C but not 2-bromopalmitate markedly increased NO release in human endothelial cells -EA.hy.926-, without changing eNOS protein expression levels. Immunofluorescence microscopy results indicated that, Triacsin C abolished the interaction between eNOS and caveolin-1 and caused the Golgi fragmentation in bovine aortic endothelial cells. Next, we determined the proportional distribution of eNOS using a discontinuous sucrose gradient. The relative localization of eNOS in two distinct pools (light membranes enriched in caveolin-1 and heavy membranes enriched in β-COP, a marker of Golgi) was not different in cells treated with Triacsin C. Triacsin C partially inhibited the cell proliferation in EA.hy.926 cells, but significantly enhanced tube formation in 3D cultures – as a model of angiogenesis 'in vitro' – in human umbilical vein endothelial cells. In myograph studies we observed that, 2-bromopalmitate and Triacsin C inhibited phenylephrine-induced contraction on aortic rings, isolated from 10 to 12 weeks old C57BL/6 mice. This effect was partially restored with NOS inhibitor L-NAME, showing the role of NO on the inhibitory effects of these acylation inhibitors ( $P < 0.05$ , Student's *t*-test). The present work provides evidences for the importance of acylation inhibitors on endothelial cell biology and vascular reactivity.

# Nitric oxide (16.00–16.45)

## C011

### Effect of 7-nitroindazole on working memory of rats in three panel runway test

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Nitric oxide (NO) is suggested to have a role in modulating cognitive functions. NO may improve (Plech *et al.*, 2003) while NO synthase inhibitors impair learning and memory (Meyer, 1998). The effects of 7-NI (2.5, 5, 10 mg/kg, i.p), a selective nNOS inhibitor and an NO precursor L-arginine (200 mg/kg, i.p) on the working memory performances of rats was investigated in the three-panel runway test. Adult male Wistar rats weighing 200–250 g were used. All results were analyzed using one-way analysis of variance (ANOVA) *post hoc* Dunnett's test. The total number of errors from the second to the sixth trial ( $7 \pm 0.81$ ,  $8.14 \pm 1.3$  and  $8.42 \pm 0.92$  respectively for 2.5, 5 and 10 mg/kg 7-NI,  $n = 7$  for each group) slightly increased but failed to reach a statistically significant value. Although there was no difference between the latencies of the animals in the first trial except in 10 mg/kg 7-NI group, the latency of the animals from the second to the sixth trial significantly prolonged in 5 and 10 mg/kg 7-NI group. The latency recorded in the first trial was  $11.42 \pm 1.7$ ,  $25.28 \pm 3.6$  and  $46.14 \pm 4.8$ ; and from second to the sixth trial of a session were  $42.28 \pm 4.2$ ,  $206.57 \pm 21.55$  and  $509.14 \pm 62.04$  respectively for 2.5, 5 and 10 mg/kg 7-NI. L-arginine (200 mg/kg,  $n = 7$ ) did not alter the number of errors and latency values of rats. L-arginine given before 7-NI ( $n = 10$ ), reversed 5 mg/kg 7-NI-induced prolongation in the latency from the second to sixth trial. Our results indicate that NO plays an essential role in formation of memory and NO-mediated mechanisms have important for developing new strategies to treat cognitive dysfunctions.

#### References:

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Plech A *et al.* *Pol J Pharm.* 2003; 55: 987–992.

## C012

### The effect of the neuronal and inducible nitric oxide synthase inhibitor TRIM on depression in the unpredictable chronic mild stress model

