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C051
COX-1 modulates the inflammatory macrophage phenotype and the host's ability to fight infection

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NSAIDs are classically used for the chronic treatment of rheumatic disorders and other degenerative inflammatory joint diseases through their inhibition of COX-derived prostaglandins, with many of their side-effects resulting from the inhibition of the constitutively expressed 'house-keeping' COX-1 enzyme. A consequence of selectively inhibiting COX-1 in an inflammatory environment and its ability to neutralise infection however, is not fully understood. In order to elucidate this, male C57/blk6 mice were orally dosed long-term with both COX-1 and COX-2 selective NSAIDs before inducing an experimental model of acute peritonitis. At specific time-points before and after the inflammatory stimulus cells within the peritoneum were analysed using FACS and the phenotype of resident and inflammatory macrophages were investigated through *ex vivo* culture, Bioplex and western blot analysis. Results demonstrate that COX-1 KO mice and wild-type mice (WTs) chronically treated with COX-1 selective NSAIDs have reduced resident peritoneal macrophages and monocytes numbers. Moreover, these tissue resident histiocytes when stimulated *ex vivo* with LPS produce more pro-inflammatory cytokines such as TNF-α, MIP-1α, MIP-1β, MCP-1 and IL-12 (p40) and less prostaglandins, cAMP, HO-1 and phosphorylated p38MAPK, STAT-1 and 3, indicating that inhibition of COX-1 polarises the phenotype of peritoneal histiocytes towards a T_H1-type immune response. 4 h after COX-1-inhibited animals were injected with group B *Streptococcus* (GBS) or zymosan, a similar profile of cytokines was observed in the cell-free exudate and in the supernatant of inflammatory leukocytes cultured *ex vivo*, with a clear reduction in IL-10. Mice chronically dosed with SC-560 or aspirin however, were unable to kill GBS as efficiently as WTs, which correlates with the depletion of tissue resident macrophages. The results therefore indicate that COX-1 is involved in tissue histiocyte cell replenishment and that it modulates the phenotype of macrophages during inflammation/infection. These discoveries have important clinical implications when considering whether to administer COX-1 selective NSAIDs to patients with chronic inflammatory diseases who are susceptible to infections.

C052
Cardioprotective doses of aspirin modulate the acute inflammatory response in humans

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Aspirin's potent and well documented anti-analgesic, anti-pyretic and anti-inflammatory properties have been exploited for centuries. Its use, albeit at high doses, for the treatment of chronic inflammatory conditions has nevertheless been associated with increased incidence of damage to the gastrointestinal tract. The search for a potent anti-inflammatory with lesser side effects is ongoing. Given the potential cardiovascular side effects which accompany the use of more recently developed selective cyclo-oxygenase 2 inhibitors and, on the basis that low-dose aspirin (75mg) is not only cardioprotective but modulates CRP levels, enhances the biosynthesis of 15-epi-lipoxin A4, a potent anti-inflammatory lipid mediator, the potential effects of cardioprotective doses of aspirin on a model of acute inflammation in humans using a well established cantharidin skin blister model were investigated. To this end, 11 healthy male volunteers between the ages to 18 and 55 were recruited, and administered a concentrated dose of cantharidin (a vesicant used in the treatment of warts) to the skin on their forearm causing blister formation. Blister content was assayed at 24h and 72h. Leukocyte recruitment and mediator release (cytokines and prostaglandins) were used as markers of the inflammatory response. The effects of low-dose aspirin were investigated using the same study design following a 10 day dosing regime with 75mg of aspirin orally. The patterns of leukocyte recruitment and pro-inflammatory cytokine release suggested a dichotomy in responses. A group of indivuduals were distinguished whose low baseline cytokine levels and leukocyte accumulation (delayed resolvers) at 24h were significantly enhanced in blisters following low dose aspirin treatment. The second group of individuals (early resolvers) presented comparatively higher baseline cytokine levels which were reduced after low-dose aspirin treatment. Interestingly, in blisters from delayed resolvers, levels of the potent anti-inflammatory mediator 15-epi-lipoxin A4 were significantly decreased following low dose aspirin treatment. The hypothesis is that this could partially account for the apparent pro-inflammatory effect of low-dose aspirin in delayed resolvers. It is demonstrated that a novel immune-modulatory effect of low-dose aspirin, which, depending on the inflammatory environment is either able to up- or down-regulate the inflammatory response in humans.

C053
Resolution-phase macrophages possess a unique inflammatory and bactericidal phenotype that is controlled by cAMP

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Macrophages are classified as either classically- (M1) or alternatively activated (M2). M1 cells express pro-inflammatory genes while M2 are typified by their protective, anti-inflammatory nature. Resolution of acute inflammation is an immune-suppressive event consequent to infection/injury characterised by the clearance of leukocytes followed by wound debridement by macrophages leading to restorative physiology. Though macrophages are crucial for determining resolution, their phenotype and the endogenous factors that control it, are unknown. To address this, we isolated macrophages from the resolution phase of acute inflammation and found that compared to M1 cells, resolution-phase macrophages (rM) possess weaker bactericidal properties and express an alternatively-activated phenotype but with elevated M1 markers including COX 2 and iNOS. Importantly, this unique phenotype is flexible being controlled by intracellular cAMP, which, when inhibited, transforms the phenotype of rM to that of M1 cells. Conversely, elevating cAMP in M1 cells alters their phenotype to that of rM. Thus, we describe rM cells as being neither classically nor alternatively activated but as a hybrid of both definitions controlled by cAMP. In doing so we provide insight in to the resolving nature of acute inflammation and put forward an explanation for host susceptibility to secondary infection and dysregulated cytokines synthesis.

Table 1. Comparison of resolving (rM) and inflammatory (M1) macrophage activities

	Comparison rM and M1 phenotype	
	rM	M1
CD206	++	+
Acute phase proteins	0	+++
Cytokines (TNF etc)	+	+++
Chemokines	+	++++
Resolving mediators (IL-10, PGD_2)	+++	+
cAMP	+++	+
Cox2, INOS	++	0
Bactericidal activity	+	+++

C054
Further studies on the rapid glucocorticoid effects in the U937 monocytic cell line and its potentiation by cromoglycate-like drugs

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We recently reported to the Society that the antiallergic drugs (AAD) sodium cromoglycate and nedocromil greatly potentiate the glucocorticoid (GC)-induced secretion of Annexin (Anx)-A1 from U937 cells (Yazid *et al.* 2007). We now report further details of this unusual mechanism. Time lapse recordings of the secretory event using U937 cells transfected with Anx-A1-GFP show that 2 nM dexamethasone (dex) triggers some Anx-A1 secretion within 3 min but that when combined with nedocromil (1–20 nM), release may be complete within 1 min. This was confirmed using EM techniques where we quantified immunogold-labelled Anx-A1 in U937 cells. Dex (2 nM) led to a four-fold increase in Anx-A1 on the cell surface ($P < 0.05$ vs. control; $n = 30$) whereas nedocromil (5 nM) alone was without effect but when given together with 2 nM dex, led to a six-fold augmentation ($P < 0.01$ vs. dex alone; $n = 30$). ELISA analysis of secreted Anx-A1 revealed that (2 nM) dex or hydrocortisone (HCT) increase Anx-A1 secretion by four-fold ($P < 0.01$, vs. control) and two-fold (vs. control) respectively, whereas when combined with (5 nM) nedocromil, Anx-A1 release was increased by a further two-threefold ($P < 0.01$ vs. dex alone) and two-fold ($P < 0.01$vs. HCT alone). In the presence of the neutralizing anti-AnxA1 antibody (20 μg/mL) thromboxane B_2 inhibition by these drugs was abolished by 95% ($P < 0.001$ vs. control). Finally to address the question of how AAD potentiated GC effects, PKCα/β activation and PKC activity were assessed and measured in the 100 000 × g membrane fraction. Dex (2 nM) alone increased PKCα/β translocation to the membrane fraction and whilst nedocromil (5 nM) had no effect on the total mass of enzyme translocated, it potentiated PKC enzyme activity by 21.3% ($P < 0.5$ vs. control, $n = 3$) and increased PKCα/β phosphorylation as revealed by Western blotting analysis. We conclude that AAG augment GC action by potentiating PKCα/β activity leading to enhanced phosphorylation and release of Anx-A1 with consequent inhibition of eicosanoid release. We thank the Research Advisory Board of St Bart's and the London for their support.

