P010
Administration of late endothelial progenitor cells enhances cerebral infarct volume 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 transplanted 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 (5 x 10^6 right femoral artery/mouse for 7 days) or control groups. The infarct volume was measured 14 days following MCAO. Seven or 14 days after MCAO, brain infarct volumes, human transplanted cell localization, apoptosis and capillary density were determined. 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). Immunohistochemical 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 7, apoptotic cell number significantly lower in transplanted rats (4.8 ± 2.0 ± 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.

P011
Effect of short-term treatment with a synthetic flavonol on rat coronary arteries: Ischemia reperfusion injury

Wine polyphenols prevent endothelial dysfunction and vascular superoxide production induced by ethanol in rat aorta


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 on rat coronary arteries with or without reperfusion after ischemia.

In vitro, coronary arteries were isolated from male Wistar rats (250-285 g). After 8 weeks of reperfusion, rats were subjected to 30 min of ischemia followed by 24 h of reperfusion. RWPs extract (10^-2 mg/ml, epicatechin, catechin or resveratrol (10^-2 M to 10^-3 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.

P010
The effects of ethanol-treatment on the neurogenic- and endothelium-dependent relaxation of corpus cavernosum smooth muscle in mouse

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Ethanol-treatment abolishes the endothelium-dependent relaxations induced by acetylcholine but failed to alter the relaxation to EFS and NO. L-arginine, a NO synthase inhibitor, inhibited 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-NAME, a NO synthase inhibitor, inhibited relaxations to EFS, NO and acetylcholine in control and ethanol-treated mice. Corpus cavernosum tissue 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 lead erectile dysfunction through reducing NO release via endothelial impairment.

P011
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 the regulator of vascular 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 vasoactivity 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 parallelly in a multi-channel 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 F2α and retinas were placed in close proximity to maintain retinal relaxation. Relaxations were expressed as % of the precontraction. Statistical analyses were determined by Students t-test.

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Antiangiogenic effects of natural compounds from medicinal plants may be due to the presence of flavonoids and other plant products. As such, these compounds, along with their mechanisms of action, are of great interest in the field of angiogenesis research. In this study, we investigated the effects of four natural compounds on angiogenesis: baicalin (from Scutellaria baicalensis), hypericin (from Hypericum perforatum), quercetin (from Quercus spp.), and kaempferol (from Quercus spp.).

The in vitro assay used a human umbilical vein endothelial cell (HUVEC) monolayer to assess the effects of the compounds on cell proliferation and migration. The concentration-dependent inhibition of HUVEC proliferation was observed for all compounds, with baicalin showing the most potent activity. In addition, the effects of these compounds on HUVEC migration were also studied using a wound healing assay. The results indicated that baicalin and hypericin significantly inhibited HUVEC migration in a concentration-dependent manner.

These findings suggest that these natural compounds may have potential therapeutic applications in the prevention and treatment of angiogenesis-related diseases. Further studies are needed to elucidate the mechanisms of action of these compounds and to evaluate their efficacy in vivo.

References:
**P116**

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.

**P117**

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 indomethacin (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.
**P118**

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 antiapoptotic potential via its free radical scavenging. Previously, we have harvested cis-beta-carotene from Dunaliella salina and tested its antioxidant property in vitro. In the present study, we aim to investigate whether cis-beta-carotene could protect murine thymocytes against 60Co 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 60Co 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. 60Co 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 µM 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.

**P119**

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 effects on the immune system, and has been transplanted in patients with autoimmune diseases. FTY720-induced apoptosis of Jurkat cells was confirmed by flow cytometry. 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 microarray. 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.

**P120**

Immunostimulatory activity of sarco/endoplasmic reticulum Ca2+-ATPases inhibitors, thapsigargin and trilobolide

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The maintenance of Ca2+ homeostasis within the cell is tightly regulated by a family of sarco/endoplasmic reticulum (SR) Ca2+-ATPases (SERCA). Inhibition of SERCA pumps leads to a release of Ca2+ from ER stores and a concomitant influx of Ca2+ extracellular space, resulting in elevation of cytosolic Ca2+ concentration. Changes in intracellular free Ca2+ 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 Ca2+-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 kinase p38 and ERK1/2.