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Nitric oxide (NO) is an intracellular messenger which plays an important role in many psychological events like depression, anxiety, learning and memory (Harkin *et al.*, 1999; Holscher, 1997; Wiley *et al.*, 1995). In many studies nitric oxide synthase inhibitors (NOSI) were shown to have antidepressant like effects in the forced swimming test (Yildiz *et al.*, 2000). The aim of this study is to investigate the effect of a new and selective neuronal and inducible NOS inhibitor, TRIM (30 mg/kg/day, 35 days), in unpredictable chronic mild stress (UCMS) (Yalcin *et al.*, 2007) exposed mice when compared with the ones of the widely used selective serotonin reuptake antidepressant drug fluoxetine (15 mg/kg/day, 35 days). 72 male inbred BALB/c ByJ mice weighed 20–30 g were used in this study. The Kruskal-Wallis H followed by Dunn's test revealed a significant difference between non-stressed NaCl ( $0.73 \pm 0.14$ ;  $n = 11$ ) and UCMS NaCl groups ( $2.27 \pm 0.25$ ;  $n = 11$ ) for the score of coat state. Fluoxetine 15 mg/kg ( $0.79 \pm 0.16$ ;  $n = 12$ ) and TRIM 30 mg/kg ( $0.67 \pm 0.14$ ;  $n = 12$ ) significantly reversed the degradation on the coat state compared with the UCMS NaCl group. Grooming latency in the splash test and the attack frequency in the resident intruder test were evaluated using one-way ANOVA followed by Tukey *post hoc* test. Both TRIM ( $181.5 \pm 8.88$ ;  $n = 10$ ) and fluoxetine

( $188.91 \pm 8.32$ ;  $n = 11$ ) blocked the stress-induced deficit in total latency of grooming in the splash test compared with the UCMS NaCl group ( $133 \pm 11.5$ ;  $n = 10$ ). TRIM ( $4.09 \pm 1.32$ ;  $n = 11$ ) and fluoxetine ( $2.27 \pm 0.94$ ;  $n = 11$ ) also significantly decreased the attack frequency compared with the UCMS NaCl group ( $9.8 \pm 2.46$ ;  $n = 10$ ) in the resident intruder test. These results support the assumption that NOS inhibitors can be a new class of antidepressant drugs which can be used in clinics effectively, possibly showing its effect on neuronal NOS

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## C013

### Influence of nitric oxide on pancreatic and pulmonary injuries of rats with severe acute pancreatitis

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Evidence from our previous studies shows that secretory phospholipases A<sub>2</sub> (PLA<sub>2</sub>s) induces acute pancreatitis when injected into the pancreaticobiliary duct of rats, which is partly dependent on both the tachykinin NK<sub>1</sub> and bradykinin B<sub>2</sub> receptors activation (Camargo *et al.*, 2005, 2008). We have now investigated the contribution of nitric oxide (NO) in this model. Male Wistar rats (220–260 g,  $n = 5–8$ ), under pentobarbital anaesthesia, were subjected to acute pancreatitis by injecting PLA<sub>2</sub> from *Naja mocambique mocambique* venom (PLA<sub>2</sub>-Nmm; 300 mg/kg) into the common bile duct. Treatment with L-NAME (20 mg/kg), a non selective inhibitor of NO synthesis, or aminoguanidine (50 mg/kg), an inducible NO synthase inhibitor, was given intravenously to the rats 30 min prior to PLA<sub>2</sub>-Nmm injection. Four hours later, serum amylase and the pro-inflammatory cytokine (TNF $\alpha$ ) were measured in addition to the increased plasma protein extravasation (PPE) and myeloperoxidase (MPO) activity assays in the pancreas or pulmonary tissue. PLA<sub>2</sub>-Nmm induced a marked increase in serum amylase (155%) and TNF $\alpha$  (260%) concentrations. This effect was associated with a significant increase of PPE (75%) and MPO activity in the pancreatic (231%) and pulmonary tissue (73%) of vehicle-treated rats. L-NAME-treated rats exhibited a significant reduction in the raised pancreatic PPE (39%) and serum TNF $\alpha$  (76%). However, L-NAME potentiated the MPO (95%) activity in the pancreas, and failed to significantly affect the serum amylase and MPO levels in the lung. Experiments with aminoguanidine had no effect on PLA<sub>2</sub>-Nmm induced-acute pancreatitis. The blockade of endothelial derived-NO can only protect the pancreas of rats with severe pancreatitis from oedema, but not from the increased serum amylase and cell influx in both pancreas and lung. Whether L-NAME-induced suppressive effect on oedema was due to the direct vascular actions of NO on pancreas microcirculation or proinflammatory cytokines production remains to be elucidated. This work was supported by FAPESP and CNPq.

