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Administration of late endothelial progenitor cells enhances cerebral infarction outcome after transient middle cerebral artery occlusion in rats

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Endothelial progenitor cells (EPCs) are a novel promising option for treatment of patients with ischemic diseases. We characterized the effect of late outgrowth EPCs transplantation in a model of transient middle cerebral artery occlusion (MCAO) in rat. EPCs were obtained from human umbilical cord blood. Adult male Sprague-Dawley rats were subjected to 1 h of MCAO and allocated 24 h after MCAO to transplanted ($4–5 \times 10^6$ late EPCs IV, $n = 12$) and control (PBS IV, $n = 13$). Body weight, adhesive-removal dot test and the mNNS test were performed over the 14 days following MCAO. Seven or 14 days after MCAO, brain infarct volumes, human transplanted cells localization, apoptosis and capillary density staining were done. Animal body weights were significantly higher in the transplanted group 7 days after MCAO ($P < 0.05$). Significantly higher neurological performance was found in transplanted rats compared to control rats ($P < 0.05$). Immunochemical staining showed that EPCs survived and were preferentially localized in the ischemic boundary zone. No difference has been observed in term of infarct volume ($P = 0.98$). At day 3, apoptotic cell number was significantly lower in transplanted rats ($48.0 \pm 18/mm^2$) compared to control rats ($130.0 \pm 50/mm^2$) ($P < 0.05$). Capillary densities showed were significantly increased ($P = <0.05$) at the boundary of the ischemic lesion in transplanted rats ($n = 4$; 2.80 ± 0.3) compared to control rats ($n = 4$; 1.72 ± 0.7) 14 days after MCAO. Late EPCs administered intravenously 24 h after MCAO enter the brain, survive, migrate, and improve functional recovery. This may be involved ischemia-induced apoptosis together with angiogenic response modulation.

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Effect of short-term treatment with a synthetic flavonol 3′,4′-dihydroxyflavonol on vascular function in early diabetes

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We investigated the effect of short-term treatment with 3′,4′-dihydroxyflavonol (DiOHF), on aortic responses to endothelium-dependent (ACh) and endothelium-independent (sodium nitroprusside (SNP)) relaxants in normal and diabetic rats. Diabetes was induced with a single i.v. injection of streptozotocin (48 mg/kg). Five weeks after STZ injection, diabetic and control rats received DiOHF (1 mg/kg sc) or vehicle (10% DMSO) daily for 7 days. After 6 weeks, the rats were killed and the thoracic aorta was removed. Lucigenin-enhanced chemiluminescence was used to measure superoxide generation in the aorta. The diabetic rats had significantly lower body weight (334 ± 14 g) and higher blood glucose levels (>33 mmol/l) as compared to control rats (body weight 502 ± 11 g and blood glucose 11.5 ± 1.3 mmol/l). Relaxation to ACh (pEC_{50} 7.36 ± 0.09, R_{max} $95 \pm 3\%$) or SNP (pEC_{50} 8.13 ± 0.14, R_{max} $100 \pm 0\%$) was unaffected by diabetes (ACh pEC_{50} 7.33 ± 0.10, R_{max} $88 \pm 5\%$; SNP pEC_{50} 8.67 ± 0.29, R_{max} $101 \pm 2\%$). ACh caused significant relaxation in the presence of the nitric oxide synthase (NOS) inhibitor L-NNA, (pEC_{50} 6.78 ± 0.06, R_{max} $54 \pm 7\%$, $P < 0.05$) or the cGMP inhibitor ODQ (pEC_{50} 6.77 ± 0.15, R_{max} $46 \pm 12\%$, $P < 0.05$) in diabetic rats whereas there was no response in the presence of these inhibitors in control rats. DiOHF treatment of control rats did not affect ACh-induced relaxation but revealed relaxation in the presence of L-NNA (pEC_{50} 6.89 ± 0.28, R_{max} $28 \pm 7\%$, $P < 0.05$) but not ODQ. DiOHF treatment of diabetic rats did not alter the responses to ACh. The cyclooxygenase inhibitor indomethacin (10 μM) had no effect on vasorelaxation in aortae from control, diabetic or DIOHF treated rats. The level of superoxide was significantly higher in aortae from diabetic (2180 ± 363 counts/mg tissue, $P < 0.05$) compared to control rats (986 ± 163 counts/mg tissue). DiOHF had no effect on superoxide levels in aortae from control or diabetic rats. Plasma total nitrite/nitrate assay showed increased amounts of NO_X in diabetic (902 ± 206 mg/l, $P < 0.05$) compared to control rats (468 ± 69 mg/l). These data indicate that early diabetes increases resistance to NOS inhibition, as does DiOHF treatment, resulting in endothelium-dependent relaxation in the presence of a NOS inhibitor by increasing nitric oxide availability.

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Wine polyphenols prevent endothelial dysfunction and vascular superoxide production induced by endothelin-1 in rat aorta

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Red wine polyphenols (RWPs) have been reported to exert antihypertensive effects and improve endothelial function *in vivo*. We have investigated the effects of RWPs extract and its main isolated components (epicatechin, catechin and resveratrol) on endothelial dysfunction and superoxide production induced by endothelin-1 (ET-1) *in vitro* in rat aorta. Rat thoracic aortic rings, obtained from male Wistar rats (250–285 g), were incubated with ET-1 (1 nM) in the presence or absence of RWPs extract (10^{-2} mg/ml), epicatechin, catechin or resveratrol (10^{-7} M–10^{-5} M) for 4 h. ET-1 reduced the relaxant responses to acetylcholine in phenylephrine contracted intact aorta, and these effects were prevented by co-incubation with RWPs extract, epicatechin, catechin, resveratrol. This endothelial dysfunction was also improved by both superoxide dismutase (100 U/ml) and the selective nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase inhibitor apocynin (10^{-4} M). No differences were observed in the endothelium-independent relaxant responses to the endothelium-independent vasodilator sodium nitroprusside in arteries from control-, control-RWPs-, ET-1- and ET-1-RWPs-treated rings. Furthermore, ET-1 increased vascular superoxide production, measured by lucigenin-enhanced chemiluminescence, and gene expression of NADPH oxidase subunit $p47^{phox}$ and $p22^{phox}$, measured by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). All these changes were prevented by both RWPs extract and by its main component epicatechin (10^{-5} M). Taken together these results indicate that RWPs prevent ET-1-induced endothelial dysfunction by inhibiting the overexpression of $p47^{phox}$ and $p22^{phox}$ and the subsequent increased O_2^- production resulting in increased nitric oxide bioavailability. Epicatechin is the main component involved on these protective effects induced by RWPs extract.

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The effects of ethanol-treatment on the neurogenic- and endothelium-dependent relaxation of corpus cavernosum smooth muscle in mouse

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Relaxation of cavernous smooth muscle is critical for inducing and maintaining penile erection. The neurogenic- and endothelium-dependent relaxation of corpus cavernosum smooth muscle and the degenerative effects of subacute ethanol-treatment on the endothelial cells of corpus cavernosum were investigated in mice. In the cavernous strips contracted with phenylephrine, electrical field stimulation (EFS), acetylcholine and exogenous nitric oxide (NO) induced relaxations in control group. Ethanol-treatment abolished the endothelium-dependent relaxations induced by acetylcholine but failed to alter the relaxation to EFS and NO. L-nitroarginine, a NO synthase inhibitor, reduced relaxations induced by EFS and acetylcholine but not those to NO in control and ethanol-treated mice. L-arginine prevented the response inhibited by L-nitroarginine. ODQ, a guanylyl cyclase inhibitor, inhibited relaxations to EFS, NO and acetylcholine in control and ethanol-treated mice. Corpus cavernosum tissues were investigated under electron microscopy and endothelial damage was observed in ethanol-treated mice. These results suggest that ethanol impairs the endothelial function of corpus cavernosum in mouse, and it may be lead erectile dysfunction through reducing NO release via endothelial impairment.

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Retina derived relaxation is preserved in carotid and mesenteric arteries of diabetic rats

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Retinal relaxing factor (RRF) is a novel transferable factor released from the retinal tissue and suggested to be included in the regulation of retinal arterial tone. Although the nature and mechanism of action of RRF has not yet been determined, its effectiveness in relaxing different types of vascular preparations suggested that it might be a general relaxant. An increased sensitivity is observed in its effectiveness under hypoxia, however, the role of RRF in pathological conditions affecting retinal vasculature has not been estimated. Herein, we aimed to investigate whether diabetes affects RRF response by determining its vasoreactivity on rat carotid and mesenteric arteries. Diabetes was induced by a single injection of streptozotocin (STZ, 65 mg/kg, i.p.) to male Wistar rats (250–300 g). After 8 weeks, carotid and mesenteric arteries of diabetic as well as control rats were isolated and mounted parallely in a multichamber wire myograph system. Following equilibration, endothelial and smooth muscle relaxant capacities of the arteries were determined. Thereafter, diabetic and control arteries were precontracted with prostaglandin $F_{2\alpha}$ and retinas were placed in close proximity to maintain retinal relaxation. Relaxations are expressed as % of the precontraction and statistical analyses were determined by Students' *t*-test. In the carotid and mesenteric arteries of STZ-induced diabetic rats, endothelium-dependent relaxations to acethylcholine were significantly reduced whereas, endothelium-independent relaxations to sodium nitroprusside were similar when compared to control arteries. Placement of the retinal tissue produced acute relaxations in rat carotid and mesenteric arteries which generally displayed a biphasic character. Diabetic retinal tissue elicited comparable relaxations to control retinal tissue in both carotid and mesenteric arteries (diabetic carotid arteries: $77.39 \pm 1.60\%$, $n = 7$ vs. control: $80.39 \pm 3.79\%$, $n = 8$, $P > 0.05$; diabetic mesenteric arteries: $91.26 \pm 4.15\%$, $n = 7$ vs. control: $91.25 \pm 2.24\%$, $n = 8$, $P > 0.05$). Our results showed that retinal relaxation is preserved in diabetic conditions where endothelial reactivity is impaired and suggested that RRF may play a regulatory role in the maintenance of retinal vascular tone in diabetes.

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Antiangiogenic effects of natural compounds

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Angiogenesis, the growth of new blood vessels from the pre-existing vasculature is associated with physiological as well as pathological conditions (e.g. tumour development). Several natural compounds, especially plant products and dietary constituents, are able to exhibit antiangiogenic activities. Chalcones are precursors of flavonoids and isoflavonoids and many studies and investigations suggested that certain chalcones can inhibit tumor initiation as well as tumor progression. On the other hand, antiangiogenic effect of chalcones have been studied only marginally. In the present work, we tested three chalcones (A-C) for their antiangiogenic effects in *in vitro* conditions. MTT cytotoxicity assay, endothelial cell migration (ECM; woun healing assay), capillary tube formation (CTF), matrix metalloproteinase activity (MMP; gelatine zymography) were performed using human umbilical vein endothelial cells (HUVECs). The level of VEGF secreted by the cancer cells in the medium was determined by ELISA kit. From compounds tested only chalcone A (*E*-2-(4′-methoxybenzylidene)-1-benzosuberone) has a significant antiangiogenic effect. The cytotoxic effect of compound A was concentration-dependent and HUVECs survival significantly decreased at c = 10^{-4}–10^{-6} mol/l. Furthermore, it completely inhibited CTF in non-toxic concentrations (10^{-7}–10^{-8} mol/l). Moreover, in concentration 10^{-7} mol/l it blocks also ECM. Gelatin zymography revealed that chalcone A reduced MMP-9 activity in HUVECs in a concentration-dependent manner. Inhibitory effect on MMP-2 activity was observed only at the highest concentration. VEGF secretion was significantly reduced in cancer cells treated by chalcone A at concetrations 10^{-6} and 10^{-7} mol/l. These findings reveal a therapeutic potential for chalcone A in angioprevention and antiangiogenic therapy.