Reference:

Yazid S, *et al.* 2007. Proceedings of the British Pharmacological Society, http://www.pa2online.org/vol5/issue2/-abst 039p, 2007.

C055

Initial characterisation of the novel transgenic, the *alx/fpr-rs2* null mouse

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The human FPR family consists of three members, namely FPR, ALX (or FPR-like 1) and FPRL2. These receptors show differential binding affinity for formyl-methionyl leucyl phenylalanine (*f*MLP); in addition they interact with a plethora of diverse and structurally unrelated ligands that transduce pro- or anti-inflammatory signals. In the mouse, the FPR family comprises seven genes of which ALX share closest homology with two mouse genes, *fpr-rs1* and *fpr-rs2*. This study describes the generation and functional characterisation of a novel *alx/fpr-rs2* null mouse colony (KO) generated using a targeting/reporter vector that contained an insert for green-fluorescent protein (GFP) to monitor the fpr-rs2 gene promoter. The *alx/fpr-rs2* KO colony was prepared by homologous recombination and confirmed by Southern blotting and PCR. GFP expression was assessed by flow cytometry in naïve and stimulated cell populations *ex vivo* as well as *in vitro*, compared to wild type littermate controls (WT). *In vitro* chemotaxis experiments, utilising several ligands, were performed with bone marrow derived macrophages (BMDM) on 5-μm ChemoTxplate™ (Receptor Technologies) and assessed by Alamar Blue (Invitrogen) staining. Two acute inflammatory models, zymosan peritonitis (1 mg i.p.; 4 h) and IL-1ß-induced air pouch (20 ng; 4 h), were used and granulocyte transmigration assessed by differential cell counts with Turk's stain and GR-1$^+$ using flow cytometry. Statistical analysis was performed using one-way ANOVA and post-hoc tests. GFP expression was observed in both naïve and stimulated cell populations in KO mice, predominantly in granulocytes and macrophages. *In vitro* BMDM derived from KO mice showed reduced capacity to migrate in response to fMLP (10 μM) and a significantly ($P < 0.01$) reduced ability to respond functionally to the ALX ligand serum amyloid protein A. *In vivo*, treatment of mice with the selective ALX agonist annexin-A1 (AnxA1) inhibited granulocyte cell influx following IL-1ß-induced air pouch in WT ($P < 0.001$, 1 μg) compared to untreated control. KO animals showed a weak response only at maximal doses ($P < 0.01$, 10 μg). In conclusion these initial analyses reveal interesting patho-pharmacological functions for the mouse orthologue of human ALX.

C056

Differential effect of PPARβ agonists on *E. coli*-induced NOSII, TNFα and IP10 in murine macrophages

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Peroxisome proliferator-activated receptor (PPAR)β is a cytosolic receptor which regulates inflammatory responses. We have previously shown that two PPARβ agonists, GW0742 and L165041, inhibit the activity of inducible nitric oxide synthase (NOSII). Here we have investigated the effects of these agonists on the expression induced by bacteria in murine macrophages of tumour necrosis factor (TNF)α and interferon-gamma-inducible 10 kD protein (IP10), as well as NOSII. Murine macrophages (J774) were incubated with GW0742 or L165041 for 4 h before addition of whole heat-killed *E. coli* and incubation for a further 24 h. NOSII activity was assessed by measuring the accumulation of nitrite in culture medium; TNFα and IP-10 production were assessed by ELISA.

Our data show that in cultured murine macrophages exposed to *E. coli* PPARβ agonists inhibit NOSII activity, increase TNFα production, but have no effect upon IP10 production. These results indicate that PPARβ agonists differentially regulate diverse inflammatory pathways.

C057

The PPAR target gene CD36 is a rate limiting factor for the sensing of gram positive *S. aureus* in vascular smooth muscle cells but not in macrophages

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Bacteria activate macrophages and vascular smooth muscle cells (VSMCs) resulting in induction of nitric oxide synthase (NOS)II activity. Here we show that the PPARγ agonist rosiglitazone (rosi) increases the sensing of Gram positive bacteria by VSMCs but not macrophages via a CD36 dependent pathway. NOSII activity was increased in rat VSMCs by Gram positive *S. aureus* (3×10^8CFU/ml) or Gram negative *E. coli* (10^8 CFU/mL). Pre-treatment of cells with rosi increased NOSII activity in cells treated with *S. aureus*, but not *E. Coli* (Fig 1A), an effect prevented by the PPARγ antagonist GW9962 (10^{-5}M; 91 ± 9% control) or a specific binding antibody to CD36 (99 ± 14%). CD36 levels in VSMCs were increased by rosi (by 8.7 ± 0.2 fold, $n = 4$). By contrast, rosi inhibited NOSII activity induced by bacteria in macrophages from wild type mice and CD36$^{-/-}$ mice (Fig 1B). *S. aureus* induced similarly hyporeactivity (mediated by NOSII) in vessels from wild type (not shown) or CD36$^{-/-}$ mice (Fig 1C).

These data reveal a mechanism by which PPARγ agonists may propagate vascular inflammation.

C058

The inflammatory effects of PAR2 activation are mediated by neurokinin-1 (NK_1) receptors in the rat temporomandibular joint (TMJ)

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The aim of the present study was to evaluate whether PAR2-induced synovial joint inflammation in the rat TMJ is mediated by neurogenic mechanisms. Male Wistar rats (200–250 g) were anaesthetised with ketamine + xylazine (80 mg/kg and 20 mg/kg, i.p., respectively) and PAR2 agonists were intra-articularly (i.art.)

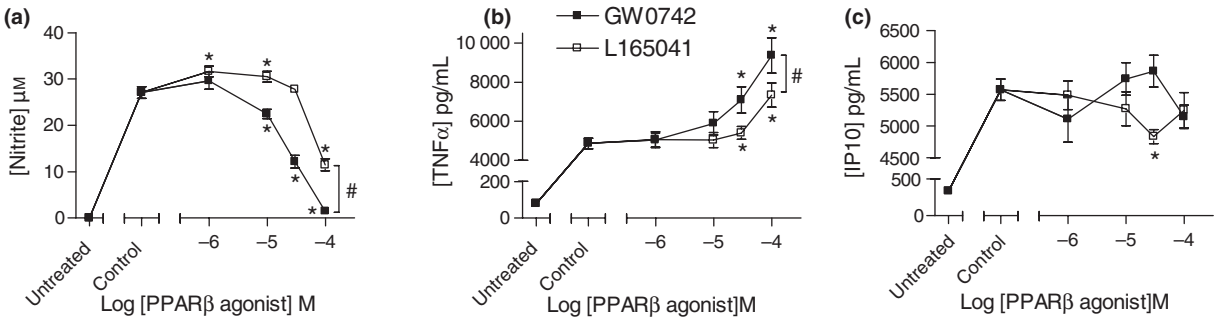

Figure 1 for C056. Effect of the PPARβ agonists GW0742(filled squares) and L165041(unfilled squares) on production of (A) NO, (B) TNFα and (C) IP10 induced by *E. coli* (3×10^7 CFU/ml).'Untreated' = no *E. coli* and no agonist; 'Control' = no agonist. Data is mean ± S.E.M of $n = 9$. # significant difference between agonists (two-way ANOVA);* significant difference to Control (Bonferroni test); $P < 0.05$.