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# Ion channels (14.30–15.30)

## C014

### Cation contents in the isolated rat heart and aorta during streptozotocin (STZ)-induced type 1 diabetes mellitus

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Cardiovascular disease is a major cause of morbidity and mortality in diabetic patients and hearts of diabetics are in a compromised position. In a previous study we have demonstrated marked voltage-dependent reductions in both force of contraction and cellular calcium ( $\text{Ca}^{2+}$ ) homeostasis and increased myofibril  $\text{Ca}^{2+}$  sensitivity in the isolated diabetic male Wistar rat heart compared to healthy age-matched control (Bracken *et al.*, 2003). Since  $\text{Ca}^{2+}$  is the main determinant of contraction, this study now investigated whether  $\text{Ca}^{2+}$  contents are reduced in the heart and aorta during diabetes mellitus compared to control. Levels of other cations ( $\text{Na}^+$  and  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{Zn}^{2+}$  and  $\text{Pb}^{2+}$ ) were also measured for comparison. Diabetes was induced using STZ (60 mg/kg body weight) in male Wistar rats. Four-six weeks after STZ injection, the animals were humanely killed and the heart and aorta ( $n = 14$  diabetic and 14 control rats) were isolated. Levels (mg/100 mg tissue) of cations in these tissues were measured using either flame photometry ( $\text{Na}^+$  and  $\text{K}^+$ ) or atomic absorption spectrophotometry ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Pb}^{2+}$ ). The results show that diabetic rats ( $222.78 \pm 7.100$  g) and their hearts ( $1.0 \pm 0.03$  g) weighed significantly ( $P < 0.05$ ) less compared to age-matched control (Body weight,  $399.10 \pm 9.84$  g and heart weight  $1.10 \pm 0.04$  g) and they have significantly ( $P < 0.05$ ) elevated blood glucose (compare  $29.33 \pm 0.84$  mM to  $5.36 \pm 0.13$  mM) and significantly ( $P < 0.05$ ) reduced plasma insulin. The levels of  $\text{Na}^+$  and  $\text{K}^+$  increased significantly ( $P < 0.05$ ) in the left ventricle of the diabetic rat compared to control. In contrast the levels of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  decreased markedly in the left and right ventricles, left and right atria as well as in the aorta of diabetic rat compared to the control. The level of  $\text{Zn}^{2+}$  was significantly increased ( $P < 0.05$ ) in the left ventricle, right atrium and aorta compared to control, whereas the levels of  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$  only increased slightly in the diabetic heart and aorta compared to control tissues. The results indicate that the diabetic rat heart contains less  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  which in turn may lead to reduced force of contraction and increased  $\text{Ca}^{2+}$  sensitivity in the myofibrils compared to age-matched control.

#### Reference:

Bracken *et al.* Mol Cell Biochem. 2003; 261: 387–408.

## C015

### TRPC3-mediated electrical remodelling of atrial myocardium

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Recent evidence suggests involvement of transient receptor potential (TRP)-related cation channels in cardiac physiology and pathophysiology, with TRPC3 as one potential key player in cardiac hypertrophy. It has been suggested that TRPC3 is upregulated in hypertrophy development and contributes to  $\text{Ca}^{2+}$  signals that govern pathological remodelling. As TRPC proteins form non selective cation channels, we hypothesized that these molecules determine not only  $\text{Ca}^{2+}$ -mediated gene expression but also excitability and basic electrical properties of the myocardium in pathological states. We utilized the patch clamp technique to characterize the electrical remodelling associated with enhanced TRPC3 expression in cardiac myocytes. The murine HL-1 atrial cell model, which was found to express both TRPC3 and TRPC6 at low levels along with TRPC1 and typical signalling partners of TRPC proteins such as  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger-1 and  $\text{Ca}_v1.2$ , was modified to overexpress a functional YFP-TRPC3 fusion protein. TRPC3 over-expressing cells displayed substantially altered electrical properties even in the absence of TRPC-activating stimuli, as evident from a significant shortening of basal action potential duration and impaired frequency-dependent adaptation of action potential shape. Action potential clamp experiments were performed to characterize the properties of the TRPC3 overexpression-induced cation conductance and to analyze the mechanism of TRPC3-mediated electrical remodelling in the heart. Our results suggest a pivotal role of TRPC channels in cardiac electrical remodelling associated with maladaptive hypertrophy.