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**P121**

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 ovarioectomy. The metabolic syndrome was induced with extra ambilateral ovarioectomy, endotoxin concentration, phagocyte and metabolic neutrophil activity. We showed that ovarioectomy was accompanied by the increase of phagocyte and decrease of metabolic and consumption activity of neutrophils (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 ovarioecrotic 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 melitin and genistein produces stimulative influences on the metabolic and consumption activity of neutrophils (P < 0.05). Melitin, genistein and 17β-estradiol administration caused normalization of phagocyte cell quantity (P < 0.05). Fensuccinal decreases that index and also the consumption activity of phagocytes (P < 0.05). The drugs failed to completely compensate the humoral chain of immunity.
P122

Attenuation of mechanical hyperalgesia in streptozocin-induced diabetic neuropathy model in rats by cerebrocrast, a mitochondria-targeted drug. L Klimavicius, D Grünvalde, J Ruņmaks, N Karavega University of Latvia, Riga, Latvia
Our hypothesis is that attenuation of diabetic neuropathy (DN) can be achieved by using mitochondria-targeted drugs. For this purpose, cerebrocrast (Cer), a novel type of atypical (non-calcium antagonist) 1,4-dihydropyridine, previously shown as mitochondria-regulating neuroprotective compound (Ye 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 algometer (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 a cerebrocrast + STZ rats was comparable with that of control rats and maintained at control level during all the experiment. However Cer did not influence glycaemia and lack of weight gain caused by STZ. The obtained data showed for the first time that atypical (non-calcium antagonist) 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 compromised mitochondria functions.

P123

The effects of water-soluble chitosan on the fracture healing in New Zealand rabbits. C Yan, B Ding, J Li, C Wang Medical College of Qinghua University, Qinghua, China
The effects of water-soluble chitosan on the fracture healing, sixty healthy 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), bescemium 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 (0.25 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 of bone suture. The mature bone morphogenetic protein (BMP) and the biochemical indexes related to fracture healing were measured. The X-ray and HE stain showed that the fracture healing in the water-soluble chitosan group was comparable with 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 was significantly higher than that of other groups (P < 0.05), the calcium concentration in serum of the water-soluble group was clearly lower than that of control groups at the seventeenth day (P < 0.05). Our results demonstrated that water-soluble chitosan can promote fracture 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 cytototoxic activities of crude extracts from the Mediterranean marine alage and sponge. A Barraquio, W Chausuch, A Choledov, F Farhat, A Dellai, M Dedi, I Mhamed et al. Unite Usage, Lab. de Pharmacologie, Faculté de Pharmacie de Monastir, Monastir, Tunisia
As part of our search for new anti-inflammatory or anticancer potential drugs, aqueous extracts of macro-algae and invertebrates collected from Mediterranean Tunisian coasts were evaluated for their anti-inflammatory and cytototoxic activities. The present study has established that the aqueous extract from the brown alga of the genus Zenaria tested at different doses (50, 100, 200 mg/kg) for their anti-inflammatory activity, using the carragenan paw edema test (Winter et al., 1962), in male albino 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 edema (P < 0.001). The % inhibition of dexamethasone (0.25 ml/kg) at 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 of bone suture. The mature bone morphogenetic protein (BMP) and the biochemical indexes related to fracture healing were measured. The X-ray and HE stain showed that the fracture healing in the water-soluble chitosan group was comparable with 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 was significantly higher than that of other groups (P < 0.05), the calcium concentration in serum of the water-soluble group was clearly lower than that of control groups at the seventeenth day (P < 0.05). Our results demonstrated that water-soluble chitosan can promote fracture 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.