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Hypoxia-reoxygenation injury to isolated porcine coronary arteries using normothermic and hypothermic models

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Hypoxia-reoxygenation (H-R) induced damage alters the vasomotor response of the coronary arteries. Different experimental models have been used in H-R studies, but comparison between protocols is difficult. Therefore, the aim of our study was to compare and evaluate normothermic and hypothermic models. Porcine coronary arterial rings were isolated and placed in an organ bath filled with Krebs-Henseleit (K-H) solution. After obtaining stable responses with 60 mM KCl and thorough rinsing, the rings were exposed to normoxic conditions and two different H-R conditions: the first induced by a 95% N_2 -5% CO_2 gas mixture (40- and 60-min hypoxia) in a normothermic protocol, and the second induced by hypothermic (4 °C) hypoxia-reoxygenation (24- and 48-hours hypoxia). Reoxygenation was applied by introducing K-H solution aerated with a 95% O_2-5% CO_2 mixture under normothermic (37 °C) conditions for 30 min. After that, the rings were pre-contracted with 50 mM U-46619 and relaxed by cumulative addition of substance P. Analysis of the maximum relaxation of the arterial rings was performed by one-way ANOVA, followed by Bonferroni's post-test. Distal segments of the coronary artery responded faster to contraction induced by U-46619 and were relaxed by substance P to a greater extent compared to proximal segments. Maximal relaxations of arterial rings induced by a 10 nM solution of substance P were significantly reduced ($P < 0.001$) after 40-min H-R (53.3 ± 5.2%, $n = 16$), 60-min H-R (35.9 ± 3.9%, $n = 19$), 24-hours hypothermic H-R (55.8 ± 2.4%, $n = 27$) and after 48-hours hypothermic H-R (37.0 ± 7.0%, $n = 10$) compared to normoxic rings (80.7 ± 1.2%, $n = 23$). The model employing 40-min normothermic H-R is as effective as 24-hours hypothermic H-R, and 60-min normothermic H-R is as effective as 48-hours hypothermic H-R in inducing endothelial injury.

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Endothelium dependant differences in 5HT responses in canine mesenteric and coronary arteries

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The 5-HT receptor profile in arteries is complex, having both contractile and relaxant actions in different species. Here we characterise the 5-HT receptor subtypes in canine coronary and mesenteric arteries by the use of agonists and antagonists in both endothelial intact and denuded arteries. Arteries were cut into ring segments and mounted in isometric organ baths under physiological conditions. Exposure to 1 μM bradykinin assessed endothelial responses. Cumulative dose response curves to the 5-$HT_{1B/1D}$ receptor agonist sumatriptan, and to 5-HT in the presence and absence of various 5-HT antagonists were performed. In coronary arteries, 5-HT caused both constriction and relaxation effects via different 5-HT receptors. At low concentrations of 5-HT (10^{-9} to 3×10^{-6} M) constriction was dominant; mediated by 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors (attenuated by 10 nM GR127935 and 1 nM ketanserin respectively). At higher concentrations of 5-HT relaxation dominated; mediated by 5-HT_7 receptors (blocked by 100 nM SB258719). Constriction responses to 5-HT and 62.5 mM KPSS were not significantly altered by removal of the endothelium; however constriction to sumatriptan was significantly increased ($P < 0.01$). Mesenteric arteries did not show this biphasic response to 5-HT; they exhibited only a constriction response at the concentrations tested (10^{-9} to 10^{-5} M). This was inhibited by 10 nM GR127935, suggesting that the 5-HT constriction response in these arteries is due entirely to 5-$HT_{1B/1D}$ receptor activation. Removal of the endothelium caused a significant increase in both the 5-HT and sumatriptan constriction responses ($P < 0.01$ and 0.05 respectively). KPSS responses of denuded and intact mesenteric arteries were not significantly different from each other, suggesting that the enhanced responses to sumatriptan in denuded mesenteric and coronary arteries are not simply due to the loss of basal EDRF's. The present results demonstrate clear differences between canine coronary and mesenteric arteries; coronary artery responses to 5-HT are complex involving 5-HT_{2A}, 5-$HT_{1B/1D}$ and 5-HT_7 receptors, whereas mesenteric artery responses appear to involve only 5-$HT_{1B/1D}$ receptors. Of particular importance is the implication that endothelial dysfunction could selectively enhance contractile responses to 5-$HT_{1B/1D}$ receptor agonists, although the mechanism by which this occurs is unclear.

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Interactions between lipopolysaccharide and hypoxia: Synergistic up-regulation of HIF-1α stabilisation in equine endothelial cells

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Lipopolysaccharide (LPS) plays a key role in laminitis, a common and painful condition of horses that can lead to permanent lameness (Hood, 1995). Reduced digital perfusion has been described in the prodromal stages and the pathology is consistent with hypoxic injury (Hood *et al.* 1993). A reduction in the supply of oxygen to cells triggers activation and transcriptional up-regulation of hypoxia inducible factor-1-α (HIF-1α). Recently, LPS-induced HIF-1α has been suggested to be important in the development of sepsis (Peyssonnaux *et al.* 2007). This study has examined the effects of LPS and hypoxia on the stabilisation of HIF-1α in equine digital vein endothelial cells (EDVEC). EDVEC were isolated as previously described (Bailey *et al.* 2003). Cells were exposed to LPS (*E. coli* 055:B5; 10 pg/ml–10 μg/mL), 5%O_2, or LPS (10 ng/ml for 1 h) then 5% O_2. HIF-1α stabilisation was determined by immunoblotting. Results are expressed as mean % increase above basal expression ± SEM ($n = 6$) and data analysed using 1- or 2-way repeat measures ANOVA with Bonferonni's *post-hoc* test. Significance was accepted at $P < 0.05$. Both LPS and 5% O_2 induced significant increases in HIF-1α (maximum increases above basal of 563 ± 102% for LPS (10 μg/ml; EC_{50}: 27.5 ng/ml) and 86 ± 29% for 5% O_2). Exposure to LPS and 5% O_2 induced a more than additive increase in HIF-1α (maximum 991 ± 20% above basal; compared to 222 ± 5% and 86 ± 29% for 10 ng/ml LPS and 5%h O_2 alone, respectively). These data demonstrate that LPS stabilises HIF-1α in equine endothelium and the increased response obtained in 5% O_2 suggests that the effect is likely to be greater in the hypoxic conditions of the laminitic hoof. The consequences of HIF-1α stabilisation in the horse endothelial cell have yet to be determined.

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Effectiveness of the vascular preconditioning by palmitoylethanolamide versus hypoxic or adenosine preconditioning in the porcine coronary artery *in vitro*

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Palmitoylethanolamide (PEA) is an endogenous cannabinoid that lacks ability of binding to CB1 and CB2 receptors, but may bind to the GPR55 receptor (Mackie *et al.*, 2006). Apart from other biological effects, PEA may be implicated in the protective effect of the ischemic preconditioning in rat coronary arteries (Bouchard *et al.*, 2003). We compared the effectiveness of the hypoxic and adenosine preconditioning with the PEA preconditioning for the preservation of the endothelium-dependent relaxation of the isolated left anterior descending coronary artery. Arterial rings (3–4 mm in diameter) were placed in 10-ml organ baths filled with oxygenated Krebs-Henseleit solution (K-H) at 37 °C. After series of contractions to 60 and 20 mM KCl, rings of the hypoxic ($n = 15$) and normoxic ($n = 15$) control groups were subjected either to 60-min hypoxia (K-H aerated with 95% N_2 + 5% CO_2) followed by 30-min reoxygenation, or to 90-min normoxia. Denuded rings ($n = 6$) were subjected to normoxia only. In intact rings, NOS and COX were inhibited by 0.36 mM L-NNA and 9.8 μM indomethacin. Subsequently, rings were precontracted with 50 nM U46619 and relaxed by the cumulative addition of the substance P (0.001 nM to 10 nM) (Kužner *et al.*, 2004). Hypoxic preconditioning consisted of two periods of 5-min hypoxia and 10-min reoxygenation. Rings incubated in 1 μM PEA ($n = 8$) or 10 μM adenosine ($n = 8$) were washed-out with fresh K-H before the hypoxia-reoxygenation. Relaxations were compared using one-way ANOVA followed by the post-test. Rings of the hypoxic and adenosine preconditioning groups relaxed significantly more ($P < 0.01$) than hypoxic and denuded rings. PEA-preconditioned rings were significantly less relaxed ($P < 0.01$) than intact normoxic rings. Hypoxic and adenosine preconditioning effectively preserved endothelium-dependent relaxation of the porcine coronary artery after hypoxia-reoxygenation *in vitro*. In contrast to that, PEA failed to preserve endothelium-dependent relaxation of the porcine coronary artery in the presence of NOS and COX inhibitors.

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The long-term resveratrol treatment increased insulin secretion and nitric oxide production in alloxan diabetic rabbit

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The phytoestrogenic compound resveratrol has been shown to possess protective effects on the cardiovascular system. Here, we investigated the effect of long-term resveratrol treatment on insulin, nitrite/nitrate, nitrotyrosine levels and superoxide dismutase activity (SOD) in diabetic rabbits. Diabetes was induced with a single dose of alloxan (100 mg/kg, i.v.) dissolved in physiological saline in male New Zealand white rabbits weighing 2.0 to 2.5 kg. Diabetes caused to decrease the plasma and aortic nitrite/nitrate productions while did not change nitrotyrosine level and SOD activity. The long-term resveratrol treatment (5 and 50 mg/l in drinking water for 10 weeks) increased insulin secretion, but slightly changed plasma glucose level, in control and alloxan diabetic rabbits (n = 6–8, $P < 0.05$). Resveratrol treatment increased nitrite/nitrate production in the samples of plasma and aortae from diabetic rabbits when compared to untreated diabetics (n = 5–7, $P < 0.05$). However, the plasma nitrotyrosine level and aortic SOD activity were not change following resveratrol treatment in diabetic rabbits. This results showed that resveratrol has some beneficial effects on diabetic experimental model in rabbit.

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Vascular reactivity to urotensin-II in hypertensive rats associated with insulin resistance

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Urotensin-II (U-II) is a peptide with a dose-dependent vasoconstrictor activity. The distribution of U-II receptors in endothelial and smooth muscle cells is dependent on the pathologic state and the vascular bed examined. Fructose feeding in normal rats provides a good model of the metabolic syndrome because it induces hypertension associated with insulin resistance, hyperinsulinemia and hypertriglyceridemia. The purpose of this study was to assess the vascular reactivity of fructose-treated rats to U-II. Male Wistar rats (n = 16, weight 250–280 g) were divided into two equal groups. One of the groups was fed standard diet and served as the control group, whereas the other group was fed a high-fructose diet for 4 weeks. Systolic blood pressure in the groups was estimated by a tail-cuff method and blood samples were obtained. Thoracic rat aorta rings were isolated and placed in organ baths filled with warmed and oxygenated Krebs-Henseleit solution. Endothelial function was assessed by pre-contraction with phenylephrine (0.1 μM) after which concentration-relaxation curves to acetylcholine (0.1 nM–10 μM) were obtained. In order to obtain U-II vascular responsiveness, all rings were contracted with 0.1 μM U-II. The same experiment was repeated in the rings with inhibited NOS and COX enzymes by application of L-NNA (0.36 mM) and indomethacine (9.8 μM), respectively. Responses were measured using an isometric mechano-electrical transducer and contraction was expressed as a percentage of 60 mM KCl pre-contracted aortic rings values. Fructose-fed rats showed higher ($P < 0.01$) systolic blood pressure levels and plasma concentrations of triglycerides and insulin than those of control. There was no significant difference in concentration-relaxation curves to acetylcholine. Contractile response to U-II was higher ($P < 0.05$) in the control (27.7 ± 5.3%) compared to fructose-fed group (14.4 ± 2.1%). Similarly, response was higher ($P < 0.01$) in the control (51.6 ± 9.1%) compared to fructose-fed group (25.5 ± 3.6%) in experiments with inhibited NOS and COX enzymes. These results suggest the possible involvement of urotensin system in the early changes of vascular responsiveness in metabolic syndrome.