## C016

### Membrane targeting of voltage-gated calcium channel $\alpha_2/\delta$ -1 subunits

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Voltage-gated calcium channels (VGCCs) are involved in diverse physiological functions, including synaptic neurotransmission, insulin secretion and skeletal muscle excitation-contraction coupling. The activity of VGCCs is partly determined by their protein subunit composition and subcellular localization. VGCCs consist of three subunits: the pore-forming  $\alpha_1$  subunit and auxiliary  $\beta$  and  $\alpha_2/\delta$  subunits. The  $\alpha_2/\delta$  subunit is required for optimal plasma membrane expression and co-expression of  $\alpha_2/\delta$  with  $\text{Ca}_v \alpha_1$  and  $\beta$  subunits lead to an increase in peak current density between 2- and 4-fold with concurrent increases in both the rates of current activation and inactivation. The  $\alpha_2/\delta$ -1 subunit is also a target of the anti-epileptic drug gabapentin and up-regulation of  $\alpha_2/\delta$ -1 expression has been observed with peripheral nerve injury, which is thought to underlie various forms of neuropathic pain. Work in our laboratory is aimed at understanding the role of the  $\alpha_2/\delta$ -1 isoform in trafficking VGCC complexes ( $\alpha_1$ ,  $\beta$ ,  $\alpha_2/\delta$ ) to the plasma membrane and determining whether  $\alpha_2/\delta$ -1 is involved in targeting VGCCs to discrete neuronal compartments. The first stage of this project has involved using an eGFP-tagged  $\text{Ca}_v2.2$  (N-type  $\alpha_1$  subunit) and multiple forms of tagged  $\alpha_2/\delta$ -1 subunits, including an mRFP-, eGFP- and HA- tagged variants. Our preliminary findings have shown that  $\alpha_2/\delta$ -1 has a punctate distribution at the plasma membrane when expressed alone. The tagged  $\alpha_2/\delta$ -1 variants are functional, as shown by whole-cell patch-clamp electrophysiology. As expected, co-expression of  $\alpha_2/\delta$ -1 with  $\text{Ca}_v2.2$  and  $\beta_{1b}$  subunits induces a significant increase ( $\sim 2.5$ -fold) in peak current density ( $P < 0.05$   $\alpha_2/\delta$ -1-mRFP;  $P < 0.05$ ,  $\alpha_2/\delta$ -1-HA), that is associated with increased rates of channel activation and inactivation. Work is in progress to assess how  $\alpha_2/\delta$ -1 is targeted to the plasma membrane and how  $\alpha_2/\delta$ -1 affects membrane targeting of  $\text{Ca}_v \alpha_1:\beta$  complexes.

## C017

### Atosiban and nifedipin for the treatment of preterm labour

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The purpose of the present study was to perform a comparison between atosiban (Oxytocin antagonist) and Nifedipin (calcium channel blocker) for acute treatment of preterm labor and their maternal safety. A randomized controlled trial study was performed on 80 pregnant women with preterm labor, between 26 and 34 weeks of pregnancy. Forty women (the atosiban group) were compared with another 40 women (the nifedipin group) for the drugs' efficacy in delaying delivery for more than 48 h in order to undergo steroid therapy, and more than 7 days or more, and also to assess their maternal safety. The duration between the drugs' administration and delivery were compared too; the statistical analysis was performed using the Statistical Package for Social Science (SPSS). There was no statistically significant difference between the two groups in the treatment of preterm labor: Atosiban was effective in 82.5% of cases and nifedipin was effective in 75% of cases ( $P = 1.000$ ), for delaying delivery for 48 h, and 75% and 65% respectively for delaying delivery for more than 7 days. The maternal side effects in the atosiban group were 17.5%, and in the nifedipin group they were 40%, which had a statistically significant difference. ( $P = 0.027$ ). The duration between treatment and delivery was  $29.03 \pm 16.12$  days in the atosiban group and  $22.85 \pm 13.9$  days in the nifedipin group with no statistically significant difference ( $P = 0.79$ ). Atosiban is an effective and safe drug for the acute treatment of preterm labor with minimal side effects, and it can be an option in the treatment of preterm labor, especially in patients with heart disease and multi-fetal pregnancies.