P125

Pharmacological evaluation of the anti-inflammatory and cytototoxic activities of extracts from the Mediterranean sponge, Petrosia ficiformis. A Dellai, A Dellai, M Mhaddibi, F Farhat, ZB Aouaz, A Bourouis Unite Usage, Lab. de Pharmacologie, Faculté de Pharmacie de Monastir, Monastir, Tunisia
As part of our search for anti-inflammatory substances from marine sponges, the anti-inflammatory and cytototoxic activities of the aqueous extract from the Mediterranean sponge, Petrosia ficiformis (Tognolini, 1985) was investigated in carragenan-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 extract (100, 250, 500 mg/kg ip) was administered intraperitoneally, and in comparison to reference drugs: Dexamethasone (1 mg/kg) and acetylsalicylic acid (200 mg/kg), exhibited, in a dose dependent manner, a significant inhibitory effect on the rats paw oedema (P < 0.001). The % inhibition of dexamethasone (0.25 ml/kg) at 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 of bone suture. The mature bone morphogenetic protein (BMP) and the biochemical indexes related to fracture healing were measured. The X-ray and HE stain showed that the fracture healing in the water-soluble chitosan group was comparable with 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 was significantly higher than that of other groups (P < 0.05), the calcium concentration in serum of the water-soluble group was clearly lower than that of control groups at the seventeenth day (P < 0.05). Our results demonstrated that water-soluble chitosan can promote fracture 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.

P126

Investigation on the anti-inflammatory properties of Oceota oxues Lam. extracts. V Ballabeni, M Tognolini, R Bruni, S Bertoni, G Girogii, E Barocelli Department Science Farmacologiche, Biologiche e Cliniche Applicate - University of Parma, Italy
P127

The role of inflammation and COX derived prostanooids in the effects of bradykinin on isolated rat aortic ring. B2 Sirmagal, FS Kilic, S Celebi, AE Dogan Eskişehir Osmangazi University, Eskişehir, Turkey
Vascular tone regulation is a complex process controlled by a variety of factors, including neurotransmitters, hormones, growth factors, cytokines, and inflammatory mediators. In the present study, we investigated the contributions of different COX-derived prostanooids and their signaling pathways to the 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 (10−7 to 10−5 M) were studied on isolated rat aorta rings contracted with
norphine (10\(^{-7}\) M) following incubation with dipyrone (100, 700 and 2000 µg). The relaxant responses of dipyrone (100, 700 and 2000 µg) 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) previously described elsewhere. Con A and LPS were all from Sigma, UK. Both the plasma and the perfusate of the aorta preparations (n = 5). The vasodilatory activity of bradykinin and des-Arg\(^{9}\)-bradykinin were significantly increased in both the plasma and the perfusate of the inflamed group. PGE\(_2\), PGF\(_1\) \(_2\), TRPV\(_{8}\) were all from Sigma, UK. These effects were blocked in the inflamed group. PGE\(_2\), PGF\(_1\) \(_2\), TRPV\(_{8}\) were all significantly high but NO\(_{2}\) activity was low in the aorta perfuse after incubation of COX-1. 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.

The study was supported by Novartis.

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 evidence as to whether intra-articular opioid receptors are present in the periphery during a FCA-induced model of inflammatory arthritis in the rat. Li et al (2005) observed a loss of antinociception of the α-opioid agonist endomorphine in a 13 day healing of the knee joint, with a differing report suggesting an opioid mediated attenuation of neural discharge evoked by noxious von Frey filaments (Strickland et al, 2007). The aim of this 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 arthropathy was induced in the left knee of adult male Wistar rats (n = 32), 90 g by 150 µl intra-articular injection of FCA (0.5 µg/ml; Sigma, UK). Rats were transiently anaesthetised using 5% isoflurane in oxygen and FCA was injected into the knee joint using a 25g 1/2 inch needle. Animals received the further intra-articular injection (100 µl) of 0.1, 0.3, 1 mg 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 (WDF) of the animal was measured as a function of the 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 mixed ANOVA, where P < 0.05 was considered significant. Results showed that the group of animals injected with 0.3 mg of morphine had significantly higher (P < 0.05) WD ratios in the time course following morphine injection, 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-injury, and novel opioids that can be restricted to the periphery may provide analgesia at the site of inflammation whilst avoiding the commonly cited, centrally mediated, undesirable side-effects of current opioid treatments.