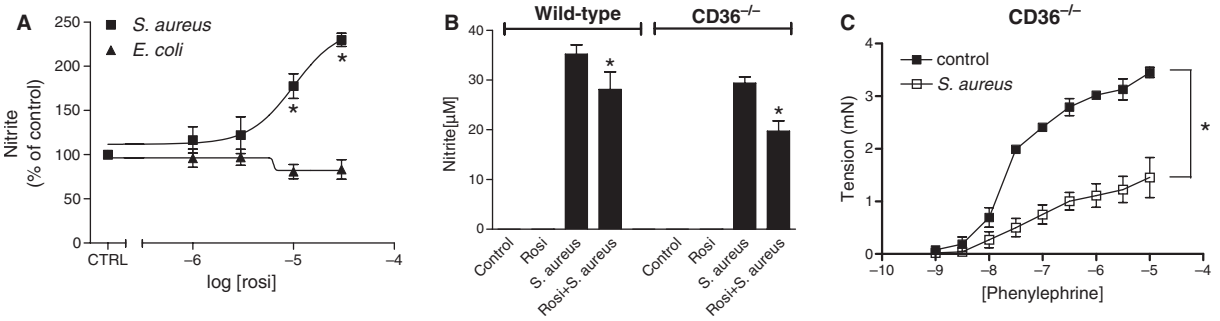

Figure 1 for C057. VSMC (A) or macrophages (B) treated with bacteria for 48 hours, NOSII activity measured as nitrite formation. C, Vascular reactivity of aorta incubated for 24 h with *S. aureus*. Results were analyzed by one-way (A and B) or two-way (C) ANOVA and * denotes $P < 0.05$.Data are mean ± SEM for $n = 4–8$.

injected in the left (ipsilateral) TMJ at doses near the ED_{50} values determined in previous experiments (i.e., 150 ng of trypsin or 100 μg of the PAR2 agonist peptide SLIGRL-NH_2 - PAR2-AP); in control animals, the same doses of boiled trypsin or the reverse peptide (LRGILS-NH2) were injected. Plasma protein extravasation in the TMJ (in μL/g TMJ) was determined by the accumulation of ^{125}I-albumin 45 min after the agonist injection; 4 h later, neutrophil influx was estimated by measuring myeloperoxidase (MPO) activity in the synovial fluid and the TMJ were dissected for histopathological examination. The neurogenic component of inflammation was evaluated by pre-treating the rats with either the non-peptide NK_1 receptor antagonist SR140333 (300 nmol/kg, i.v.) 15 min prior to PAR2 agonists. Co-localization of PAR2 and TRPV1 receptors in trigeminal ganglion neurones was assessed by double immunofluorescence. Both PAR2-AP and trypsin elicited significant plasma extravasation compared to the contralateral joint (116 ± 5 vs. 20 ± 3; $P < 0.01$, $n = 4$). In addition, PAR2-AP also increased MPO activity (6.9 ± 1.4 1.6 ± 0.5; $P < 0.01$, $n = 6$), and these markers were significantly inhibited by SR140333 ($P < 0.05$). Evidences of tissue proteolysis (presence of resident cells in synovial lavages and synovial damage) were observed 4 h after trypsin. Immunofluorescence microscopy revealed a high degree of PAR2 and TRPV1 receptor co-localization in trigeminal ganglion neurones. Based on these results, we suggest that PAR2-induced inflammation of the rat TMJ is mediated by a neurogenic component involving the activation of NK_1 receptors. The authors thank CNPq and FAPESP for the financial support and fellowships.

C059

Effect of L-carnitine and DL-α-lipoic acid on neurotransmitters and antioxidant status in the discrete brain regions related to parkinsonism in MPTP induced neurotoxicity in aged rats

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Increase in reactive oxygen species (ROS) production and imbalanced antioxidant defence status and repair mechanisms, which may lead to membrane molecular neurovascular damages, leads to cell death during ageing and age related neurodegenerative disorders such as Parkinson's disease (PD). PD is characterized by signs of major oxidative stress and mitochondrial damage in the pars compacta of the substantia nigra is uniquely vulnerable to oxidative damage. Biochemical evidence suggests that PD involves multifactorial oxidative neurodegeneration and that L-dopamine (L-DOPA) therapy further adds to the oxidative burden. Ageing is associated with several deficits in the brain, which have been correlated with the impaired synthesis, uptake, release of neurotransmitters, number of receptors and their functions. Vascular oxidative stress is the key mechanism involved in the age-related alteration in the expression of nitric oxide synthase (NOS) and an ultimate synthesis of the endothelium derived relaxing factor nitric oxide radical (NO). The present study was designed to investigate whether the combined application of L-carnitine (LC) (300 mg/kg b.wt/day) and DL-α-Lipoic acid (ALC) (100 mg/kg b.wt/day) (LC and ALC started 3 days prior to MPTP) for 21 days when administered intraperitoneally would prevent the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg body wt/i.p administration twice in 1 h interval, six animals in each group) induced neurotoxicity and the concentration of neurotransmitters such as dopamine, serotonin and norepinephrine in the discrete brain regions related to parkinsonism such as striatum and hippocampus of aged albino rats. Aged MPTP-challenged rats elicited a significant decline ($P < 0.05$) in superoxide dismutase, catalase, glutathione peroxidase activities, glutathione content and neurotransmitters and increased lipid peroxidation, xanthine oxidase (XO) and NOS activities when compared with control aged rats which is reversed by the combined application of LC+ALC. The results of this study provide evidence that LC and ALA by acting as a potent ROS scavengers and iron-chelating antioxidants exerted protective effect against MPTP and proved efficacious in augmentation of neurotransmitters and protecting brain against neurogenotoxic-xenobiotics, ageing related neurodegeneration itself and relevant disorders such as parkinsonism.

C060

Minocycline reduces inflammatory markers but not acute axonal injury following traumatic brain injury in mice

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Minocycline has shown to exert anti-inflammatory and neuroprotective effects in several animal models of neurodegenerative diseases and acute brain injuries. However, the effect of minocycline on the consequences of traumatic brain injury (TBI) is still not fully investigated. Therefore, our aim was to study the effects of minocycline on TBI-induced inflammatory markers (MMP-9 and IL-1β) and traumatic axonal injury (TAI) in a murine model of diffuse brain injury, which mimics severe TBI. Closed head injury was performed in anesthetized male Swiss mice (28–30 g) under 2% halothane (Hellal *et al.,* 2003). Brain MMP-9 and IL-1β levels were evaluated by Zymography/Western blot and ELISA, respectively. TAI was evaluated by accumulation of β-amyloid precursor protein (β-APP) revealed by immunohistochemistry. Minocycline was administered twice, 5 min (90 mg/kg, i.p.) and 6 h (45 mg/kg, i.p.) following TBI, whereas the control group received vehicle (PBS 0.1M pH 7.4) with the same protocol (n = 6–10 per group). Diffuse brain injury leaded to 1) acute and persistent MMP-9 activity up to 72 h post-TBI, 2) acute and transient elevation of brain IL-1β levels with a maximum 6 h after TBI, 3) acute accumulation of axonal β-APP at 6 h which declined up to 72 h post-TBI. Treatment with minocycline markedly decreased the TBI-induced MMP-9 and IL-1β with no effect on the TBI-induced axonal accumulation of β-APP at 24 h. Minocycline exerts anti-inflammatory effects in our model of diffuse brain injury. However acute treatment with minocycline is not able to reduce TAI suggesting that under our experimental conditions the neuroinflammation does not play a crucial role in the development of acute TAI. Future studies have to be investigated to test the effect of chronic administration of minocycline on the late TAI following TBI.

Reference:

Hellal F *et al.* J Neurotrauma. 2003; 20(9): 841–51.

C061

Simvastatin, a hydroxymethylglutaryl-coenzyme A reductase inhibitor, promotes neurological recovery and anti-oedematous effect in traumatic brain injury

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Traumatic brain injury (TBI) leads to a deleterious neuroinflammation including brain oedema, matrix metalloprotease 9 (MMP9) activation, and parenchyma infiltration by leukocytes. In addition, inhibition of HydroxyMethylGlutaryl-Coenzyme A (HMG-CoA) reductase by statins mediates anti-inflammatory effects. This led us to study the effect of simvastatin on the consequences caused by TBI. TBI was induced by lateral fluid percussion of the temporoparietal cortex on male Sprague-Dawley rats (290–320 g). Sham-operated rats (n = 14) underwent the same surgery except for percussion. Rats were given orally vehicle (n = 14) or simvastatin at 25, 37.5, 50, 75 or 100 mg/kg (n = 5-8) 1 and 6 h after brain injury. At 24 h, neurological assessment was done by two tests: one for reflexes and sensorimotor responses (ranging from zero = worst to nine = best), and the second untitled beam walking test for motor coordination (ranging from zero = worst to four = best). Then cerebral oedema was determined using brain water content. Thereafter, the effect of simvastatin on MMP9 activity was evaluated by zymography at 24 h after TBI. TBI decreased both the neurological and beam walking scores (4.9 ± 0.3 and 1.0 ± 0.3, $P < 0.001$). Simvastatin at 37.5 mg/kg improved both scores (6.6 ± 1.1, $P = 0.05$; 2.8 ± 0.8, $P < 0.05$). Other doses were devoid of any effect. TBI induced a brain oedema (82.6 ± 0.4%, $P < 0.001$) that was reduced by three doses of simvastatin (37.5 mg/kg: 80.6 ± 0.5%, $P < 0.01$; 50 mg/kg: 81.3 ± 0.4%, $P < 0.05$; 75 mg/kg: 81.1 ± 0.5%, $P < 0.05$). Simvastatin at 37.5 mg/kg, which decreased neurological deficit and brain oedema, failed to reduce post-traumatic MMP9 activity. Our study shows that simvastatin reduces neurological deficits and brain oedema after TBI. Its anti-oedematous effect is independent of MMP9 decrease, indicating mechanism(s) remains to be elucidated.