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Anti-apoptotic effect of beta-carotene from *Dunaliella salina* on 60Co-irradiated murine thymocytes

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Natural antioxidants have been postulated to be antiradiation potential via its free radical scavenging. Previously, we have extracted cis-beta-carotene from *Dunaliella salina* and tested its antioxidant property *in vivo*. In the present study, we aim to investigate whether cis-beta-carotene could protect murine thymocytes against ^{60}Co γ-ray radiation. The cis-beta-carotene was extracted from *Dunaliella salina* and analyzed by high performance liquid chromatography (HPLC). Murine thymocytes were pretreated with cis-beta-carotene, and followed by ^{60}Co γ-ray radiation (3 Gy). Cell viability was determined by MTT assay. Brdu assay was used to detect cell proliferation. Apoptosis was examined by annexin V/PI double staining and by gel electrophoresis. Mitochondria membrane potential was measured by flow cytometry. The ultrastructure of thymocyte was also observed by electron microscope. The phosphorylation of p38 protein was examined by western blot. ^{60}Co γ-ray radiation (3Gy) caused significant damage to murine thymocytes. Treatment of thymocytes with cis-beta-carotene showed that this compound could inhibit radiation-induced apoptosis. A study of kinetics showed that addition of 0.32 μg•mL-1 cis-beta-carotene after irradiation could decrease DNA fragmentation even when it was added 2–3 h after irradiation. Moreover, cis-beta-carotene could prevent the radiation-induced loss of mitochondrial membrane potential and the alteration of cell ultrastructure. The phosphorylation of p38 kinase was also blocked by cis-beta-carotene as shown by Western blot. Our data suggested that cis-beta-carotene protected murine thymocytes from radiation-mediated apoptosis, maintained mitochondrial membrane potential, and further inhibited the activation of p38 kinase.

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Analysis of gene expression during FTY720 induced apoptosis in Jurkat cells by cDNA microarray

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FTY720 (2-amino-2-[2-(4-octylphenyl) ethyl]-1, 3-propane-diol hydrochloride) has immunosuppressive activity in experimental organ transplantation and shows a marked decrease of peripheral blood T lymphocytes upon oral administration. The lymphocytes decrease was mainly a result of FTY720-induced apoptosis. However, this apoptotic mechanism is not well understood. We examined the mechanism of FTY720-induced apoptosis in human lymphoid T cell line Jurkat. Reduction of viability, Hoechst 33258 staining, Annexin V binding and DNA fragmentation revealed that FTY720 induced Jurkat cell apoptosis in a dose and time dependent manner, and most of cell apoptosis was observed when incubated with 10 μM FTY720 for 6 h. To define the apoptotic signal pathways mediated by FTY720, we compared the transcriptional profiles of Jurkat cells untreated with treated with 10 μM FTY720 for 6 h by cDNA microarrys. Of the 458 genes related to apoptosis in the microarray, we identified 54 significantly up-regulated and 10 down-regulated genes in FTY720 treated cells. Microarray difference expression was confirmed by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) and Western blotting for two selected significantly up-regulated genes MOAP-1 and VEGF. Data were expressed as mean ± SD for the number of experiments indicated. All statistics were generated using SPSS 12.0 software, and all figures were drawn using Origin 7.5 software. Statistically significant differences between groups were made using a multivariate analysis of variance with *post hoc* testing. Comparisons between two groups were performed by Student's *t* test for parametric data and Mann–Whitney *U* test for nonparametric data. Correlation was calculated using Spearman's non-parametric test. $P < 0.05$ was considered statistically significant.

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Immunostimulatory activity of sarco/endoplasmic reticulum Ca^{2+}-ATPases inhibitors, thapsigargin and trilobolide

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The maintenance of Ca^{2+} homeostasis within the cell is tightly regulated by a family of sarco/endoplasmic reticulum (ER) Ca^{2+}-ATPases (SERCA). Inhibition of SERCA pumps leads to a release of Ca^{2+} from ER stores and a concomitant influx of Ca^{2+} from extracellular space, resulting in elevation of cytosolic Ca^{2+} concentration. Changes in intracellular free Ca^{2+} levels are known to modulate cellular signaling and gene expression, and may thus lead to the activation of immunocompetent cells. We have therefore investigated possible *in vitro* effects of the SERCA inhibitors thapsigargin and trilobolide on immune functions of animal macrophages and human peripheral blood mononuclear cells (PBMC). Both these compounds are sesquiterpene lactones of guianolide type, isolated from plants *Thapsia garganica* L. and *Laser trilobum* L., respectively. Thapsigargin has become a powerful and most frequently used means to study Ca^{2+} signaling pathways because it is an inhibitor with high specificity and affinity. We have found that both thapsigargin and trilobolide remarkably stimulated production of nitric oxide by rat peritoneal macrophages, the effect being enhanced in the presence of lipopolysaccharide. Typical feature of NO production was a bell-shaped dose-response dependency curve. The mechanism of NO activation is obviously via the ability of these SERCA inhibitors to greatly stimulate secretion of interferon-g (IFN-g). Not only rat cells but also human PBMC respond readily to the IFN-g-stimulatory potential of both thapsigargin and trilobolide. The stimulatory effects are mediated by nuclear factor kappaB and mitogen activated protein kinases p38 and ERK1/2.

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The condition of non specific immune resistance in postcastration metabolic syndrome and within its pharmacological correction

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The aim of this research was to determine the influence of pharmacological drugs on metabolic changes correction caused by hypoestrogenia on the phagocyte and humoral links condition of non specific immune resistance. The studies were carried out on 42 white non breed female rats which at the age of 3 months underwent ambilateral ovarioectomia. The metabolic syndrome was induced with extra carbohydrate diet of 30% of saccharose solution. Over 5 days, the influence of the antidiabetic drugs fensuccinal, meletin flavonoid, genistein isoflavonoid and natural estrogen 17β-estradiol was investigated. In blood serum we defined the level of the natural hemolysin antibodies and heterophil agglutinins, endotoxin concentration, phagocyte and metabolic neutrophil activity. We showed that ovarioectomia was accompanied by the increase of phagocyte and decrease of metabolic and consumption activity of neutruphils ($P < 0.05$), by the decrease of natural antibodies ($P < 0.05$), and by the increase of androgenic intoxication ($P < 0.05$). The extra carbohydrate diet with ovarioectomic rats produces metabolic and phagocyte activity of granulocytes but with a much bigger decrease in their consumption activity ($P < 0.05$) and the titer of hemolysins. We showed that meletin and genistein produces stimulative influences on the metabolic and consumption activity of neurophils ($P < 0.05$). Meletin, genistein and 17β-estradiol administration caused normalization of phagocyte cell quantity ($P < 0.05$). Fensuccenal decreases that index and also the consumption activity of phagocytes ($P < 0.05$). The drugs failed to completely compensate the humoral chain of immunity.

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Attenuation of mechanical hyperalgesia in streptozocin-induced diabetic neuropathy model in rats by cerebrocrast, a mitochondria-targeted drug

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Our hypothesis is that attenuation of diabetic neuropathy (DN) can be achieved by use of mitochondria-targeted drugs. For this purpose, cerebrocrast (Cer), a novel type of atypical (non-calcium antagonist) 1,4-dihydropyridines, previously shown as mitochondria-regulating neuroprotective compound (Velena *et al.*, 1997) was investigated in streptozocin (STZ) diabetic model in male Wistar rats (200–220 g). STZ (60 mg/kg ip) was administered for two consecutive days, Cer at 0.1 mg/kg ip was injected *per se* and in combination with STZ for 6 weeks. The pain intensity was measured by algesimeter (Ugo Basile, Italy). The rats having glucose level above 15 mmol/l were used for experiment. The body weight was measured every three days. Results were analyzed by ANOVA followed by *post-hoc* Dunnett test and expressed as the mean ± SEM values, and the significance was set at $P < 0.05$. The results showed that STZ-induced peripheral neuropathy developed already at the first week after STZ injections and was maintained for all experimental period. Cer *per se* did not influence the pain threshold, while it showed a markedly expressed lowering of hyperalgesia caused by STZ: the pain intensity in cerebrocrast + STZ rats was comparable with that of control (saline) values and maintained at control level during all the experiment. However Cer did not influence hyperglycemia and lack of weight gain caused by STZ. The obtained data showed for the first time that atypical (non-calcium antagonistic) 1,4-dihydropyridine compound Cer successfully protected the development of peripheral neuropathic pain in STZ diabetic rats, indicating its (or its analogues) usefulness in the treatment of pathologies caused by compromized mitochondrial functions.

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The effects of water-soluble chitosan on the fracture healing in New Zealand rabbits

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Chitin and its derivatives possess many biological activities. In order to investigate the effects of water-soluble chitosan on the fracture healing, sixty health male New Zealand rabbits (weight range: 2.1 ± 0.4 kg) were made into models with 3 mm bone defect in the middle segment of radius, and randomly divided into three groups, normal saline group ($n = 20$), bonesetting group ($n = 20$) and water-soluble chitosan group ($n = 20$). They were respectively given normal saline (1.00 ml/kg), jiegupian (1.00 ml/kg) and water-soluble chitosan (0.28 g/kg) daily through gastric tube. Each group was divided into four intervals of 9, 17, 30 and 42 days postoperatively. X-ray and HE staining was used to detect the healing effect; immunohistochemisty was applied to observe the expression of bone morphogenetic protein (BMP) and the biochemical indexes related to fracture healing were measured. The X-ray and HE stain showed that the fractures healing of water-soluble chitosan group was better than that of the other two groups in all intervals ($P < 0.05$). The expression of BMP in water soluble group of 9 and 17 days intervals were significantly higher than that of other two groups ($P < 0.01$). The ALP concentration in serum of the water-soluble group was clearly lower than control groups at the seventeenth day ($P < 0.05$), while the calcium concentration of the water-soluble group was higher significantly than the control groups at the seventeenth day ($P < 0.05$). Our results demonstrated that water-soluble chitosan can promote facture healing, with the possibility that it could increase calcium concentration in serum and could promote osteoblast to synthesize BMP and increase the expression of BMP to accelerate fracture healing.

P124

Pharmacological evaluation of the anti-inflammatory and cytotoxic activities of crude extracts from the Mediterranean marine algae and sponge

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As part of our search for new anti-inflammatory or anticancer potential drugs, aqueous extracts of macro-algae and invertebrates collected from Mediterranean Tunisia coasts were evaluated for their anti-inflammatory and cytotoxic activities. The present study has established that the aqueous extract from the brown algae of the genus *Zonaria* tested at different doses (50, 100, 200 mg/kg) for their anti-inflammatory activity, using the carrageenan paw oedema test (Winter *et al.*, 1962) in male albinos Wistar rats (150–170 g) and in comparison to reference drugs: dexamethasone (1 mg/kg) and aspirin (300 mg/kg), exhibited, in a dose dependent manner, a significant inhibitory effect on the rats paw oedema; the % inhibition of oedema, 3 h after carrageenan injection ranged from 66% to 86%. We also established that the aqueous extract of the marine sponge of the genus *Spongia*, showed a strong cytotoxic activity against three human tumour cell lines (A-549, MCF-7, HCT-15), using a MTT cytotoxicity assay (Bissery *et al.*, 1991). At concentration of 0.50 to 1.25 mg/ml, this extract suppressed, dose dependently, the proliferation of the three cell lines by more than 75%. The IC50 values ranged from 0.80 to 0.90 mg/ml. The pharmacological activities of these active extracts are discussed in accordance with the secondary metabolites present in the brown algae, Zonaria (Puntip *et al.*, 2003) and spongia (Grassia, *et al.*, 1994). Purification these extracts are under investigation.