# Ion channels (16.00–16.30)

C018

## Inhibitory effect of cromakalim on muscarinic receptor-stimulated spontaneous activity in diabetic rat bladder

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Potassium (K) channels have re-emerged as potential targets for treatment of overactive bladder and other urological diseases in recent years. ATP-sensitive K ( $K_{ATP}$ ) channels play an important role in mediation of smooth muscle membrane potential and spontaneous activity (SA) (Gopalakrishnan & Shieh, 2004). The aim of this study was to investigate the effect of  $K_{ATP}$  channel modulators on muscarinic-stimulated SA of bladder detrusor muscle from normal and streptozotocin-induced diabetic rats. Strips of detrusor muscle isolated from male Wistar rats, (250–400 g), 1 week following streptozotocin (65 mg/kg, i.p.) administration or from weight matched controls, were mounted under 2 g tension in Krebs-bicarbonate solution at 37°C and SA was recorded. Tissues were stimulated with carbachol (CCH) (0.05–0.5  $\mu$ M) to induce SA. The effect of the  $K_{ATP}$  channel opener cromakalim, (0.3–10  $\mu$ M), on this SA was assessed. The effect of the  $K_{ATP}$  channel blocker glibenclamide, (1–10  $\mu$ M), on basal SA was also investigated. Statistical analysis was performed using Student's *t*-test. Cromakalim decreased the amplitude of SA in control tissues ( $n = 16$ ) at 3  $\mu$ M ( $10.80 \pm 15.67\%$ ) and 10  $\mu$ M ( $29.26 \pm 17.19\%$ ) and at the same concentrations in 1-week diabetic tissues ( $n = 14$ ) ( $13.97 \pm 8.89\%$  and  $19.66 \pm 10.91\%$  respectively). There was no significant difference in the percentage decrease in amplitude of SA between control and diabetic tissues. However, there was a significant difference ( $P < 0.05$ ,  $n = 10$  in both groups) in percentage inhibition of the frequency of SA between control and diabetic tissues at 3  $\mu$ M (control =  $41.90 \pm 5.25\%$  vs. diabetic =  $16.26 \pm 6.24\%$ ) and 10  $\mu$ M (control =  $53.93 \pm 6.30\%$  vs. diabetic =  $21.61 \pm 5.84\%$ ) cromakalim. Glibenclamide did not significantly affect basal SA in either group. Opening of  $K_{ATP}$  channels seems to be important in modulating SA in control and diabetic bladder strips. Diabetic bladder is less sensitive to cromakalim than control tissues. This requires further investigation, but could play a role in the bladder dysfunction associated with diabetes mellitus.

### Reference:

Gopalakrishnan M and Shieh CC. *Expert Opin Ther Tar*. 2004; 5: 437–458.

C019

## Enhanced expression of vascular $K_{ATP}$ channels in an *in vitro* model of sepsis

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Activation of vascular ATP-sensitive potassium ( $K_{ATP}$ ) channels is implicated in the pathogenesis of septic shock. Upregulation of channel function may relate to increased activity and/or channel number. We used a cell model to investigate the effect of an inflammatory insult on  $K_{ATP}$  gene expression and whether this involved overproduction of nitric oxide (NO). Quiescent primary cultures of rat aortic smooth muscle cells were treated with *S. typhosa* lipopoly-saccharide (LPS: 1  $\mu$ g/mL) and interleukin (IL)-1 $\beta$  (10 ng/mL) for 48 h, with or without 1400 W (10  $\mu$ M), a selective inhibitor of inducible NO synthase. Levels of gene expression of the pore-forming Kir6.1 subunit and the regulatory SUR2B subunit (the two components of the  $K_{ATP}$  channel) were determined using RT-PCR. Densitometry quantified mRNA levels with respect to the housekeeping gene,  $\beta$ -actin, rubidium efflux (a surrogate marker of  $K^+$  efflux) and the membrane potential-sensitive fluorescent dye, DiBAC<sub>4</sub>(3) assessed functional responses to levromakalim, a specific  $K_{ATP}$  channel opener. In LPS/IL-1 $\beta$  treated cells, Kir6.1 subunit expression increased  $6.6 \pm 1.5$  fold ( $P < 0.05$ ,  $n = 5$ ), an effect reversed by 1400 W. By contrast, SUR2B levels did not change. Rubidium efflux significantly increased, as did the response to 1  $\mu$ M levromakalim (from  $12 \pm 0.1\%$  to  $16.7 \pm 0.1\%$ ,  $P < 0.001$ ,  $n = 12$ –15). Levromakalim caused membrane hyperpolarization in a dose-dependent manner, an effect potentiated in the presence of LPS/IL-1 $\beta$  and reversed by 1400 W. Increased expression and function of the  $K_{ATP}$  channel was seen in this *in vitro* model of sepsis. This increase in activity may be an important factor underlying the vascular hyporeactivity seen in septic shock.