C062

Neuroprotective effect of dizocilpine on acute phase changes induced by partial global cerebral ischemia in mice

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The rationale of the study was to investigate for the possible neuroprotective effect of dizocilpine, a NMDA antagonist, on acute phase changes in mice model of cerebral ischemia induced by Bilateral Carotid Artery Occlusion (BCAO). Balb/c male mice (n = 72) weighing 20–30 g were used in study. BCAO model was used to induce partial global cerebral ischemia. Animals were divided into three groups (sham operated, surgery group and treatment group). Morphological assessment included infarct size measurement and brain oedema measurement. Post ischemic seizure susceptibility was assessed using sub convulsive dose of PTZ (30 mg/kg i.p.). Short term memory impairment and motor coordination was assessed. All the biochemical estimations were done including TNF- alpha level along with protein estimation. BCAO induced significant infarct size (87.65 ± 1.95 %; $P < 0.001$) and oedema (76.28 ± 0.51%; $P < 0.01$) in saline treated control group along with high increase in oxidative stress shown by increased lipid peroxidation and decreased levels of antioxidants like SOD, CAT, GPx. Dizocilpine administration at the dose 0.1 mg/kg i.p. showed neuroprotective effects by reducing cerebral infarct size significantly as compared to control group. Post ischemic seizure susceptibility was not reduced as number of positive responders and mean seizure score was not decreased to a significant level. Brain oedema did not subside and remained almost at the same level as in control group. Dizocilpine has improved the short term memory and grip strength to a significant level. Dizocilpine had reduced the TNF-alpha levels as compared to ischemia group but not significantly. Dizocilpine has improved all the antioxidants levels showing activity against oxidative stress induced by BCAO. In conclusion, dizocilpine showed neuroprotection against ischemia except post ischemic susceptibility suggesting a role of NMDA antagonists as neuroprotective agents.

C063

Does primary absence epilepsy have an antagonistic effect on kainic acid-induced limbic seizure generalization?

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Previous studies of kindling in models of genetic absence epilepsy have shown a resistance to secondary generalization of focal limbic seizures. In this study, an interaction between temporal lobe epilepsy by using intra amygdaloid injection of kainic acid and typical absence epilepsy was investigated in adult genetic absence epilepsy rats from Strasbourg (GAERS). We examined behavioral and EEG changes in Wistar control rats and GAERS in acute short-term periods during status epilepticus and chronic long-term periods. Male Wistar rats ($n = 6$) and GAERS ($n = 6$) were used in the experiments. A guide cannula was stereotaxically implanted into the right basolateral amygdala for the kainic acid injection under the anaesthesia. Animals were given a single intraamygdaloid injection of kainic acid (750 ng) dissolved in 300 nl of artificial cerebrospinal fluid. The behavior of rats after the kainic acid injection was evaluated on the basis of a 6-stage scale. The times to the first seizures and number of convulsive and non-convulsive seizures in acute and chronic periods were evaluated from video recordings. In the acute period the first convulsive seizure was significantly delayed in GAERS compared to control animals. There was no difference in the number of convulsive seizures per hour between the Wistar and the GAERS groups. Thereafter, the animals in both groups became epileptic although convulsive seizures were delayed for GAERS relative to Wistar rats. Intensive basal spike-and-wave discharge activity in GAERS was completely suppressed immediately after kainic acid injection and gradually reappeared. Duration of the disappearance of spike-and-wave discharges ranged from 24 h to 3 days. Our findings demonstrate an interaction between two types of epilepsy and further a mutual cross inhibition of circuits involved in absence epilepsy and temporal lobe seizures.

C064

Pharmacotherapy resistance in patients undergoing resective epilepsy surgery due to hippocampal sclerosis is not associated with C3435T polymorphism in the ABCB1 (MDR1) gene

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The pathogenesis underlying resistance to pharmacotherapy in epilepsy is not fully clarified. One of the candidate mechanisms that has attracted considerable attention recently is the limitation of anticonvulsant medication access to the seizure focus by a number of efflux transporters, the most well known of which is P-glycoprotein (P-gp), encoded by the multidrug resistance ABCB1 (or MDR1) gene. The C3435T single nucleotide polymorphism is a common mutation in the gene coding for ABCB1. This polymorphism has been correlated with drug response. There are conflicting results about the association between C3435T polymorphism and resistance to treatment in epileptic patients. We investigated the frequency of C3435T polymorphism in epileptic Turkish patients who underwent resective epilepsy surgery due to resistance to antiepileptic therapy. The results were compared with those of healthy controls. DNA samples were obtained from 100 healthy controls and 89 consecutive adult patients who underwent resective brain surgery due to refractory seizures at our epilepsy center. Genotypes for the C3435T polymorphism were determined by PCR and restriction analysis. Comparison of drug resistant patients and healthy controls revealed no significant difference in allele frequency (C vs. T, $P = 0.90$) and genotype frequency ($P = 0.36$). The findings in the pure hippocampal sclerosis (HS) group ($n = 73$) were not significantly different from the control subjects (allele frequency: $P = 0.59$; genotype frequency: $P = 0.34$). Our findings failed to demonstrate an association between C3435T polymorphism and drug resistance in a sample of Turkish patients with refractory epilepsy who underwent resective brain surgery. Further studies are warranted in larger patient populations.

C065

Oral clonidine inhibits visceral pain-related visceromotor and cardiovascular responses to colorectal distension in rats

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The α2-adrenegic agonist, clonidine, modulates colorectal motor and sensory functions in humans and, given intrathecally, has analgesic effects in the colorectal distension (CRD) model in rats. We evaluated the effects of systemic clonidine on the visceral pain-related viscerosomatic and autonomic cardiovascular responses to CRD in rats. Activity of the abdominal musculature (viscerosomatic responses), monitored by intracolonic manometry, and changes in mean arterial blood pressure (MABP) and heart rate (HR), monitored by telemetry, were assessed simultaneously in conscious rats during CRD (repetitive noxious: 12 × 80 mmHg, or ascending phasic: 10–80 mmHg). Clonidine or vehicle was administered systemically (po or i.v.) 30 min before CRD. Clonidine (50, 100 or 200 nmol/kg, po, $n = 7$ each) inhibited the viscerosomatic response to repetitive noxious CRD by 6 ± 7%, 18 ± 7% ($P = 0.06$ vs. vehicle) and 29 ± 8% ($P < 0.05$ vs. vehicle), respectively. In telemetrized rats, clonidine (200 nmol/kg, po, $n = 6$) attenuated phasic, noxious CRD-evoked increase in MABP (70 ± 7% inhibition) and HR (67 ± 16% inhibition) and reduced the viscerosomatic responses to CRD by 40 ± 6% (all $P < 0.05$ vs. vehicle). Similar effects were observed after i.v. administration. Likewise, clonidine (200 nmol/kg, po, $n = 8$) reduced the response to ascending phasic CRD by 55 ± 5% and increased the threshold for pain (vehicle: 32.5 ± 4.5 mmHg; clonidine: 65.0 ± 6.3 mmHg) (both $P < 0.05$ vs. vehicle). At the doses tested, no gross side-effects were observed. Systemic clonidine, at doses devoid of visible side-effects, has analgesic effects in the CRD model of visceral pain in rats. These observations confirm the analgesic activity of clonidine on visceral pain and support the translational value of the rat CRD model to humans.