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P125

Pharmacological evaluation of the anti-inflammatory and analgesic activities of the aqueous extract from the Mediterranean sponge, *Petrosia ficiformis*

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As part of our search for anti-inflammatory substances from marine sponges, the anti-inflammatory and analgesic activities of the aqueous extract from the mediterranean sponge, *Petrosia ficiformis* were investigated in animal models using the carrageenan-induced paw oedema assay (Winter *et al.*, 1962) in male albino Wistar rats (150–170 g) and acetic acid writhing test in mice (Koster *et al.*, 1959). The aqueous extract (50, 100 and 200 mg/kg) administered intraperitoneally, and in comparison to reference drugs: Dexamethasone (1 mg/kg) and acetylsalicylic acid (300 mg/kg), exhibited, in a dose dependent manner, a significant inhibitory effect on the rats paw oedema ($P < 0.001$). The % inhibition of oedema, 3 h after 0.05 ml of 1% carrageenan injection were higher than 50% and ranged from 51% to 64%. In addition, this aqueous extract (200 mg/kg), S/C administered in mice and in comparison to a reference drug: acetylsalicylic acid (200 mg/kg), significantly reduced the nociception induced by the 1% acetic acid intraperitoneal injection ($P < 0.01$). The % inhibition of writhing, 30 min after acetic acid injection, was 53%. The pharmacological activities of this active extract are discussed in accordance with the secondary metabolites present in the sponge of the genus *Petrosia* (Giner, 1999; Shin *et al.*, 1998; Lim *et al.*, 1999). Purification and determination of the chemical structures of compound(s) in this extract are under investigation.

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P126

Investigation on the anti-inflammatory properties of *Ocotea quixos* Lam. essential oil

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In the context of the development of novel therapeutics from natural sources useful as anti-inflammatory agents, we focused our attention on the pharmacological profiling of *Ocotea quixos* essential oil which, in previous studies, demonstrated antiplatelet, vasorelaxant and antithrombotic effect (Tognolini *et al.*, 2006, Ballabeni *et al.*, 2007). In this work, the anti-inflammatory activity of the essential oil and of its main components, trans-cinnamaldehyde and methylcinnamate, was evaluated in experimental *in vitro* and *in vivo* assays and their gastric tolerability was assessed in rats. Experiments were performed in cultured cells (J774 or SK-N-MC) and in female Wistar rats (200 g) applying experimental procedures supervised and approved by the Ministero della Salute (DL116/92). *In vitro*, *Ocotea* essential oil and trans-cinnamaldehyde but not methylcinnamate significantly ($P < 0.01$) reduced LPS induced NO release from J774 macrophages at non-toxic concentrations, in the range 1–10 microg/ml. At the same concentrations the essential oil also inhibited COX-2 expression induced by LPS in the cell line. Among the tested compounds, only the essential oil increased ($P < 0.05$) forskolin-induced cAMP production in SK-N-MC cells without modifying the intracellular mediator levels in unstimulated cells suggesting that only the overall phytocomplex possesses phosphodiesterase inhibitory effect. *In vivo*, the essential oil as well as trans-cinnamaldehyde but not methylcinnamate demonstrated acute anti-inflammatory activity in carregeenan-induced hindpaw edema when orally administered in rats at the doses of 3 and 10 mg/kg without provoking gastric mucosal damage. These data represent the first evidence for a favourable anti-inflammatory gastro-sparing activity of *Ocotea quixos* essential oil. On the basis of *in vitro* studies we speculate that this antiphlogistic activity is mainly related to trans-cinnamaldehyde and involves the reduction of both NO production and COX-2 expression. On the whole, the present findings support a reviving interest in trans-cinnamaldehyde as drug lead suitable to design new chemical entities endowed with optimized anti-inflammatory profile.

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P127

The role of inflammation and COX derived prostanoids in the effects of bradykinin on isolated rat aorta and urinary bladder

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Bradykinin, a vasoactive peptide increases during inflammation and induces the formation of prostaglandins through specific receptor activation. Two types of receptors mediate the biological effects of bradykinin, B_1 and B_2 receptors. Although B_2 receptors are present in most tissues, B_1 receptors are expressed after inflammatory stimuli or tissue injury. Bradykinin has high affinity for B_2 and low affinity for B_1 receptors, whereas the opposite occurs for des-Arg^9-bradykinin. Recently, it has been reported that nonsteroidal antinflammatory drugs have different inhibitory activities on cyclooxygenase isozymes; COX-1, COX-2 and COX-3. In the present study, we have investigated the contributions of different COX isozyme inhibitions and inflammation on bradykinin induced effects of isolated rat aorta and urinary bladder smooth muscle contractions. Male Sprague Dawley rats weighing 200–250 g were used in the study. The vasodilatory responses to bradykinin (10^{-9}–10^{-6} M) were studied on isolated rat aorta rings contracted with

norepinephrine (10^{-7} M) following incubation with dipyrone (100, 700 and 2000 μM). The relaxant responses of dipyrone (100, 700 and 2000 μM) were also compared on the isolated rat urinary bladder contracted with bradykinin ($n = 8$). Bacterial lipopolysaccharide (LPS) was used for the induction of inflammation ($n = 8$). The levels of PGE_2,$PGF_{1\alpha}$,TXB_2,NOS, IL_{10} and TNFα? were all determined in both the plasma and the perfusate of the aorta preparations ($n = 5$). The vasodilatory activities of bradykinin and des-Arg^9-bradykinin were significantly increased upon the inhibition of COX-3 (dipyrone at 100 μM). These effects were blocked in the inflamed group. PGE_2, $PGF_{1\alpha}$, TXB_2 were significantly high but NOS activity was low in the aorta perfusate after inhibition of COX-3. Dipyrone increased the relaxant activity of the urinary bladder contracted with bradykinin. The vasodilatory activity of des-Arg^9-bradykinin was higher in inflamed group than that of uninflamed group. Bradykinin did not contract the urinary bladder in the inflamed group. The results suggest that COX induced products may play an important role in the bradykinin induced rat aortic smooth muscle contractions.
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P128

Intra-articular morphine attenuates knee hypersensitivity in a rat model of unilateral joint inflammation

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Intra-articular injection of morphine produces a long lasting analgesia in both rheumatoid and osteoarthritis (Likar *et al.*, 1997; Stein *et al.*, 1999). Electrophysiological recordings of the afferent fibres innervating the rat knee joint have provided conflicting evidence as to whether functional opioid receptors are present in the periphery during an FCA-induced model of inflammatory arthritis in the rat. Li *et al.* (2005) observed a loss of antinociception of the μ-opioid agonist endomorphin-1 to hyper-rotation of the knee joint, with a differing study reporting an opioid mediated attenuation of neural discharge evoked by noxious von Frey filaments (Strickland *et al.*, 2007). The aim of the current study was to determine whether an intra-articular injection of morphine would attenuate FCA-induced knee joint hypersensitivity observed at 14 days post-FCA injection. On day 0 a unilateral joint arthritis was induced in the left knee of adult male Wistar rats ($n = 32$, 150–200 g) by a 150 μl intra-articular injection of Freund's complete adjuvant (FCA; 1 mg/ml, *Mycobacterium tuberculosis*, Sigma, UK). Rats were transiently anaesthetised using 3% isoflurane in oxygen and FCA was injected into the knee joint space. On day 14 animals received a further intra-articular injection (100 μl) of 0.1, 0.3, or 1 mg morphine or vehicle (saline) into the left knee joint. Prior to, and at 30, 60, 90, 120 and 180 min post-morphine, the weight distribution (WD) of each animal was measured. WD data were expressed as a ratio of the weight placed through the left limb divided by the weight placed through the right limb. Differences in group mean WD ratios were analysed using a two-way ANOVA, where $P \leq 0.05$ was considered significant. Results showed that the group of animals injected with 0.3 and 1 mg of morphine had significantly higher ($P < 0.05$) WD ratios in the time course following morphine injection, when compared to vehicle. Peripherally administered morphine reduced FCA-induced joint hypersensitivity in a pre-clinical model of inflammatory joint pain. This behavioural data provides further evidence that functional opioid receptors are present in the periphery following FCA-insult, and novel opioids that can be restricted to the periphery may provide analgesia at the site of inflammation whilst avoiding the common, centrally mediated, undesirable side-effects of current opioid treatments.

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P129

Preliminary research on the effects of some serotonin re-uptake inhibitors in a visceral pain model

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This study investigated serotonin reuptake inhibitors antinociceptive effects on an accepted visceral pain model (behavioural models of inflammatory cystitis). The experiment was carried out, with white male mice (20–25 g), divided into 8 groups of 7 animals each, treated orally for 14 days, with the same volume of solution, as follows: Group I: saline solution 0.3 mL (control); Group II (IMP): imipramine 10 mg/kbw; Group III (CMP): clomipramine 10 mg/kbw; Group IV (FLX): fluoxetine 10 mg/kbw; Group V (FVM): fluvoxamine 50 mg/kbw; Group VI (VLX): venlafaxin 25 mg/kbw; Group VII (RBX): reboxetine 25 mg/kbw. Morphine (2 mg/kbw) administered subcutaneously (0.1 ml) is used as positive control drug with a known analgesic effect on this visceral pain model. The model of visceral pain consists of inflammatory cystitis after intraperitoneal injection of cyclophosphamide (200 mg/kbw). Data were statistically analyzed with SPSS for Windows version 10.0. Statistical analysis of the results obtained in cyclophosphamide cystitis shows that: administration of imipramine, venlafaxin resulted in a significant reduction of behavioural manifestations of inflammatory cystitis due to chemical irritant agent ($P < 0.05$) comparing with control group. The effects of these substances were less intense than those of morphine. The most intense effect was observed for imipramine. clomipramine, fluvoxamine, reboxetine, fluoxetine also caused diminution of behavioural score in mice with inflammatory cystitis but this was insignificant compared with the control group. In our experimental conditions in this visceral pain model, most of serotonin reuptake inhibitors manifest antinociceptive effect in different time intervals, except fluoxetine. The analgesic effects of these substances ware less intense then those of morphine. Imipramine and clomipramine determine the most intense antinociceptive effect in this experimental study. Imipramine administration, resulted in a significant antinociceptive effect in cyclophosphamide cystitis test ($P < 0.05$), in all time intervals of the experiment.

P130

Tramadol-agmatine interaction and investigation of possible mechanisms in an experimental acute pain model

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A central analgesic agent tramadol has been proposed to act by partially opiatergic, noradrenergic and serotonergic mechanisms. Although ongoing studies show that some other mechanisms may also be involved in the effect of tramadol, but the precise mechanism has not been determined yet. On the other hand, agmatine, suggested as a new neurotransmitter in the brain has been shown to elicit antiallodynic and antihyperalgesic effects in neurophatic pain and to enhance morphine antinociception. The aim of the present study was to investigate the possible effects of agmatine and agmatine-tramadol combination on nociception by using the tail-flick method, an acute analgesic test in mice. We also evaluated the mechanism of the potentiating effect of agmatine on tramadol-induced antinociception by pretreatment with nitric oxide modulators such as L-arginine and L-NAME and the NMDA receptor blocker MK-801. Male Balb/c mice weighing between 20–25 g were used in all experiments. The antinociceptive effect was determined in mice by using tail-flick test. Drugs were administered in 30 min interval. The Tail-flick test was performed 30 min after the last drug injection. The Tail-flick latencies (TFL) were record as second and the results were expressed as the mean ± SEM. One-way analysis of variance followed by a *post-hoc* Student Newman–Keuls test was used for the statistical analysis. Tramadol and agmatine significantly increased the TFL of mice when compared to the control group. Agmatine enhanced the antinociceptive effect of tramadol. L-arginine and L-NAME did not change the TFL of agmatine, but L-arginine decreased whereas L-NAME enhanced the antinociceptive effect of agmatine + tramadol combination. MK-801 pretreatment did not change the TFL of agmatine, tramadol or agmatine-induced potentiation of the tramadol effect. Our results demonstrate that agmatine combination with tramadol produces an antinociceptive enhancement and this effect does not seem to be mediated via the nitrergic system or NMDA receptors. Administration of agmatine-tramadol and/or agmatine-tramadol-L-NAME combinations may also provide an effective therapeutic strategy for future medical treatment of pain.