# Pharmacogenomics/pharmacokinetics/drug safety (14.30–15.45)

## C020

### Trends (1995–2004) in propoxyphene consumption in the elderly: is its use justified?

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There is a lack of consensus on whether the use of propoxyphene is appropriate or not in the elderly in France. The aim of this study was to estimate the trends over 10 years (1995–2004) of propoxyphene use in the elderly and to assess factors associated with propoxyphene use in 2004. We carried out a repeated cross-sectional study using data collected among people aged  $\geq 65$ , examined in the Center for Preventive Medicine in the east of France. Studied variables were sociodemographic (gender, age), number of visits to the physician, medication use and the self-health status. The Joinpoint regression analysis was used to estimate the temporal changes in propoxyphene rate. Factors predicting propoxyphene use were identified by multiple logistic regression analysis. 30 683 participants were included in the study. 51.2% were women and the mean age was  $70.1 \pm 4.3$  years [65–99]. 83.8% of participants used at least one drug. Propoxyphene use concerned 2.9% of participants. The annual rates of the propoxyphene consumption increased significantly from 1.3% in 1995 to 3.3% in 2004. The Annual Percentage Change (APC) was 6.9% per year [2.9–11.1];  $P < 0.003$ . The risk to consume the propoxyphene was higher among women (OR = 2.46), participants with poor health status (OR = 1.73) and those consuming psychoactive drugs (OR = 2.05). This study has shown an unjustified increase of the propoxyphene consumption in France from 1995 to 2004. With the lack of consensus the question remains: is its use justified?

## C021

### Ablation of primary afferent neurons by neonatal capsaicin treatment reduces the susceptibility of the portal hypertensive gastric mucosa to ethanol-induced injury in cirrhotic rats

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Primary sensory afferent neurons modulate the hyperdynamic circulation in cirrhotic rats with portal hypertension. The stomach of cirrhotic rats is prone to damage induced by ethanol, a phenomenon associated with reduced gastric hyperemic response to acid-back diffusion (Ferraz *et al.*, 1997). The aim of this study was to examine the impact of ablation of capsaicin-insensitive neurons and the tachykinin NK1 receptor antagonist A5330 on the susceptibility of the portal hypertensive gastric mucosa to ethanol-induced injury and its effects on gastric cyclooxygenase (COX) and nitric oxide synthase (NOS) mRNA expression. Capsaicin was administered to neonatal, male, Wistar rats and the animals were allowed to grow. Cirrhosis was then induced by bile duct ligation in adult rats while controls had sham operation. Ethanol-induced gastric damage was assessed using *ex vivo* gastric chamber experiments. Gastric blood flow was measured as well as COX/NOS mRNA expression. Topical application of ethanol produced significant gastric damage in cirrhotic rats compared to controls ( $76 \pm 4\%$  vs.  $19.5 \pm 3\%$ ), which was reversed in capsaicin- and A5330-treated animals ( $21 \pm 5\%$  and  $34.8 \pm 1\%$ , respectively). Mean arterial and portal pressure was normalized in capsaicin-treated cirrhotic rats ( $92.54 \pm 3.7$  to  $126.7 \pm 4.9$  and  $17.52 \pm 0.9$  to  $9.4 \pm 1.3$ , respectively). Capsaicin and A5330 administration restored gastric blood flow responses to topical application of ethanol followed by acid in cirrhotic rats. Differential COX and NOS mRNA expression was noted in bile duct ligated rats relative to controls. Capsaicin treatment significantly modified gastric eNOS/ iNOS/ COX-2 mRNA expression in cirrhotic rats. Capsaicin-sensitive neurons modulate the susceptibility of the portal hypertensive gastric mucosa to injury induced by ethanol via tachykinin NK1 receptors and signaling of prostaglandin and NO production/release. Supported by FAPESP and CNPq.