C066

Deficit on learning and working but not on reference memory in rats treated with harmane

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Harmane have a wide spectrum of pharmacological actions. We investigated the effect of harmane on learning and memory by using passive avoidance (PA) and three panel runway paradigm [reference memory (RM) and working memory (WM)]. Harmane (2.5, 5, 7.5 mg/kg) was administered i.p. 30 min prior to experiments. The experiments were performed with adult male Wistar rats (200–250 g). All results were expressed as the mean ± SE and analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. The number of error values (NOE) in the WM task recorded in the one trial were 2.9 ± 0.28 in the vehicle ($n = 8$), in the harmane groups, 3.89 ± 0.59 (2.5mg/kg, $n = 8$), 4.8 ± 0.49 (5 mg/kg, $n = 7$) and 5.3 ± 0.22 (7.5 mg/kg, $n = 9$). Recorded from the 2–6 trial were 4.5 ± 0.78 (vehicle, $n = 8$), for harmane groups 4.45 ± 0.85 (2.5 mg/kg, $n = 8$), 10.2 ± 0.68 (5 mg/kg, $n = 7$), 15 ± 2.08 (7.5 mg/kg, $n = 9$). In the WM task there was no change in the NOE in the one trial while there were statistically significant values in 5 and 7.5 mg/kg harmane doses from the 2–6 trial. The latencies in the one trial were 8.58 ± 0.56 (vehicle, $n = 8$), the harmane groups were 8.92 ± 0.77 (2.5 mg/kg, $n = 8$) and 11.09 ± 0.61 (5 mg/kg, $n = 7$), 9.14 ± 0.88 (7.5 mg/kg, $n = 9$) and the latency recorded from 2–6 trial were 22.86 ± 1.13 (vehicle, $n = 8$), in the harmane groups, 21.99 ± 1.02 (2.5 mg/kg, $n = 8$), 24.94 ± 1.36 (5 mg/kg, $n = 7$), 29.48 ± 1.78 (7.5 mg/kg, $n = 9$) in the WM. The latency of the animals from the 2–6 trial was significantly prolonged in harmane 7.5 mg/kg group.There were no differences in the latency and NOE values in the RM task. The passive avoidance results were 251.43 ± 17.20 (control, $n = 16$), 189.44 ± 31.45 (2.5 mg/kg, $n = 18$), 92.34 ± 25.19 (harmane, 5 mg/kg, $n = 18$), 58.77 ± 17.68 (harmane, 7.5 mg/kg, $n = 22$). Impaired PA performance was observed in doses 5 and 7.5 mg/kg of harmane. We conclude that harmane can modulate learning and WM but not RM.

C067

Effect of delphinidin pre-treatment on endothelial cell survival and vascular function

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We tested the hypothesis that delphinidin, an anthocyanin in berries, protects endothelial cells against oxidant-induced death and has antioxidant effects that protect nitric oxide (NO) (Stoclet *et al*, 2004). Human umbilical vein endothelial cells (HUVECs) were pre-treated with delphinidin (100 nM–100 μM) 24 h prior to induction of oxidative stress by co-incubation (a further 24 hr) with H_2O_2 (10 μM), pyrogallol (O_2^- generator; 100 μM) or menadione (OH generator; 10 μM). Cell survival was assessed by trypan blue exclusion. Rings of porcine coronary artery (domestic pig; male/female; ~60–70 kg) were also incubated with delphinidin (100 nM–100 μM; 24 h) prior to endothelial denudation and pre-constriction using the thromboxane A_2 analogue, U46619 (100 nM). Cumulative concentration-response curves to a NO donor (DETA-NONOate) were subsequently obtained before and after the addition of the Cu/Zn superoxide dismutase inhibitor, diethyldithiocarbamate (DETCA; 10 mM, 15 min incubation). ANOVA was used throughout to determine statistical significance. All oxidising agents significantly increased cell death in HUVECs ($P < 0.05$, $n = 5$ for all). Delphinidin pre-treatment significantly reduced ·OH-mediated cell death, but only at a concentration of 100 nM ($P < 0.05$, $n = 5$). Pre-treatment had no significant effect on cell death initiated by H_2O_2 or pyrogallol. Functional studies revealed a different pattern, whereby only 10 μM delphinidin significantly reversed DETCA-induced loss of sensitivity to NO ($P < 0.05$; $n = 6$); pre-treatment with either 100 nM-1μM or 100 μM delphinidin failed to reproduce the beneficial effects of the 10 μM intermediate concentration. These results suggest that pre-incubation of HUVECs with physiologically relevant concentrations of delphinidin protect against ·OH-induced cytotoxicity, but not that induced by $\cdot O_2^-$ or H_2O_2. A supra-physiological concentration of delphinidin is required to protect NO in a vascular model of oxidative stress, suggesting that dietary intake of delphinidin is likely to have a greater impact on endothelial function through improved cell survival than via direct protection of NO.

Reference:

Stoclet *et al.* Eur J Pharm. 2004; 500: 299–313.

C068

Bioactive tripeptides Ile-Pro-Pro and Val-Pro-Pro protect endothelial function *in vitro* in normotensive and hypertensive rats

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Milk drink containing casein-derived bioactive tripeptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) has been shown to decrease blood pressure both in animal models and clinical studies. This effect can be attributed to the tripeptides. It has been suggested that one possible blood pressure lowering mechanism of the tripeptides could be angiotensin-converting enzyme (ACE) inhibition. However, not all studies support this finding. Thus, *in vitro* studies using isolated rat mesenteric arteries were performed to elucidate the effects of IPP and VPP on vascular function. Experiments were carried out with male normotensive Wistar-Kyoto (weight range 317–412 g) and spontaneously hypertensive (SHR, 191–269 g) rats. Isolated superior mesenteric arteries were incubated in Krebs solution containing 1 mM of the peptide (either IPP or VPP) in +4°C for 48, 24, 12 or 1 h. After incubation mesenteric artery rings were mounted in an organ bath chamber and extensive vascular reactivity measurements were performed. Acetylcholine-induced endothelium-dependent relaxation was better preserved ($P < 0.05$, repeated measures ANOVA followed by Bonferroni's test) in mesenteric arteries of both strains incubated with IPP or VPP compared to the control. This was not the case in sodium nitroprusside-induced endothelium-independent relaxation. The ACE-inhibitory activity of IPP and VPP was studied by measuring the response to angiotensin I and II. Proportioned to KCl-induced contraction, no clear reduction in angiotensin I -contraction was seen. Thus, ACE-inhibition may not be the main mechanism for the long-term effects of IPP and VPP in the protection of endothelial function. We suggest that the tripeptides do not affect smooth muscle but they protect endothelium during incubation indicated as preserved acetylcholine-induced endothelium-dependent relaxation.

C069

Neurotrophin-3 (NT-3) promotes reparative neovascularization and blood flow recovery in a mouse model of limb ischaemia

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Neurotrophins (NTs) are a class of growth factors which shape the development of the nervous system by regulating neuronal survival and differentiation. Recently a non-neural function of NTs has been shown: nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) promote angiogenesis and endothelial cell (EC) survival. Moreover neurotrophin-3 (NT-3) is involved in mammalian heart development. The angiogenic potential of NT-3 has not been evaluated, yet. This study investigated whether NT-3 is implicated in reparative angiogenesis in response to ischaemia. We preliminary found that EC of capillaries and arterioles express trkC in skeletal muscles, thus suggesting their responsiveness to NT-3. Unilateral limb ischaemia was performed in anesthetised CD1 mice by femoral artery resection and NT-3 was over-expressed in the ischaemic adductor by intramuscular injection of an adenoviral vector carrying the rat NT-3 gene (*Ad.NT-3*, 10^8 p.f.u.). Controls received *Ad.Null*. Capillary and arteriole densities and blood flow (BF) recovery were evaluated by immunohistochemistry (IHC) and color Laser Doppler, respectively on 14 days post-ischaemia. To study the effect of *Ad.NT-3* on EC proliferation, IHC for BrdU and proliferating cell nuclear antigen (PCNA) was performed on adductors harvested after 3 days from the surgery. Finally, in ischaemic muscles, the levels of phosphorylated (active) trkC, Akt and eNOS were analyzed by western blot and the content of VEGF-A mRNA and protein was measured by RT-PCR and ELISA, respectively. *Ad.NT-3* improved BF to the ischaemic foot ($P < 0.05$ vs. *Ad.Null* at 14 days), enhanced EC proliferation ($P < 0.05$ vs. *Ad.Null* for both BrdU and PCNA analyses) and increased capillary and arterioles densities ($P < 0.01$ and $P < 0.05$ vs. *Ad.Null*, respectively) in ischaemic adductor muscles. *Ad.NT-3* increased phosphorylation of trkC, Akt and eNOS, without changing VEGF-A expression. Our data show that local NT-3 delivery may provide a new strategy for inducing therapeutic neovascularization, thus improving BF to ischaemic limbs. NT-3 could be considered as a novel candidate for the treatment of ischaemic vascular disease.