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Effects of spinal administration of amitryptiline, venlafaxine and fluoxetine on C-fibre and A-fibre evoked responses of dorsal horn neurones

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Antidepressants are among the most useful drugs used to relieve many different types of chronic pain including neuropathic pain, arthritis, low back pain, fibromyalgia and central pain. The modulation of nociception by antidepressants appears to be exerted at supraspinal sites through the activation of descending monoaminergic/opioid pathways to the dorsal horn. Nevertheless, spinal actions of antidepressants may also contribute to their analgesic effects. In this study, we have evaluated and compared the effects of spinally administered amitryptiline (tricyclic antidepressant), venlafaxine (noradrenaline/serotonin re-uptake inhibitor) and fluoxetine (selective serotonin re-uptake inhibitor) upon the responses of dorsal horn neurones. Extracellular recordings of electrically-evoked responses of single wide-dynamic-range (WDR) neurones were made in isofluorane anaesthetised rats (Sprague-Dawley, male, 2 months old). The effect of each dose of antidepressant ($n = 5$–7; cumulative doses: 10–20–40 μg/50 μl, i.t.) on Aβ-, Aδ- and C-fibre evoked responses and postdischarge responses was followed for 1 h Aβ-fibre evoked responses of neurons were not altered by any of the antidepressants. However, Aδ-fibre evoked responses were dose-dependently and highly inhibited by amitryptiline and venlafaxine (40 μg dose: 39.9 ± 12.6% and 34.4 ± 19.3% of control values, respectively) whereas fluoxetine was less effective (40 μg dose: 56.4 ± 12.9% of control). A similar pattern of inhibition was observed upon the C-fibre evoked responses (40 μg dose: 42.0 ± 15.3%, 34.6 ± 16.3% and 53.5 ± 14.7% of control, for amitryptiline, venlafaxine and fluoxetine, respectively). The evoked C-fibre mediated post-discharge responses were almost abolished with the highest dose of amitryptiline and venlafaxine (13.5 ± 4.6% and 6.8 ± 3.1% of control, respectively) while fluoxetine was devoid of effect. These results demonstrate that antidepressants inhibiting noradrenaline/serotonin re-uptake (amitryptiline and venlafaxine) exhibit greater spinal antinociceptive activity than those acting as selective serotonin re-uptake inhibitors (fluoxetine).

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Rip2 mediates NOSII-induction by NOD1 activation in vascular smooth muscle cells but not by TLR4 activation in macrophages

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Lipopolysaccharide activates TLR4 whilst peptidoglycan products activate NOD1. We have recently shown that activation of NOD1 by the specific agonist FK565 results in a profound induction of NOSII in vascular smooth muscle cells (VSMCs) but not in macrophages. Our findings also suggest that the signalling pathways are separate, but converge at the level of (NF)-κB activation for NOSII induction (Moreno *et al.* 2007). Here, we have analysed the effect of a number of pharmacological inhibitors to characterise the role of MAPK pathways in the induction of NOSII following TLR4 or NOD1 receptor activation. Rat VSMCs and murine macrophages (J774) were stimulated with LPS (1 μg/ml) or FK565 (10 nM) for 24 h before NOSII activity was assessed by the accumulation of nitrite using the Griess reaction. The ERK1/2 inhibitor PD98059 inhibited both TLR4 and NOD1-induced NOSII activity in both cell types similarly. The JNK inhibitor SP600125 inhibited the responses induced by TLR4 but not NOD1. The Src inhibitor PP2

(0.03 or 1 μM) selectively inhibited NOSII induction in VSMCs by NOD1 activation. Our findings show that the MAPK pathway provides an added level of regulation for both TLR4 and NOD1 signalling and suggest an important role for Rip2 in the induction of NOSII following NOD1 activation in VSMCs.

	Control (μM)	Control (100%)	PD98059 (10 μM)	SP600125 (10 μM)	PP2 (1 μM)	PP2 (0.03 μM)
VSMCs (FK565)	18.8 ± 3	100	36.1 ± 7*	103 ± 2	3.2 ± 3.2*	52.3 ± 8*
Macrophages (LPS)	38.2 ± 2.5	100	33.2 ± 11*	85 ± 5*+	79.3 ± 9 *+	89 ± 5 *+

Effect of signalling pathway inhibitors in the induction of NOSII activity by TLR4 and NOD1 activation in VSMC vs. macrophages. Results were analyzed using one sample and Students T-test, * and + denotes $P < 0.05$ vs. control or VSMCs responses respectively. $n = 6–9$.

Moreno *et al.* 2007. Relative roles of TLR4 vs. NOD1 in the sensing of *E. coli* by vascular smooth muscle cells and macrophages: identification of distinct signalling pathways.

P133

Analgesic activity of bradykinin B1 receptor antagonists in rats and rabbits

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The objective of this study was to compare analgesic efficacy of two orally active non-peptide bradykinin B1 receptor (B1R) antagonist model compounds on subacute phase of monoarthritic pain test in rats and rabbits with those of currently used analgesics. The two antagonists had low nanomolar IC_{50} at rabbit and 50–100-fold lower potency at rat B1 receptors. SPRD rats and NZ rabbits were injected with Freund's complete adjuvant (FCA) into right knee joint and right wrist joint, respectively. Compounds were administered on Day 3 (rat) or Day 1 (rabbit) following FCA injection. The pain was measured based on the decreased weight bearing capacity of the affected limb using an 'Incapacitance tester'. Measurements were taken pre-dose and at several time points (0.5, 1, 2, 3 and 4 h) after drug administration. Both B1R antagonists effectively alleviated the FCA induced pain in both species in a dose dependent manner. In rabbits, they produced maximum pain reversal of 70–77% at doses of 1–3 mg/kg, which represents similar efficacy to that of selective COX-2 inhibitor valdecocib (79%) but somewhat less than that of NSAIDs (diclofenac and indomethacin 85 and 94%, respectively) and morphine (100%). In rats, the B1R antagonists produced 55–75% reversal at 10 mg/kg and the comparison to the reference compounds yielded similar relative efficacies to those in rabbits. The current results suggest that B1 receptor antagonism, targeted with orally acting non-peptide B1R antagonists, may be an effective mechanism in alleviating arthritic or inflammatory pain, whereas it is hoped that B1R antagonists might have better side effect profile than current therapeutic agents.

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Synthesis of novel heterocyclic curcumin derivatives as non-steroidal anti-inflammatory drug candidates

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Non-steroidal anti-inflammatory drugs (NSAIDs) do not solve the underlying problem of inflammation; also their long term use is associated with serious gastrointestinal side-effects (Fiorucci *et al.* 2005). Curcumin, Figure A (1) the yellow pigment isolated from the rhizomes of *Curcuma longa*, has a well established role as an anti-inflammatory agent (Nurfina *et al.*, 1997). Using curcumin as a lead compound, our aim is to discover a new class of drugs which have anti-inflammatory properties and which act on the production of pro-inflammatory cytokines; interleukin-1, CXCL-8, tumour necrosis factor-α and reactive oxygen species nitric-oxide; without the side-effects associated with the conventional NSAIDs. In this study, we have successfully synthesized and characterized a series of novel heterocyclic curcuminoids Figure A (2-5) through the condensation of the

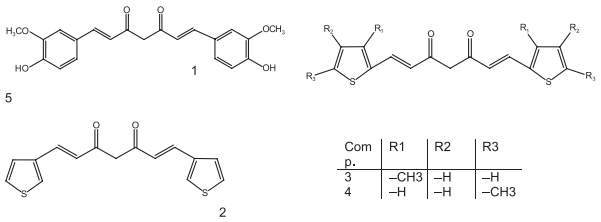

Figure A for P134. Curcumin and its novel heterocyclic analogues

acetylacetone-boron complex with the corresponding thiophenealdehyde followed by the acid hydrolysis of the complexed curcumin (Deng *et al.*, 2006). In order to enhance the aqueous solubility of these lipohilic drugs, the non-covalent inclusion complexes with hydroxypropyl-γ-cyclodextrins have also been synthesized using the co-precipitation method (Tang *et al.*, 2002). The effects of these drugs on the production of pro-inflammatory cytokines are currently being studied using both human intestinal epithelial cell line CACO2 and human monocytic cell line THP1.

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P135

Effects of gabapentin on secretion of nNOS and PGE_2 from rat hippocampus slices

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It is desirable to show that antiepilectic gabapentin has analgesic effects, especially at clinical studies conducted at neuropatic pain syndroms. In this study, the effects of gabapentin (G) on secretion of nNOS and PGE_2 from rat hypocampus slices are investigated. All experiments for animal testing were approved by the Eskişehir Osmangazi University School of Medicine Animal Use and Care Committee. In our study, Male albino rats were used (250 ± 25 g). Hippocampi were isolated and sliced into 0.6'μ pieces at rat brain. These slices are oxidized at incubation system with a mixture of 95% O_2 and 5% CO_2 at 37 °C. Our material (gabapentin and additonal indomethacin, cyproheptadin, L-NAME, naloxone) are added to the incubation system and nNOS and PGE_2 are measured in the obtained perfusate and homogenate. In all control groups, gabapentin decreased PGE_2 and increased nNOS for all doses used. L-NAME; decreased PGE_2 for different doses in all control groups; when used with G it decreased according to control group. When G+L-NAME are given together PGE_2 level decreased compared to L-NAME is given alone. In all concentrations, it increased nNOS level. When used with G, nNOS level increased more compared with control and G groups of Indomethacine; decreased PGE_2 level, while there was no change when it is used with G. It also increased nNOS level, while when used with G it increased nNOS level according to control and G groups cyproheptadin; increased PGE_2 in all concentrations, while when used with G it decreased PGE_2 compared with the control group; it did not change when compared with the G group. It increased nNOS levels dose dependently, when it decreased when used with G. Naloxon; increased PGE_2 level to control values which was decreased by G. Besides, there was not an important change in nNOS values for different G groups. The significance of differences was analyzed using Dunnett *t* test and one-way analysis of variance (ANOVA). Values of $P < 0.05$ were taken to indicate statistical significance. It can be concluded that in the antinosiseptive activity of G, except for opioidergic system, nitrergic and serotonergic systems have an important role and they affect nNOS and PGE.