References:


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: (I) impiramine 10 mg/kg/day Group III: imipramine 10 mg/kg/day Group IV: fluoxetine 10 mg/kg; Group V (FVM): fluvoxamine 50 mg/kg; Group VI (VLM): venlafaxin 25 mg/kg; Group VII (RBX): reboxetin 25 mg/kg. Morphine (1 µg/50 µl) was administered subcutaneously as a 0.1 mL injection, with a known analgesic effect on this visceral pain model. The model of visceral pain consists of inflammatory cystitis after intraperitoneal injection of cyclophosphamide (50 mg/kg). The body weight of all animals were statistically analyzed with the software of P < 0.05 of 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 venlafaxin, clomipramine while clomipramine also caused diminution of behavioural score in mice with inflammatory cystitis but this was insignificant compared with the control group. In our experimental conditions, serotonin re-uptake inhibitors have shown antinociceptive effect in the manifestation antinociceptive effect in different time intervals. except Fluoxetine. The analgesic effects of these substances were less intense than those of morphine. Imipramine administered in different concentrations determine the most intense antinociceptive effect in this experimental study. Imipramine administration, resulted in a significant antinociceptive effect in cyclophosphamide cystitis (P < 0.05), in all time intervals of the experiment.

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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 opiateergic, noradrenergic and serotonergic mechanisms. Although ongoing studies show that serotonin and noradrenaline are also involved in the effect of tramadol, 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 inhibitory effects on the inflammatory and anti-inflammatory pain. The present study was to investigate the possible effects of tramadol and agmatine-tramadol combination on nociception by using the tail-flick test in mice. The results indicated that tramadol decreased the mechanism of the potentiation effect of agmatine on tramadol-induced antinociception by pretreatment with nitric oxide modulators such as L-arginine and L-NAMe, which demonstrated that tramadol significantly increased the tail-flick latency and tail-flick response. 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 test was used for the statistical analysis. Treatment 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 tramadol + tramadol combination. MK-801 pretreatment did not change the TFL of tramadol, tramadol-induced 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 nitricergic 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 neurons

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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 is beneficial in a wide variety of conditions and may involve monoaminergic/opioid pathways to both the dorsal horn. Nevertheless, spinal actions of antidepressants may also contribute to their analgesic effects. In this study, we have evaluated the spinal and comparator effects of an antidepressant (a serotonin and noradrenergic/ opioid antagonist), venlafaxine (noradrenaline/retron serotonin re-uptake inhibitor) and fluoxetine (selective serotonin re-uptake inhibitor) upon the responses of dorsal horn neurons in mice to mechanical and chemical inputs. We also evaluated the possible effects of agmatine and agmatine-tramadol combination on nociception by using tail-flick method in mice. The results indicated that tramadol increased the antiallodynic and antihyperalgesic effects in neurophatic pain and to enhance the nociception by tramadol. L-arginine and L-NAMe are also important role in the bradykinin induced rat aortic smooth muscle contractions. The study was supported by Novartis.
(0.03 or 1 μl) 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.

<table>
<thead>
<tr>
<th></th>
<th>Control (μM)</th>
<th>Control (100%)</th>
<th>P00805 (10 μM)</th>
<th>P00601 (10 μM)</th>
<th>PP2 (1 μM)</th>
<th>PP2 (0.03 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSMCs (PKX65)</td>
<td>18 ± 3</td>
<td>100</td>
<td>36 ± 1 ±*</td>
<td>103 ± 2</td>
<td>3.2 ± 3.2</td>
<td>52.3 ± 8.8</td>
</tr>
<tr>
<td>Macrophages (S5)</td>
<td>38 ± 2 ±5</td>
<td>100</td>
<td>32 ± 2.1 ±*</td>
<td>85 ± 5</td>
<td>7.3 ± 9 ±*</td>
<td>89 ± 5 ±*</td>
</tr>
</tbody>
</table>

Effect of signalling pathway inhibitors in the induction of NOSII activity by TLR4 and NOD1 activation in VSMCs vs. macrophages. Results were analyzed using one sample and Students T-test. * and ** denote P < 0.05 vs. control of 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 IC50 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 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 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 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 50–100-fold lower potency at rat B1 receptors. SPRD rats and NZ rabbits were treated with 250 ± 25 g. Hippocampi were isolated and sliced into 0.6 µm slices. Slices were oxidised at incubation system with a mixture of 95% O2 and 5% CO2 at 37 °C. Our material (gabapentin 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 antinociceptive activity of G, except for opioidergic system, nitricergic and serotonergic systems have an important role and they affect nNOS and PGE.