C070

Comparison of thyroid hormones between normal and pre-eclamptic pregnancies

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The purpose of this study was to investigate thyroid function in pre-eclamptic patients in comparison with normal pregnant women. Cross-sectional study. Akbar Abady Women's Hospital at the Iran University of Medical Sciences. 100 preeclamptic patients were compared with 100 normal pregnant women in third trimester of pregnancy. Free T4 and TSH levels were measured and compared in two groups. Patient sampling was done by convenience method. Patients with thyroid disorders were excluded from study. A significant decrease in concentrations of free T4 (0.729 ± 0.324 μg/dl vs. 0.929 ± 0.314 μg/dl $P< 0.001$) were observed in the preeclamptic group when compared with the normotensive group. But statistical significance was not seen in concentration of TSH (2.935 ± 2.16 μIU/ml vs. 2.339 ± 1/15 μIU/ml, $P = 0.170$). Pregnant women with pre-eclampsia may have transiently lower freeT4 levels, without evidence of a thyroid disorder. TSH regulates angiogenesis that is required for early implantation and placentation. Consequently, it is possible that disruption of these early vascular events may cause pre-eclampsia.

C071

1-methylnicotinamide, a major metabolite of nicotinamide afford anti-thrombotic, anti-inflammatory and vasoprotective activity, a possible involvement of Cox-2/pgi$_2$-pathway

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1-methylnicotinamide (MNA) has been for a long time considered to be an inactive metabolite of nicotinamide. Here for the first time we describe anti-thrombotic, anti-inflammatory, vasoprotective and anti-atherosclerotic activity of MNA *in vivo*. The antithrombotic action of MNA was studied in normotensive rats with extracorporeal thrombus formation (thrombolysis), anti-inflammatory activity in the contact sensitivity reaction (CS) to oxazolone (OX) in CBA/J inbred mice, and anti-atherosclerotic activity in ApoE/LDLR$^{-/-}$ mice. MNA (3–100 mg/kg) induced a dose-dependent and sustained thrombolytic response, associated with a rise in 6-keto-$PGF_{1\alpha}$ in blood, while the levels of PGE_2 and TXB_2 did not change. The COX-2 inhibitor, rofecoxib (0.01–1 mg/kg), dose-dependently inhibited the thrombolytic response of MNA, indomethacin (5 mg/kg) abrogated it, while L-NAME (5 mg/kg) were without effect. Nicotinamide itself, or various compounds structurally related to MNA were either inactive or weak stimulators of COX-2/PGI2-dependent pathway and did not shared the profile of thrombolytic activity of MNA *in vivo*. In mice challenged with OX (recipients of OX-specific T cells), chronic treatment with MNA (100 mg/kg), resulted in a remarkable anti-inflammatory effect. The anti-inflammatory effects of MNA was inhibited by IP receptor antagonist RO-324479 (10 mg/kg). In addition chronic treatment with MNA (100 mg/kg) had a vasoprotective activity as it reversed endothelial dysfunction in hypertriglyceridemic or diabetic rats and inhibited progression of atherosclerosis progression (lipid deposition and macrophages accumulation) in apoE/LDL$^{-/-}$ mice. In conclusion we demonstrated that MNA, displays a unique profile of vasoprotective activity. Endogenous MNA, produced in the liver by nicotinamide N-methyltransferase, appears to be an endogenous activator of the COX2/PGI_2 pathway and may play an important regulatory role in the cardiovascular system providing anti-thrombotic, anti-inflammatory and vasoprotective activity. Since not only nicotinamide, but also nicotinic acid is metabolized to MNA, our results open a new avenue for understanding of vascular effects of nicotinic acid.

C072

Nuclear factor (NF)-kappa B inhibition improves vascular reactivity on aortas of 7 day isoproterenol-treated rats

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Previous data from our group demonstrated that chronic activation of beta-adrenoceptors (beta-AR) with isoproterenol (ISO) enhances vasoconstrictor response to phenylephrine (PHE), induces oxidative stress and increases nuclear activity of NF-kappaB. However, an interrelationship between up-regulation of NF-kappaB and altered vascular function has not been shown. Thus, in the present study we investigated the effect of NF-kappaB inhibition on the vascular reactivity on aortas from ISO-treated rats. For this, male Wistar rats were treated with vehicle (CT, $n = 8$) or ISO (0.3 mg/kg/day, s.c., $n = 7$) or with ISO plus thalidomide (Thal, 150 mg/kg/day, p.o., $n = 6$) for 7 days. At the end of treatment, aortic rings were isolated to evaluate the vasoconstrictor response to PHE (10^{-10}–10^{-4}M) and the relaxation to acetylcholine (ACh, 10^{-5}M). Acute NF-kappaB inhibition on PHE-induced contraction was evaluated in aortic rings from CT and ISO incubated with sodium salicylate (NaSal, 5 mM) for 1 h p65 subunit of NF-kappaB protein expression was quantified by Western-blot. ISO-treatment enhanced maximal contraction to PHE (Emax) compared to CT (ISO: 93 ± 1 vs. CT: 55 ± 6 % contraction to KCl 75 mM; $P < 0.05$, *t*-test). Co-treatment with Thal partially inhibited this ISO effect (ISO: 93 ± 1 vs. ISO+Thal: 71 ± 6 %; $P < 0.05$, *t*-test). NaSal did not affect PHE response in aortas of CT but normalized it in ISO rats (ISO+NaSal: 69 ± 10 vs. CT: 59 ± 13 %, $P > 0.05$, *t*-test). Neither, ISO, Thal nor NaSal significantly modified ACh relaxation. ISO increased p65 protein expression (+53%) in aortas. On the other hand, Thal significantly reduced p65 protein expression compared to ISO (-30%). In conclusion, the present results suggest the involvement of up-regulated NF-kappaB in the altered vascular function induced by chronic hyperactivation of beta-AR by ISO-treatment. We were supported by FAPESP/ CNPq.

C073

The anorexigenic peptides, neuromedin U-25 and neuromedin S, are present in the human cardiovascular system and function as potent vasoconstrictors

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Neuromedin U (NMU), shown to elicit a pressor response in rats (Minamino *et al.*, 1985), was paired with the 'orphan' G-protein-coupled receptors, NMU1 and NMU2 (Howard *et al.*, 2000). This peptide has an important role in energy regulation; *NMU* knock-out mice developed obesity (Hanada *et al.*, 2004) and *NMU* Arg165Trp amino-acid variant was shown to co-segregate with childhood obesity (Hainerova *et al.*, 2006). Recently, neuromedin S (NMS) was paired with the same receptors and also has pressor (Mori *et al.*, 2005) and anorexigenic actions (Ida *et al.*, 2005) in rats. However, little in known about the direct effects of these peptides on vascular beds and, particularly, on their vascular effects in humans. Using a novel RIA, NMU-25-like immunoreactivity was detected in human plasma, left ventricle (LV), coronary artery (CA), saphenous vein (SV) and cardiac adipose tissue. Reverse-phase HPLC established that both NMU-25 and NMS were present in LV and plasma. Quantitative receptor autoradiography, using [^{125}I]-NMU-25, demonstrated binding to the myocardium of LV and the medial smooth muscle layer of small and large diameter blood vessels. Binding sites were specific and saturable, and [^{125}I]-NMU-25 bound with high affinity (K_D = 0.26 ± 0.06 nM). Western blotting indicated that both NMU1 and NMU2 receptors were present in human LV, CA and SV. In accordance with binding distribution, we have shown NMU-25 to be a potent vasoconstrictor of human artery and vein *in vitro*. In CA, NMU-25 caused vasoconstriction with potency and maximum contractile response similar to angiotensin II. NMS constricted SV with similar potency to NMU-25 but a significantly lower maximum response, whereas the Arg165Trp variant was without effect. Our identification of specific binding sites, presence of endogenous peptide and discovery of function fulfil some of the criteria for NMU-25 as an emerging transmitter in the human cardiovascular system.