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P136

Glucocorticoids are a cause for memory deficit induced by naloxone precipitated morphine withdrawal in mice

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Morphine withdrawal leads to an increase in corticosterone concentration in plasma (Zelena *et al.* 2005), and cognitive deficits are found, after withdrawal. Since previous studies have shown that glucocorticoid hormones could affect memory, the aim of current study was to evaluate the effects of metyrapone and mifepristone on memory deficit following naloxone precipitated morphine withdrawal. Male NMRI mice weighing 25–30 g were made dependent by increasing doses of morphine (30–90 mg/kg) twice daily for 3 days. Withdrawal was elicited by injection of naloxone (0.1 mg/kg) 3 h after last morphine injection. Mifepristone (50 and 100 mg/kg) and metyrapone (6.25, 12.5 and 25 mg/kg) were used before the first trial and effects were compared with control values. To assess memory performance the object recognition task was used as described by Bertaina-Anglade, *et al.* (2006). The test was comprised of three sections; habituation for 15 min, first trial for 12 min and test trial for 5 min. In this learning paradigm, the difference in exploration between a previously seen object and a novel object is taken as an index of memory performance (recognition index, RI). Mean level of corticosterone was significantly increased from 29.4 ± 0.7 ng/mL in controls to 33.9 ± 0.3 ng/ml in morphine withdrawn animals. Metyrapone at 12.5 mg/kg, significantly improved RI (42.7% ± 4.6, $n = 6$) in dependent animals after withdrawal but it did not show a dose response relationship. Mifepristone at 100 mg/kg improved RI (18.3% ± 8.5) in mice after morphine withdrawal ($n = 6$), since they spent more time in exploring the new object than the familiar one. Therefore, metyrapone, by inhibiting glucocorticoid formation, and mifepristone by inhibiting glucocorticoid receptors are effective in preventing memory deficit following morphine withdrawal. Increased glucocorticoid concentration might be involved in memory deficit caused by morphine withdrawal.

References:

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P137

Memantine ameliorates the amyloid-beta 1-42 peptide-induced decrease in [^{3}H]dopamine release in rat corticostriatal slices

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Extracellular deposition of amyloid-beta peptides seriously affects cholinergic neurotransmission and cognitive functions in Alzheimer patients. Cholinesterase inhibitors have been used for treatment as the only available medications for a long time. Clinical investigations, however, have suggested the beneficial effect of the weak NMDA-receptor antagonist memantine. The glutamate-NMDA receptors play a pivotal role in the glutamate-dopamine interactions in the striatum. Enhanced prefrontal cortical activity triggers the release of glutamate from the corticostriatal axons in the striatum, which in turn modulates the release of dopamine from the nigrostriatal afferents (Leviel *et al.* 1990). It was previously reported that the release of dopamine decreased in beta-amyloid infused rats (Itoh *et al.* 1996). We have investigated the effect of memantine on the release of [^{3}H]dopamine in the presence of amyloid-beta 1-42 peptide. Male SPRD rat (200–250 g) corticostriatal slices were loaded with [^{3}H]dopamine, submerged in a two-compartment bath so that the corticostriatal glutamatergic afferentation was preserved between the cortical and striatal regions (Juranyi *et al.* 2003). Electrical stimulation of cortical regions increased the release of [^{3}H]dopamine in the striatal parts. Amyloid-beta peptide (10 nM) significantly decreased the electrically-evoked release of [^{3}H]dopamine. It has been suggested that amyloid-beta peptide binds to nicotinic acetylcholine receptors containing α7 subunits and blocks them in a non-competitive way. Memantine (1 μM, 10 μM) alone did not change either the spontaneous or the electrically-evoked release of [^{3}H]dopamine. Application of memantine in the presence of amyloid-beta peptide (10 nM) partially reversed the suppressed release of [^{3}H]dopamine. These results suggest that the amyloid-beta peptide impedes the cortically-evoked release of [^{3}H]dopamine in the striatal region of corticostriatal slices and this depressed release can be partly restored by the weak NMDA blocker memantine.

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Juranyi *et al.* J Neurosci Methods. 2003; 126: 57.
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P138

Antagonism of melanin concentrating hormone receptor does not alter sleep electroencephalogram parameters in rats despite high central receptor occupancy

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Melanin concentrating hormone (MCH) has been implicated as a powerful hypnogenic peptide in rats (Verret *et al.* 2003). The aim of this study was to establish a *ex-vivo* receptor occupancy assay for MCH_1 and identify if high receptor occupancy (RO) with a MCH_1 antagonist, 4′-{[1-(Cyclopropylmethyl)piperidin-4-ylidene][5-fluoro-6-(trifluoromethyl)-1H-benzimidazol-2-yl]methyl}biphenyl-3-carbonitrile (compound A), results in any changes in electroencephalogram (EEG) in rats. With local ethical approval male rats (250–300 g) were orally administered with compound A (3, 10, 30 and 100 mg/kg) or vehicle and subsequently sacrificed at 6 h post dose. Blood samples were collected immediately after decapitation to determine free plasma concentrations. Following optimisation studies, coronal brain sections (20 μm) capturing the caudate putamen were pre-incubated in buffer for 15 min followed by a 45 min incubation with 50 pM [^{125}I]-S36057 (Perkin Elmer, UK) For EEG studies, rats were orally dosed with compound A (3 or 30 mg/kg) or vehicle ($n = 7$) at light on-set in a cross-over design. Recording of EEG and electromyogram (EMG) signals began immediately after dosing. Percentage RO was calculated using specific binding from vehicle and compound treated animals. Significance was set at $P < 0.05$. To obtain >60% specific binding of [^{125}I]-S36057 ex vivo, it was essential to have 15 min pre-incubation. The mean ED_{50} in RO was 9.3 ± 1.4 mg/kg ($n = 4$), equivalent to a free plasma concentration of 40 nM, approximately 11 fold the primary Ki value for this compound (Wu *et al.*, 2006). At 30 mg/kg (free plasma concentration of 100 nM), the RO measured was 90 ± 8.2%. For EEG analysis every 12 second epoch was automatically classified as either WAKE, NREM or REM sleep. Compound A had no effect on the sleep-wake pattern measured over the first 6 h after administration. In summary, we report a novel *ex-vivo* RO assay in sections of rat brain that can be used for compound screening and as a target biomarker for central occupancy of small molecular MCH_1 antagonist. Despite high central MCH_1 RO, we saw no significant changes in EEG parameters and conclude that EEG may not be a suitable translatable biomarker for central MCH_1.

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P139

Repeated low dose MDMA protects against a subsequent neurotoxic dose of MDMA and reduces IL-1β release

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3,4-Methylenedioxymethamphetamine (MDMA) induces persistent serotonergic neurotoxicity in rat brain reflected as loss of serotonin (5-HT) content and reduction in 5-HT transporter (5-HTT) density. In addition, MDMA administration produces interleukin-1beta (IL-1β) release (Orio *et al.* 2004) which is related to the hyperthermic response induced by the drug. In ischemia and several other neurotoxicity models, previous exposure to a low degree of insult induces tolerance against a subsequent more severe insult (Dirnagl *et al.* 2003). We aimed to study if exposure to repeated low dose MDMA protects against a subsequent neurotoxic dose of MDMA and decreases IL-1β release. Male Dark Agouti rats (175–200 g) were given MDMA (3 mg/kg, i.p.) or saline for 4 days at a room temperature (RT) of 22 °C or 4 °C. On the fifth day, rats were injected with a neurotoxic dose of MDMA (12.5 mg/kg, i.p.) at RT22 °C and rectal temperature was monitored for 6 h. Different groups of animals were killed at 3 h for the quantification of IL-1β (ELISA) and at 7 days to evaluate the density of [^{3}H]-paroxetine labelled 5-HTT and concentration of 5-HT (HPLC) in the cortex and hippocampus. Repeated low dose MDMA pretreatment administered at both room temperatures attenuated the decrease in 5-HT content and 5-HTT density produced by the neurotoxic dose of MDMA without modifying the hyperthermic response induced by the drug. Furthermore, repeated low dose MDMA pretreatment administered at both room temperatures reduced the increase in IL-1β release produced by neurotoxic MDMA in both cortex and hippocampus. Similar protective results were obtained when the 4 day pretreatment regimen was replaced with a single low dose exposure either 24 h or 4 d before neurotoxic MDMA. In summary, prior exposure to single or repeated low dose MDMA at RT22 °C protects against the serotonergic neurotoxicity produced by a subsequent neurotoxic dose of MDMA. This protective regimen also attenuates the IL-1β release produced by the drug and is mediated by mechanisms independent of ambient temperature. Financial support: SAF2006-07045 (MEC), PI07/0892 (FIS), SAF2007-65175 (MEC).

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P140

Effect of ethanol on GABA turnover in the brain of mice treated with a neurotoxic dose of MDMA

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Mice administered a neurotoxic dose of MDMA are less sensitive to the hypnotic effect of acute ethanol (EtOH) and are resistant to the development of rapid tolerance to hypothermia (Marchant *et al.* 2005). GABAergic transmission plays a relevant role in the behavioural effects of EtOH. In fact, acute EtOH reduces GABA turnover (Hellevuo & Kiianmaa, 1989) and enhances the activity of GABA uptake in mouse brain (Cai *et al.* 2006). We have now examined GABA turnover in discrete brain areas of mice exposed to a neurotoxic regimen of MDMA and then challenged with EtOH. Adult male C57BL/6J mice (25–30 g) were injected with saline or MDMA (30 mg/kg, i.p. × 3, 3 h intervals). Seven days later animals received aminooxyacetic acid (AOAA, 12 mg/kg, i.p.) 10 min after EtOH (3 g/kg, i.p.). GABA accumulation was determined in the striatum, hippocampus and frontal cortex 1 h after inhibition of GABA-transaminase (GABA-T) by AOAA. To prevent non-specific postmortem synthesis of GABA, 3-mercaptopropionic acid (100 mg/kg, i.p.) was injected 3 min before decapitation. GABA was measured by h.p.l.c. using a precolumn o-phthaldehyde/sulfite derivatization method. Seven days after MDMA mice showed a pronounced reduction (80%) in striatal dopamine concentration and dopamine transporter density. There was no difference in GABA levels between mice lesioned and not lesioned with MDMA. While EtOH significantly reduced GABA accumulation in the striatum of non-lesioned mice, no effect was observed in the MDMA-treated group. In hippocampus and frontal cortex EtOH reduced GABA concentration in both saline and MDMA-injected mice. These findings indicate that the effect of EtOH on striatal GABA turnover differs between saline and MDMA-treated mice and that this may contribute to the lower sensitivity to the EtOH-induced sedative effect and the resistance to the development of rapid tolerance to hypothermia that are evident 7 days after drug injection in the

lesioned mice. Financed by: PR75/06-15077, RD06/0001 (MSC), 910258 (UCM-CAM).
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P141

Cocaine withdrawal internalizes DAT in the endosome and prevents MDMA-induced dopaminergic toxicity in mouse striatum

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Dopamine transporter (DAT) is an important target for psychostimulant drugs such as 3,4-methylenedioxymethamphetamine (MDMA) and cocaine. In mice, MDMA produces long-term dopaminergic toxicity (Colado *et al.* 2004), reflected as a decrease in striatal dopamine (DA) content and DAT density. Cocaine can induce PKC-mediated DAT trafficking between plasma membrane and endosome (Little *et al.* 2002; Loder *et al.* 2003). Since MDMA produces neurotoxicity by a mechanism involving DAT (Camarero *et al.*, 2002), we studied the effect of repeated cocaine pretreatment and withdrawal on MDMA neurotoxicity. Adult male NIH/Swiss mice (25–30 g) were given cocaine (20 mg/kg, i.p., twice daily for 3 days) and either killed 24 h or 4 days later (for determination of [^{3}H]WIN35,428-labelled striatal DAT density in plasma membrane and endosome) or given MDMA (20 mg/kg, i.p., 2 injections separated 3 h) 24 h or 4 days after cocaine and killed 7 days after MDMA for the determination of neurotoxicity parameters (loss of DA content by HPLC and reduced DAT density in striatum). A separate group of mice were given the PKC inhibitor, NPC15437, 30 min before each cocaine injection and during the 4 days of cocaine withdrawal before MDMA and killed 7 days after MDMA treatment. Repeated cocaine pretreatment 4 days but not 24 h before MDMA prevented the MDMA-induced decrease in striatal DA and DAT density. NPC15437 attenuated this protection induced by cocaine. Four days after repeated cocaine pretreatment, DAT density was lower in plasma membrane and higher in endosome. No such changes in DAT distribution were observed 24 h after cocaine pretreatment. In conclusion, cocaine pretreatment protects against the dopaminergic neurotoxicity induced by MDMA through a mechanism requiring withdrawal and involving PKC-mediated DAT trafficking. Financial support: PNSD 3SI/04/01 (MSC), 910258 (UCM-CAM), RD06/0001 (MSC).