#### Reference:

Ferraz *et al.* Am J Physiol. 1997; 272: G809–G814.

## C022

### The ASPOC project on perioperative chemoprophylaxis in general surgery: preliminary results from eight European countries

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The ASPOC Project (ASPPOC, Antibiotic Surveillance Project on Perioperative Chemoprophylaxis) is a multicentric study carried out in European countries, aiming at improving the quality and establishing a successful and evidence-based perioperative chemoprophylaxis. The aim of this study, which is part of ASPPOC, was to check conformance to the guidelines for perioperative chemoprophylaxis in general surgery, and to trace out changes that must be made in European countries

to improve its quality. Thirteen general surgery departments from eight European countries participated in the study. The participating countries were: Greece, Italy, Denmark, Hungary, Serbia, Croatia, Slovenia and Montenegro. Conformance to the recommended guidelines and quality of perioperative chemoprophylaxis were checked by use of the same questionnaire in each country. The following criteria of quality in chemoprophylaxis were checked: application of perioperative chemoprophylaxis, duration and time of initiation of chemoprophylaxis, and the kind of antibiotics used. A different practice on application and quality of perioperative chemoprophylaxis was observed in each country. Chemoprophylaxis was usually initiated during preinduction to anesthesia but its duration exceeded the maximum recommended time of 24 hours in many cases (Bratzler and Houck, 2005; Bratzler and Hunt, 2006; Fry, 2006). The recommended  $\beta$ -lactams were used in most cases; metronidazole or clindamycin was used in cases of anaerobic contamination. 3rd generation cephalosporins, gentamicin, amikacin and ciprofloxacin were also used. Non conformance to the recommended guidelines was observed in all countries, with serious discrepancies in application of perioperative chemoprophylaxis in most of them. Dynamic changes are required in order to improve the quality and establish an evidence-based perioperative chemoprophylaxis in general surgery.

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## C023

### Association between $\beta_2$ -adrenoceptor polymorphisms and receptor function in Malaysian asthmatics

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The  $\beta_2$ -adrenoceptor gene, a candidate gene in asthma, has been shown to have several single nucleotide polymorphisms (SNPs) that affect the receptor function and sensitivity to pro-inflammatory stimuli. Three of these SNPs are found at positions Arg-19Cys (influences the expression of receptor), Arg16Gly (influences airway hyper-responsiveness) and Gln27Glu (protects against receptor down-regulation) (Brodde *et al.*, 2005). These SNPs have the potential to affect lung function and bronchial inflammatory response and may act as disease modifiers in asthmatics (Lipworth *et al.*, 2002). This is a pilot study to determine the frequency of these SNPs and its effects on lung function and inflammatory responses in Malaysian asthmatics. Lung function test was performed and blood samples were collected from 150 asthmatics and 152 healthy volunteers. Plasma IgE levels were quantified by ELISA and direct sequencing was performed to detect the SNPs. Allelic frequencies of the SNPs are given in the table below. The SNPs at position Arg-19Cys and Gln27Glu were in strong linkage disequilibrium. There was no significant difference in the allelic frequencies of these SNPs between the two groups. These results differ from the published data for Caucasians (Leineweber *et al.*, 2004), indicating that the frequencies of these polymorphisms are different in the Malaysian population. The mean percentage predicted FEV<sub>1</sub> of asthmatics ( $63.91 \pm 1.60\%$ ) was significantly ( $P < 0.001$ ) lower than of the volunteers ( $89.51 \pm 0.67\%$ ). The plasma IgE levels were significantly ( $P < 0.001$ ) higher for asthmatics ( $301.4 \pm 16.72$  ng/ml) compared to the volunteers ( $206.31 \pm 13.45$  ng/ml). There was no significant association between the mean percentage predicted FEV<sub>1</sub> and plasma IgE levels for the different alleles and genotypes in both groups of subjects. Our observations suggest that these polymorphisms of  $\beta_2$ -adrenoceptor do not affect the lung function and plasma IgE levels in Malaysian asthmatics.