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C074

Concentration - dependent contractile effect of methylene blue in human internal mammary artery: A quantitative approach to its use in vasoplegic syndrome

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The vasoplegic sydrome that occurs early after cardiac surgery with cardiopulmonary bypass is characterized by a severe and persistent form of hypotension and is poorly responsive to volume increase with fluid infusion. Recently it has been shown that preoperative methylene blue (MB) administration reduces incidence and severity of vasoplegic sydrome in the high-risk patients (Ozal *et al.*, 2005). Previously, pharmacokinetics of MB in human *in vivo* and time-dependent concentration of MB in plasma has been reported (Peter *et al.*, 2000), but its concentration-dependent vascular effects *in vitro* is not known. The aim of this study is to determine the concentration-dependent effects of MB in human isolated internal mammary artery (IMA). IMA segments were collected from 24 patients undergoing coronary artery bypass graft surgery. The arteries cut into 3–4 mm rings. The rings were mounted in organ baths, containing Krebs–Henseleit solution gassed with 95% O_2 and 5% CO_2 at 37°C. Changes in arterial tensions were recorded isometrically by a force-displacement transducer. After the equilibration period, the arteries were contracted with 68 mM potassium chloride (KCl) solution. After a washout period the arteries were challenged with MB (10 nM to 100 μM). MB produced concentration–dependent contraction in the arteries. The maximal contraction to MB was 44.2% ± 3.8 of 68 mM KCl maximum contraction. pEC_{50} ($-\log_{10}$ of 50% effective concentration) value was 5.5 ± 0.1. This study is the first quantitative approach to the contractile effect of MB in human isolated arteries. According to a pharmacokinetic study in human, MB can produce plasma concentrations which are used in the present study (Peter *et al.*, 2000). The internal mammary artery is a commonly used conduit for coronary artery bypass graft surgery. According to our results, MB can cause vasoconstriction in this artery, this may impair graft function. So the patients should be followed up for this complication.

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C075

FE 202158, a novel, short-acting, potent, and selective vasopressin type 1a receptor (V1aR) agonist

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FE 202158, [Phe^2,Ile^3,Hgn^4,Orn(iPr)^8]vasopressin, where Hgn is homoglutamine and iPr is isopropyl, is a novel vasoconstrictive agent, specifically designed for intravenous use in vasodilatory hypotension conditions such as septic shock (Wisniewski *et al.*, 2008). FE 202158 was purposely engineered to be a potent, selective and short-acting vasoconstrictive agent. *In vitro* potency, efficacy, and selectivity were assessed in reporter gene assays with recombinant human vasopressin type 1a, 1b, or 2 receptors (hV1aR, hV1bR, hV2R) or oxytocin (hOTR) receptors, or with rat, sheep, or pig V1aR or V2R. Vasoconstrictive potency and efficacy were assessed *ex vivo* in isolated rat common iliac artery rings by measuring isometric contractility and *in vivo* using intravenous (i.v.) infusion administration in anesthetized rats by measuring skin blood flow with a laser Doppler probe. Duration of action was determined after i.v. bolus administration in dibenamine-pretreated anesthetized rats by measuring the decay of increased arterial pressure in the carotid artery. FE 202158 was a potent and full V1aR agonist *in vitro* at all the cloned human, rat, pig, and sheep V1aRs with EC50s ranging from 0.55 to 3.8 nM. It was 142-, 1107- and 440-fold selective vs. the hV1bR, hV2R, and hOTR, respectively, and it was also selective vs. the V2Rs from rat, sheep, and pig; this contrasts with the endogenous hormone arginine vasopressin (AVP), which is a mixed V1aR/V2R agonist. FE 202158 was a potent vasoconstrictor in rats, both *ex vivo* and *in vivo* (ED_{50} = 4.0 pmol/kg/min, calculated EC_{50} = 0.33 nM). It was also short-acting, with the vasopressor response to a submaximally effective dose disappearing within minutes. FE 202158 is a selective, potent and full V1aR agonist. It has virtually no activity at the V2R. Thus, unlike AVP, it is not expected to induce antidiuresis or the release of coagulation factors at therapeutic plasma concentrations. Its short-acting nature allows for a rapid rise to steady-state, dose titration, and rapid onset and offset of action by intravenous infusion, all essential features for its intended uses in critical care medicine.

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C076

Novel vasoconstrictor action of the inflammatory chemokine MIP-1β in human vasculature *in vitro*: antagonism by the HIV/AIDS drug maraviroc

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The chemokine receptor, CCR5, a major co-receptor for the HIV virus, is expressed with its ligand MIP-1β in human vascular smooth muscle (Schecter *et al.*, 2000). Recently CCR5 ligands have been shown to be transiently raised during periods of unstable angina (Kraaijeveld *et al.*, 2008), however, a direct effect on vascular tone has not been reported. Our aim was to determine whether MIP-1β has constrictor or dilator actions in human saphenous vein *in vitro* and confirmed that this is a CCR5 mediated action using the selective antagonist maraviroc (Dorr *et al.*, 2005). Human saphenous vein, obtained with ethical approval, were cut into 4 mm rings, mechanically denuded of their endothelium and mounted in 5 mL organ baths, containing Krebs' solution at 37°C, for isometric tension recording. The CCR5 antagonist, maraviroc (300 nM) or vehicle were added to the bathing medium and 30 min later cumulative concentration-response curves (CRC) were constructed to MIP-1β. CRC were repeated in veins preconstricted with 10 nM endothelin-1. MIP-1β responses were expressed as phenylephrine or reversal percentage of ET-1, respectively. Data (mean ± SEM) were analysed to determine MIP-1β potency (pD_2). MIP-1β had no direct dilator actions over the concentration range tested. In contrast MIP-1β contracted saphenous vein with pD_2 = 7.73 ± 0.17 (n = 12). This was abolished by maraviroc (n = 4). These data reveal an as yet unidentified role for MIP-1β as a potent constrictor of human saphenous vein, an effect mediated via the smooth muscle CCR5 receptor.

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C077

Vasodilatory prostaglandins down-regulate transcription of the thrombin receptors PAR-1 and PAR-3 in human vascular smooth muscle cells

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Thrombin is a potent mitogen for vascular smooth muscle cells (SMC) and stimulates extracellular matrix formation. These effects are mediated via protease-activated receptors (PARs). Thrombin also increases the generation of COX-2-derived prostaglandins PGI_2 and PGE_2, which have opposite effects. This study examines whether these vasodilatory prostaglandins counteract the mitogenic effects of thrombin in human SMC and whether regulation of PAR-receptor expression is involved. Human saphenous vein SMC (passage 4–7) were synchronised by serum-deprivation prior to incubation (1–24 h) with study drugs. Mitogenesis was determined by [^{3}H]-thymidine incorporation, mRNA and total protein expression by quantitative real-time PCR and Western blotting, respectively. The stable PGI_2 analogue iloprost (10–100 nM) significantly attenuated PAR-1 and PAR-3 mRNA expression at 6–24 h and total protein levels at 24 h by up to 50% (all $n = 5–6$, $P < 0.05$). Pretreatment (24 h) with iloprost (10 nM) also blunted the mitogenic response to thrombin (3 U/ml) or selective peptide ligands for PAR-1 (TFLLRN) and PAR-3 (TFRGAP, both 200 μM, $n = 4$, $P < 0.05$). Comparable down-regulation of PAR-1 and PAR-3 mRNA and protein was also observed with cicaprost (10 nM) and butaprost (1 μM), which selectively activate Gs-coupled IP- and EP2-prostanoid receptors, respectively (both $n = 3–6$, $P < 0.05$). Significant inhibitory effects of PGE_2 on PAR-1 and PAR-3 expression were observed only at micromolar concentrations (all $n = 6$). The adenylate cyclase activator forskolin (10 μM) and the cyclic AMP analogue db-cAMP (1 mM) mimicked the regulatory effects of prostanoids on PAR-1 and PAR-3 expression (both $n = 6$, $P < 0.05$) while the protein kinase A inhibitor myr-PKI (5 μM) prevented iloprost-induced down-regulation of PAR-1 and PAR-3 mRNA and protein levels ($n = 3–6$, $P > 0.05$). In conclusion, vasodilatory prostaglandins acting at receptors coupled to Gs and cyclic AMP/protein kinase A signaling regulate PAR-1 and PAR-3 expression and thereby suppress the mitogenic response to thrombin in human VSMC. This may represent a negative feedback mechanism to control thrombin-mediated SMC proliferation and secretory function following vascular injury.