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Colado *et al.* Psychopharmacology. 2004; 173: 249–263.
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P142

The improved cognition induced by huprine X may be related to APP processing via PKCα and MAPK in middle aged mice

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Cognitive enhancing properties and putative adverse effects of huprineX ((-)-12-amino-3-chloro-9-ethyl-6,7,10,11-tetrahydro-7,11methanocycloocta[*b*]quinoline hydrochloride [HX]), a new anticholinesterasic and its effects on the regulation of PKC, MAPK and α-secretases (ADAM10 and TACE) activities related to amyloid precursor protein (APP) processing remain to be established. Thus, correlates between behavioural effects of HX with the above mentioned molecular substrates were performed. In our study, 28 middle aged C57b/6 male mice which received chronic i.p. treatment with either saline or HX (0.04 and 0.12 μmol/kg), were submitted to a battery of behavioural tests. Afterwards both cytosolic and membrane fractions obtained from the hippocampus and the cortex of saline and HX treated mice were obtained and its effects on the expression and distribution of PKCα, MAPK activation as well as its effects on APP processing and the trafficking of both ADAM10 and TACE by means of immunoblotting were determined. The results show that, in a dose dependent manner, HX improved learning and memory in the Morris water maze and some indicators of emotionality without inducing adverse effects affecting no motor activity or anxiety-like behaviours. The increases of both phospho-PKCα and phospho-42/44 MAPK could participate in the rise of membrane holoAPP processing through the induction of α-secretase activity and thus may underlie the cognitive benefits. These results suggest that HX constitutes a promising therapeutic agent for the treatment of dementias caused by cholinergic dysfunction.

P143

Investigating the protective properties of the Nrf2-Keap1 regulatory pathway using small interfering RNAs

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Oxidative stress is one of the main contributors to neurodegeneration in Parkinson's disease. The transcription factor, nuclear factor-erythroid 2-related factor 2 (Nrf2) and its negative binding regulator, kelch ECH associated protein 1 (Keap1) mediate the transcriptional activation of a variety of genes involved in cellular defence against stimuli such as oxidative stress. The hypothesis is that cells with decreased Nrf2 are more susceptible to, and cells with increased Nrf2 are protected from oxidative stress. Nrf2 protein size was determined using a combination of Western blotting and immunoprecipitation. Nrf2 levels were manipulated in HEK cells using small interfering RNA (siRNA) against Nrf2 or Keap1 transcripts, and chemical inducers were also used. The effects were assessed using a variety of real time PCR, Western blotting and a luciferase-reporter gene assay for Nrf2-mediated gene transcription. Oxidative stress was induced using 6-hydroxydopamine and cytotoxicity assays were performed to measure apoptosis. True Nrf2 bands were identified at 58 and 78 kDa. siRNA against Keap1 significantly knocked down Keap1 expression but failed to induce Nrf2-mediated gene transcription. However, an optimized concentration of tert-butylhydroquinone (tBHQ) activated Nrf2-phosphorylation and its mediated gene transcription. siRNA against Nrf2 transcripts also showed knock down at the protein level and subsequent transcriptional activity. Preliminary results suggest that pretreatment with siRNA against Nrf2 caused cells to be more susceptible to oxidative stress, and cells pretreated with tBHQ were protected from the effects of 6-hydroxydopamine. These initial findings suggest that the Nrf2-Keap1 pathway could be a potential therapeutic target in preventing further neurodegeneration caused by oxidative stress in Parkinson's disease.

P144

Effects of sertraline on experimental models of psychosis in mice

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Recently, it has been reported that treating negative symptoms of schizophrenia with a combination of a typical antipsychotic and a selective serotonin reuptake inhibitor was more effective than the treatment with antipsychotic alone (Chertkow *et al.* 2007). The present study was designed to study the effects of sertraline on neuroleptic-induced catalepsy; apomorphine-induced climbing behaviour and amphetamine or MK-801-induced locomotor activities in female Swiss albino mice weighing 30–35 g. Catalepsy was induced by haloperidol (1 mg/kg i.p.), apomorphine (1.5 mg/kg s.c.) was used for studying climbing behaviour, d-amphetamine (3 mg/kg i.p.) or MK-801 (0.3 mg/kg i.p.) was used for testing locomotor activities. Eight animals were used in each group. Sertraline (10 mg/kg i.p.) was injected acutely or as 5 days repeated treatment. Single dose of sertraline enhanced locomotor activity, while it inhibited catalepsy and climbing behaviour when it was used 5 days repeated doses. These results suggest that selective serotonin reuptake inhibitors may have beneficial effects to treatment of negative symptoms of psychosis.

Reference:
Chertkow *et al.* J Neural Transm. 2007; 114(11): 14433–54.

P145

Chronic fluoxetine regulates beta-catenin expression in dentate gyrus of an animal model of depression

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Bilateral olfactory bulbectomy (OB) is a validated animal model of depression showing behavioural, neurochemical and structural features similar to those observed in human major depression, that are reversed by chronic antidepressant treatment. It has been proposed that neurogenesis in the dentate gyrus of the adult hippocampus is required for the behavioural effects of antidepressants. Interestingly, the OB rat also exhibits impaired neurogenesis. On the other hand, the β-catenin pathway plays a crucial role in cell proliferation and fate of adult hippocampal stem/progenitor cells (AHPs). Thus, we have investigated in the OB model of depression whether this proliferative pathway is altered and its regulation by the chronic administration of the serotonin re-uptake inhibitor (SSRI) fluoxetine (10 mg/kg/day, 14 days; s.c). Male Sprague–Dawley rats (n = 24), 2 months old, were randomized in four groups: sham-operated + vehicle; sham-operated + fluoxetine; OB + vehicle; OB + fluoxetine. Chronic fluoxetine fully attenuated hyperactivity in the 'open-field' test in the OB + fluoxetine group ($P < 0.01$ vs. OB + vehicle) whereas had no effect in sham-operated animals. Brain sections (45 μm) were immunostained for β-catenin by the avidin-peroxidase technique. The number of β-catenin immunopositive cells decreased (-36 ± 2%) in subgranular zone of hippocampal dentate gyrus (SGZ) of OB + vehicle group ($P < 0.01$ vs. sham-operated + vehicle). Chronic fluoxetine treatment increased β-catenin expression in SGZ on both sham-operated + fluoxetine ($P < 0.01$ vs. sham-operated + vehicle) and OB + fluoxetine animals ($P < 0.05$ vs. OB + vehicle). These results suggest the implication of this proliferative pathway in the mechanism of action of fluoxetine that might represent a putative new target for antidepressant development.
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P146

Long-term treatment with fluoxetine induces desensitization of 5-HT4 receptors in rat hippocampus

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Treatment with selective serotonin reuptake inhibitors (SSRIs) benefits many patients with major depression disorders. It has been proposed that the therapeutic effects of fluoxetine might be related to adaptive changes in serotonergic neurotransmission, through the activation of the different serotonin receptor subtypes. 5-HT$_4$ receptors are mainly located in several areas of central nervous system, mediating neuronal excitability of hippocampal CA1 pyramidal cells. However, the information about the involvement of this subtype in the mechanism

of action of antidepressants is still limited. The aim of this study was to evaluate the effect of a 21-day treatment with two doses of the SSRI fluoxetine (5 and 10 mg/kg/day p.o.) in both density and functionality of 5-HT_4 receptors in rat hippocampus using receptor autoradiography ([^{3}H]GR113808) and electrophysiological recordings. Chronic fluoxetine decreased the density of 5-HT_4 receptor in the CA1 field of hippocampus (38.3 ± 3.6%) only with the 10 mg/kg dose. However, treatment of rats with both doses of fluoxetine resulted in an attenuation of 10 μM zacopride-induced stimulation of population spike in the pyramidal cells of CA1 of hippocampus (% red = 57.3 ± 8.1% and 41.4 ± 6.4%, for 5 and 10 mg/kg, respectively). In conclusion, these results support the concept that a net decrease in the signalization pathway of 5-HT_4 receptors occurs after chronic fluoxetine, this effect being already evident at relatively low dose. They also suggest that the interaction with these receptors could be of relevance in the mediation of the clinical effects of the drug.

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P147

Pyrimidine analogue 1(4-nitrophenyl)4, 4, 6 trimethyl, (1H, 4H) pyrimidine – 2 thiol: A potential new antiepileptic

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Epilepsy is a disease with significant morbidity. Despite the availability of a large number of antiepileptic drugs, none of the drugs is effective in curing the disease. Pyrimidine derivatives are well established agents with a wide range of therapeutic uses. Most of the pyrimidine compounds show some central nervous system activity. 1(4- nitro phenyl) 4, 4, 6 trimethyl, (1H, 4H) pyrimidine – 2 thiol, is a new pyrimidine analogue with structural resemblance to phenobarbitone. It was studied at different dose levels (10, 20, 40 and 80 mg/kg body weight) for its effects on MES (maximal electroshock seizure) and PTZ (pentylene tetrazole) induced convulsions in mice and compared with equivalent doses of phenobarbitone. With the test compound, for PTZ induced convulsions, 20 mg, 40 mg and 80 mg per kg body weight dose produced mean percentage protection of 48.34% ($P < 0.05$), 60% ($P < 0.05$) and 90% ($P < 0.001$). Mean percentage protection for MES seizure was 30%, 41.67%, 42.5% and 81.67% with doses of 10, 20, 40 and 80 mg/kg respectively. Only the 80 mg/kg body weight dose produced statistically significant protection ($P < 0.05$). The mean percentage protection offered by phenobarbitone for MES at doses 10, 20, 40 and 80 mg/kg body weight of mice were 48.34%, 60%, 80% and 98.34% respectively which was statistically significant ($P < 0.05$) at all dose levels. For the PTZ induced convulsions, statistically significant ($P < 0.05$) results were seen at doses 40 mg and 80 mg per kg body weight with mean percentage protection offered being 53.34% and 61.67% respectively. The compound was further tested for its effects on skeletal muscles, cardiac muscle and smooth muscle. Effects on heart rate and cardiac contractility were studied using frog's heart *in situ*. There was no significant change in heart rate and cardiac contractility. There was an increase in skeletal muscle contraction in frog rectus abdominis muscle. However there was a significant decrease in smooth muscle tone and contractile response to spasmogens in rabbit ileum model. This new pyrimidine analogue may thus be potentially tested for its further activities in epilepsy.

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[^{3}H]CGP 54626 binding to $GABA_B$ receptors in the rat brain during cocaine self-administration and withdrawal

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Till now, there has been no reports showing if voluntary (active) administration of cocaine affects $GABA_B$ receptors in the rat brain and whether the changes are due to direct pharmacological actions of cocaine or are related to motivational states dependent on active drug-seeking behavior. In the present study, we used *in vitro* autoradiography to investigate if cocaine self-administration and its withdrawal produces changes in [^{3}H]CGP 54626, a $GABA_B$ receptor antagonist, binding to $GABA_B$ receptors in a variety of brain regions in rats. We used a 'yoked' procedure in which rats were tested simultaneously in groups of three, with only one rat actively self-administering cocaine while the other two received yoked injections of either cocaine or saline. Male Wistar rats (300 g) were trained to intravenously self-administer cocaine (0.5 mg/kg/infusion) Following a stabilized cocaine self-administration, the rats underwent the 10-day extinction (cocaine was replaced by saline). The animals were sacrificed after the last maintenance or withdrawal sessions. We found a significant (ca. 20%) decrease in [^{3}H]CGP 54626 binding in the nucleus accumbens in rats actively and passively administered cocaine. Moreover, only passively administered cocaine produced a decrease in the binding in the frontal and prefrontal cortices, septum and dorsal striatum. Following 10-day withdrawal in a group of rats previously actively self-administering cocaine, a 20–40% decrease in [^{3}H]CGP 54626 binding to $GABA_B$ receptors was found in almost all investigated brain areas, except for the paraventicular thalamic nucleus where a 25% increase was shown. Summarizing, decreases in [^{3}H]CGP 54626 binding to $GABA_B$ receptors during cocaine self-administration are probably related to the pharmacological effects of cocaine, while the reduction in the $GABA_B$ receptor binding following cocaine withdrawal may be linked to neuroadaptive changes due to previous active administration of cocaine.