Allele	Arg-19	Arg16	Gln27
Healthy volunteers	0.832	0.457	0.832
Asthmatics	0.904	0.497	0.894
P value	0.059	0.568	0.162

#### References:

Brodde *et al.* Pharmacogenetics & Genomics. 2005; 15: 267–275.  
Leineweber *et al.* Life Sciences. 2004; 74: 2803–2814.  
Lipworth *et al.* Thorax. 2002; 57: 61–66.

## C024

### Pharmacokinetics of L-carnitine and its metabolic product after single oral administration of L-carnitine oral solution in Chinese healthy volunteers

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The specific, sensitive and simple HPLC-UV and HPLC-FL methods were developed to determine L-carnitine(LC) and its metabolic product acetyl-L-carnitine(ALC) and propionyl-L-carnitine(PLC) levels in human plasma and urine. The HPLC-FL method was successfully used in the pharmacokinetic study of L-carnitine and its metabolic product after single oral administration of L-carnitine oral solution in Chinese healthy volunteers. HPLC-UV: The analytes were extracted by protein precipitation and then precolumn derivatization with 2,4-dibromoacetophenone(PBPB) were performed. The fluorescent derivatives were separated on a Hypersil SiO<sub>2</sub> column, and the mobile phase consisted of acetonitrile-citrate buffer solution (10 : 90), the flow rate was 1.2 ml/min, the derivatives were monitored with a UV detector set at 260 nm. HPLC-FL: The analytes were extracted by protein precipitation and then precolumn derivatization with L-aminoanthracene (L-AA) were performed. The fluorescent derivatives were separated on a Hypersil C<sub>18</sub> column, and the mobile

phase consisted of acetonitrile-0.1 mol/l ammonium acetate (34 : 66), the flow rate was 1.0 ml/min. The derivatives were monitored with a fluorimetric detector set at 248 nm (excitation wavelength) and 418 nm (emission wavelength). The concentration of LC, ALC and PLC in plasma and urine were measured by HPLC-FL after single oral administration of L-carnitine oral solution, and the pharmacokinetics parameters were calculated by DAS software. The good linearity of the assay was observed over the concentration ranges investigated, 2.5~500  $\mu\text{mol/l}$  for LC, 0.5~50  $\mu\text{mol/l}$  for ALC, 0.1~20  $\mu\text{mol/l}$  for PLC in plasma and 2~1000  $\mu\text{mol/l}$  for LC, 1~500  $\mu\text{mol/l}$  for ALC, 0.2~50  $\mu\text{mol/l}$  for PLC in urine. The recoveries,

precision and stability test of LC, ALC and PLC correspond the requirement in plasma and urine. This method is proved to be stable, accurate and convenient to study the concentrations of LC, ALC and PLC in clinical plasma and urine. After single oral administration of 2 g L-carnitine oral solution, the main pharmacokinetic parameter of LC  $C_{\text{max}}$  is  $(84.73 \pm 25.23) \mu\text{mol/l}$ ,  $t_{1/2\beta}$  is  $(60.33 \pm 14.97) \text{h}$ ,  $\text{AUC}(0-t)$  is  $(2676.41 \pm 708.33) \mu\text{mol/h}$ ,  $T_{\text{max}}$  is  $(3.4 \pm 0.46) \text{h}$ . The accumulated excretion of LC, ALC and PLC were  $613.47 \pm 161.72 \mu\text{mol}$ ,  $368.25 \pm 134.77 \mu\text{mol}$ ,  $61.29 \pm 37.75 \mu\text{mol}$  in 24 h, respectively. And the accumulated excretion rate of LC was 6.05% in 24 h after administration.