C078

Putative role of the beta3 adrenergic receptor in ischemic heart disease using a model of neonatal rat cardiomyocytes exposed to hypoxia and chronic noradrenaline stimulation

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β_3 adrenergic receptors (β_3ARs) increase cardiac function through G_s-protein coupling, whereas β_3ARs coupled to G_i-protein decrease contractility. Circulating cathecholamines are elevated in heart disease, leading to an alteration of the cardiac function, development of hypertrophy and cells death. The aim of this work was to determine the role of β_3ARs in cardiomyocytes of neonatal Wistar rats exposed to normoxia (NX), NX and 100βM noradrenaline (NX/NA), hypoxia (HX, 0.5% O_2) or HX/NA for 24 h. Functional studies were performed using dobutamine (Dob, β_1 agonist) on cAMP accumulation and Cl 316243 (Cl31, β_3 agonist) on forskolin-induced cAMP response. β_1 and β_3 mRNA levels were determined by real time PCR. In addition, cell death was investigated for necrosis by LDH assay and for apoptosis by measurement of caspase 3 activity in presence or absence of 2βM Cl31. Hypertrophy was evaluated by investigating ANF expression by real time PCR and Western blotting, and protein synthesis using [^{3}H] leucine incoproration. HX downregulated β_1 response by 45% (vs. NX, $P < 0.001$, $n = 6$) and NA treatment decreased further by 44% ($P < 0.001$, $n = 6$) Dob-induced cAMP accumulation. Likewise, chronic NA treatment inhibited β_1 response by 78% in NX ($P < 0.05$, $n = 6$). No functional β_3 response was observed in NX and HX. NA treatment increased Cl31-mediated inhibition of forskolin response in NX and HX, with a higher maximal inhibition in HX/NA group (Imax: 43 ± 3% vs. 23 ± 4%, $P < 0.05$, $n = 6$). The modulation of β_1AR and β_3AR mRNA expression correlated well with the changes in functional response. Chronic NA treatment induced an increase in LDH activity in NX (100% vs. 170 ± 18%, $n = 4–5$, $P < 0.05$) and HX (230 ± 23 vs. 332 ± 23%, $P < 0.05$). β_3 treatment had no effect on LDH level in all the groups, whereas this treatment reduced caspase 3 activity by 52% in NX/NA group and 29% in HX and HX/NA groups ($n = 5–6$, $P < 0.05$). ANF mRNA expression increased by 29% (NX/NA) and 52% (HX). β_3 treatment inhibited ANF mRNA and protein levels in all groups. Protein synthesis was increased in HX group (100 vs. 164 ± 11 %, $P < 0.01$, $n = 5$) likewise in all NA treated groups (NX vs. 189 ± 7; HX vs. 218 ± 16, $n = 5$, $P < 0.05$), which were counteracted significantly by chronic β_3 stimulation ($P < 0.05$). In conclusion, the further upregulation of the β_3 function following chronic NA stimulation in HX in comparison to NX, the anti-apoptotic and anti-hypertrophic properties indicate that the β_3 subtype plays an important role in heart disease.

C079

Pleiotropic effects of atorvastatin and simvastatin: A prospective randomized eight week comparative study on erythrocytic and plasma antioxidant properties, and platelet aggregation inhibition in hypertensive, hyperlipidemic Indian patients

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Hyperlipidemia increases oxidative stress and lowering may be beneficial (Araujo *et al.*, 1995).The present study is a prospective randomized study to assess the effects of hyperlipidemia on erythrocytic and plasma oxidative stress and its modulation by low dose simvastatin and atorvastatin in Indian hypertensive patients. The effects of these drugs are also studied on platelet aggregation inhibition using ADP and epinephrine as aggregants. Forty statin naïve hyperlipidemic, hypertensive patients were prospectively randomized into two equal groups to receive 10 mg daily dose of either atorvastatin or simvastatin. Assessment was done for formation of thiobarbituric acid reactive substances (TBARS) levels, superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes and plasma besides measuring platelet aggregation using ADP and epinephrine before the start of treatment and after eight weeks. There was a significant increase in baseline erythrocytic TBARS levels (mean ± SD) 294.1 ± 126.8 units in hyperlipidemics vs. 99.4 ± 22.50 units in normolipidemics, $P = 0.002$. After 8 weeks of therapy erythrocytic TBARS levels decreased 38.3% in atorvastatin group and 28.2 % in simvastatin group ($P = 0.005$). Erythrocytic superoxide dismutase, catalase and glutathione peroxidase levels increased by 32.1%, 90.9%, 19.8% ($P = 0.01$, 0.005, 0.005) respectively in atorvastatin group. The increase in simvastatin group was 32.9%, 12.6% and 36.9% ($P = 0.01$, 0.01, 0.05) respectively. Similar results were obtained for plasma oxidative and antioxidative parameters. ADP and epinephrine induced platelet aggregation decreased by 38.7% and 58% respectively for atorvastatin group (P-value 0.008 for both). The decrease in simvastatin group was 14.8% and 24.5% for ADP and epinephrine respectively (P-value 0.005 and 0.008). The decrease in cholesterol levels was also significant in both the groups. There was no correlation between lipid lowering effects and antioxidative effects or platelet aggregation inhibition effects. Erythrocytic antioxidant activity correlates well tissue antioxidant activity (Simon *et al.*, 1998). The results of this preliminary study are highly favourable in demonstrating pleiotropic effects of low dose statins in Indian patients besides having safety of low dose use.

References:

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C080

Reduction of paraquat-induced renal dysfunction in rats by a manganese complex of ethylenebis(hydroxyphenylglycine) (EHPG)

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Paraquat (PQ) nephrotoxicity is mediated via the production of reactive oxygen species such as superoxide anions. Metal complexes of the contrast agent ethylenebis (hydroxyphenylglycine) (EHPG) possess superoxide dismutase activity (Fisher *et al.*, 2004). We have recently reported that the manganese(II) (Mn) complex of EHPG provides significant protection against PQ-induced toxicity in rat NRK-52E renal cells via reduction of superoxide generation (Samai *et al.*, 2008). The aim here was to investigate if Mn(II)-EHPG could also provide protection against PQ-induced renal dysfunction in rats. Twenty-one male Wistar rats (200–250 g) were divided into 4 groups (Table 1). Rats were either administered saline or 10 mg/kg PQ followed by either saline or 4 mg/kg Mn(II)-EHPG. After 24 h, serum creatinine levels (sCr) were measured and used to assess renal (or glomerular) function.

Table 1. Effects of PQ and Mn(II)-EHPG on sCr levels in rats after 24 h

Sham (saline only)	PQ only	Mn(II)-EHPG only	PQ + Mn(II)-EHPG
64.5 ± 2.47 μmol/L #	120.43 ± 10.70 μmol/L*	59.17 ± 3.70 μmol/L #	62.30 ± 6.40 μmol/L #

Data are expressed as mean ± SEM for 3–6 rats, analysed using one-way ANOVA followed by a Dunnett's post test. # $P < 0.05$ vs. 10 mg/kg PQ only group, *$P < 0.05$ vs. Sham (saline only) group.

These results suggest that the Mn complex of EHPG can significantly reduce the renal dysfunction caused by PQ. This novel SODm could therefore be beneficial as a future therapy for PQ nephrotoxicity.

References:

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