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Chronic treatment with the acetylcholinesterase inhibitor huprine X (HX) decreases insoluble Abeta1-40 in 3xTg mice

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Deposition of beta-amyloid (Abeta) is an early and crucial event in the pathogenesis of Alzheimer's disease (AD). In addition, it is well-known that acetylcholinesterase (AChE) co-localizes with Abeta deposits in the brains of AD patients and accelerates the assembly of Abeta peptides through the peripheral anionic site of the enzyme. Huprine X (HX), an anticholinesterase hybrid of tacrine and huperzine A, has been shown to have high affinity for AChE as well as agonist activity on muscarinic M1 receptors and nicotinic receptors. Recently, it has also been demonstrated that HX is able to block the peripheral anionic site of AChE, inhibiting the amyloidogenic process. The aim of the present work was to investigate the ability of HX to modify Abeta levels in the brain of 7-month-old 3xTg (PS1M146V, APPSwe and tauP301L) after chronic drug treatment (0.12 μmol/kg; i.p.; 21 days). The effects of huperzine A (HUP A, 0,8 μmol/kg; i.p.; 21 days) were studied under the same experimental conditions. Cortex and hippocampus were dissected, homogenized and centrifuged (100 000 x ***g*** for 1 h at 4 °C). Tris soluble (soluble) Abeta1-40 and Abeta1-42 were obtained from the supernatant, while insoluble forms of Abeta were extracted from the pellet using a guanidinium-HCl (5 M) solution. The levels of Abeta peptides were analyzed by using comercial sandwich ELISA kits (BioSource). The regional distribution of Abeta in the brain of the mice was also analyzed by immunohistochemistry using the 6E10 antibody. Our preliminary findings show that the hippocampus exhibits the highest levels of Abeta whereas cortical Abeta was below detection levels. In the hippocampus, the levels of Abeta1-40 were in the picomolar range while the levels of Abeta1-42 were around the femtomolar range. Treatment with HX significantly decreased the levels of insoluble Abeta1-40 (40%, $P < 0.05$) in the hippocampus of the 3xTg mice, whereas no changes could be found after HUP A treatment. Moreover, the levels of soluble and insoluble Abeta1-42 were not significantly altered after neither HX nor HUP A treatment. These findings suggest that effects of HX on cholinergic receptors and on the peripheral site of AChE could be involved in the decrease of insoluble Abeta1-40 observed after drug treatment.

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Neuroprotective action of mildronate in anti-HIV drug-induced toxicity models *in vivo*

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Nowadays a rational therapy of neurodegenerative diseases is suggested to be focused on reducing both the neuroinflammatory and the apoptotic events. In this context, we investigate mildronate (MIL), a [3-(2,2,2-trimethylhydrasine) propionate dihydrate], which previously was found to act as anti-inflammatory agent in azidothymidine (AZT)-cardiotoxicity model in mice (Klusa *et al.* 2006); it also was capable of reducing AZT-induced mitochondrial dysfunction (Pupure *et al.* LR Patent, 2007). In the present study, AZT and stavudine (STA) were used as model-compounds (both at 50 mg/kg ip). In male mice, MIL (50, 100 and 200 mg/kg ip) *per se* and MIL + AZT were administered for 2 weeks; histopatologic changes and caspase-3 expression were examined in the brain cortical tissue. In male rats, MIL (100 mg/kg ip) was injected daily for 4 weeks after STA administration for 5 weeks. The ischiadic nerve and myelin tissue were examined histologically and immunohistochemically (anti-myeline antibodies). Statistics: the data were calculated as mean ± SEM and significance evaluated at $P < 0.05$ (unpaired *t*-test or Mann–Whitney *U* test). The results: i) In mice brain cortical tissue, MIL considerably reduced AZT-induced glyosis, focal apoptosis and caspase-3 activity. ii) In rats' schiatic nerve tissue, MIL completely normalised the STA-induced increase (by 80%) in the number of degenerative cells, and decrease (by 30%) in myelin expression. The data obtained show a markedly expressed neuroprotective and anti-neuroinflammatory actions of MIL. These activities can be explained at least in part by MIL mitochondria-protecting action, indicating its beneficial effectiveness also in other mitochondrial pathologies (e.g. Parkinson's disease, diabetes).

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Experimentally-induced diabetes attenuates CCAAT-enhancer binding protein levels in peripheral and central nervous systems

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CCAAT-enhancer binding protein (C/EBP) is one of the transcription factors and has a role in regulation of inflammation (Poli, 1998). It is known that C/EBP activates variety of genes that are related with acute phase of inflammation and immune response. The aim of our study was to investigate the effects of short- and long-terms of experimental diabetes on C/EBP in peripheral (sciatic nerve) and central (hippocampus) nervous systems. For this purpose male Sprague-Dawley rats (300–320 g) were injected with streptozotocin (45 mg/kg) to induce diabetes. Age-matched non-diabetic rats were used as control. At the end of 6- and 12-weeks, sciatic nerves and hippocampus tissues were isolated after the rats were sacrificed. Tissue homogenates were prepared and evaluated for C/EBP proteins by Western blot. Student's *t* test was used for statistical analysis and $P < 0.05$ was considered significant. C/EBP levels did not alter in 6-weeks but decreased significantly in both sciatic nerve and hippocampal extracts of 12-weeks experimentally-diabetic rats ($P < 0.05$, $n = 4$). In conclusion long-term experimental diabetes attenuated the levels of C/EBP both in peripheral and central nervous systems. According to these results it could be suggested that C/EBP may have a role in diabetic complications. This study is supported by Novartis.

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Age related changes in glial fibrilary acidic protein expression in cortex of genetic absence epilepsy rats from Strasbourg

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Genetic absence epilepsy rat from Strasbourg (GAERS) is a well validated genetic model of absence epilepsy. In GAERS spike-and-wave discharges (SWD) first appear in the EEG at around the 30th postnatal day (PN30) and mature gradually with age. GAERS shows a resistance to kindling development which is in parallel to SWDs maturation. In this study we investigated age related expression of glial fibrilary acidic protein (GFAP), a marker for astrocyte activity, in the cortex of Wistar rats and GAERS to evaluate astrocytic reaction in relation to the development of SWD. Young (PN30 and PN 60) and adult (4 months-old) male Wistar rats and GAERS were used in the experiments for GFAP immunostaining. The brains were removed after perfusion and brain slices (20 μm) were prepared. The sections were immunostained using the anti-GFAP. Peroxidase activity was visualized by incubation with 0.03% 3-3-diaminobenzidine and 0.003% hydrogen peroxide in PBS. There was an increase in GFAP staining of cortex in GAERS compared to Wistar animals; however there was not a clear difference in astrocytic reactivity between young and adult GAERS. The increased GFAP staining in GAERS suggests that astrocytic reactivity is related with the development of SWDs. However this relation needs further investigation to understand whether it is the cause or the result of SWDs.

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Neuropeptide VIP attenuated aromatase expression in cholesterol-deprived astrocytes

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Aromatase is a key enzyme in the conversion of androgens to estrogens and has an important role in maintaining a homeostatic balance and neuroprotection (Negri-Cesi *et al.* 1996). Recently, it has been shown that aromatase expression in hippocampal astrocytes was enhanced after serum (FBS - ; main cholesterol source) deprivation (Azcoitia *et al.* 2003). In this study, Sprague–Dawley rat primary cortical astrocyte cultures were established from cortices of newborn rats (P3). Serum-deprived astrocytes were incubated with neuropeptide vasoactive intestinal polypeptide (VIP; 1 μM). Cellular extracts were subjected to SDS-PAGE and aromatase, ERK and p-ERK were detected by immunoblotting. Blots were measured by Scion Image 4.0.3.2. and data were analyzed by one-way ANOVA and Bonferroni as *post hoc* test for statistical significance at $P < 0.05$. All tests were performed using Prism 3.0. Each experiment of independent groups ($n = 3$) was performed in triplicate. In the extracts of astrocytes exposed to serum-deprived (FBS −) medium, aromatase increased in 6 h compared to the control (FBS +) extracts. With VIP supplementation, increased aromatase level has been attenuated. Similarly, cholesterol-supplementation brought the aromatase protein back to the control level in this cell line ($P < 0.05$; $n = 3$).VIP incubation decreased aromatase levels in the serum-deprived rat cortical astrocytes compared to the extracts from which the cells exposed to serum-deprived medium, only ($P < 0.05$; $n = 3$). Therefore, we concluded that aromatase compensates cell defence where there is a lack of neuropeptides such as VIP. When VIP is supplemented to the media, aromatase expression is attenuated so that, VIP provides cell protection against stress conditions such as serum deprivation. The disturbance of cholesterol homeostasis appears to be an important factor in the pathogenesis of Alzheimer's disease (AD) (Koudinov *et al.* 2002). These findings may have important implications for understanding the possible neuroprotective roles of neuropeptides on aromatase gene in disease states such as AD.

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P154

Head twitches in serotonin transporter (SERT)-deficient mice: 5-HT_{1A} and 5-HT_{2A} receptor interactions

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Alterations in serotonin transporter (SERT) expression are associated with altered 5-HT_{2A}-mediated responses. SERT overexpressing mice show increased head twitches after the 5-$HT_{2A/2C}$ agonist DOI (Jennings *et al.*, 2008), whereas SERT knockout (−/−) mice show an 86% reduction in this response (Qu *et al.*, 2005). 5-HT_{1A} antagonists also induce head twitches, likely via an indirect mechanism (Darmani and Reeves, 1996). Despite decreased 5-HT_{1A} and 5-HT_{2A} expression and function, we recently noted that the selective 5-HT_{1A} antagonist WAY 100635 induced head twitches in SERT −/− mice. Using the head twitch response, we examined 5-HT_{1A} and 5-HT_{2A} receptor interactions in SERT +/+, heterozygous (+/-) and −/− mice (Bengal *et al.*, 1998). WAY 100635 induced significantly more head twitches in SERT −/− mice compared to SERT +/+ mice. DOI induced similar numbers of head twitches in SERT +/+ and +/- mice, with 85% fewer twitches in SERT −/− mice compared to SERT +/+ mice. DOI-induced head twitches were decreased by the 5-HT_{1A} agonist 8-OH-DPAT in SERT +/+ and +/- mice, and increased by WAY 100635 in SERT +/- and −/− mice. All effects were completely blocked by the 5-HT_{2A} antagonist MDL 11,939. In SERT −/− mice, pretreatment with the serotonin synthesis inhibitor *para*-chlorophenylalanine (PCPA) decreased tissue serotonin levels and abolished the head twitch response induced by WAY 100635. These studies show that despite previous reports of decreases in 5-HT_{1A} and 5-HT_{2A} receptor expression and function in SERT −/− mice, an interaction exists between these receptors, one likely associated with elevated extracellular levels of serotonin previously noted in SERT −/− mice (Mathews *et al.*, 2004).

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