This study was supported by Osmangazi University Research Foundation.

**P135**

**Effects of gabapentin on secretion of nNOS and PGE2 from rat hippocampus slices**

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It is desirable to show that antiepileptic 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 PGE2 from rat hippocampus slices are investigated. All experiments for animal testing were approved by the Eskişehir Osmaniye 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 µm slices at rat brain. These slices are oxidised at incubation system with a mixture of 95% O2 and 5% CO2 at 37 °C. Our material (gabapentin 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 antinociceptive activity of G, except for opioidergic system, nitricergic and serotonergic systems have an important role and they affect nNOS and PGE.

This study was supported by Osmangazi University Research Foundation.
Glucocorticoids are a cause for memory deficit induced by naloxone precipitated morphine withdrawal in rats
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Morphine withdrawal leads to an increase in corticosterone concentration in plasma (Zelená et al. 2005), and cognitive deficits are found, after withdrawal. Since previous studies have shown that glucocorticoid hormones could affect memory, the aim of the present study was to investigate the role of corticosterone withdrawal on memory deficit following naloxone precipitated morphine withdrawal. Male NMRI mice weighing 25–30 g were made dependent by increased doses of morphine (30 mg/kg i.p.) for 10 days. Male daily for 30 min before injection of naloxone (0.1 mg/kg) 5 h after last morphine injection. Mifepristone (50 and 100 mg/kg, i.p.) and Melanin-concentrating hormone (MCH 1 antagonist, 4 lL of compound A, results in any changes in electroencephalogram (EEG) in 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, mifepristone, 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:

Memantine ameliorates the amyloid-beta 1-42 peptide-induced decrease in [1H]dopamine release in rat corticostriatal slices
Z. Juranyi1,2,3, B. Hofmann1,2, K. Sunaga4, E. Torres1, E. Colado1, M. Izco1, N. Llopis1, I. Peraile1, A. Mayado1, E. O'Shea2, M. Colado1, E. O'Shea1

Dpto. Farmacología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain

3-Methylendioxymethamphetamine (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 inter- and intra-individual variability (P136). We aimed to study if exposure to repeated low dose MDMA protects against a subsequent neurotoxic dose of MDMA and decreases IL-1 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 5-HT nerve terminals labelled 5-HTT with the membrane staining and a screen as a target biomarker for central occupancy of small molecular MCH1 antagonist. Despite high central MCH1, we saw no significant changes in EEG parameters and conclude that EEG may not be a suitable translatable biomarker for central MCH1.

References:

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

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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 ethanol (EIHO), and are resistant to the development of rapid tolerance to hypothermia (Marchant et al. 2005). GABAergic transmission plays a relevant role in the behavioral effects of EIHO. In fact, acute EIHO reduces GABAturnover (Hellevuo & Kiasmäki, 2002) and reduces neuronal activity by the activation of GABA A receptors in mouse brain (Cal et al. 2006). We have now examined GABA turnover in discrete brain areas of mice exposed to a neurotoxic dose of MDMA and then challenged with EIHO. Adult male C57BL/6J mice (25–30 g) were injected i.p. 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.) 1 h before EIHO (1 ml/kg, i.p.). GABA turnover was determined in the striatum, hippocampus and frontal cortex 1 h after administration of GABA-transaminase (GABA-T) by AOAA. To prevent non-specific postmortem synthesis of GABA, 3-mercaptoacrylic acid (100 mg/kg, i.p.) was injected 3 min before decapitation. GABA was measured by h.p.l.c. using a precolumn o-phthaldehydesulfite derivatization method. Seven days after MDMA, we observed 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 EIHO sensitized sham-operated control mice to the sedative effect of ethanol, no effect was observed in the MDMA-treated mice. In hippocampus and frontal cortex EIHO reduced GABA concentration in both saline and MDMA-injected mice. This may contribute to the lower sensitivity to the EIHO-induced sedative effect and the resistance to the development of rapid tolerance to hypothermia that are evident 7 days after drug injection in the...
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Cocaine withdrawal internalizes DAT in the endosome and protects MDMA-induced dopaminergic toxicity in mouse striatum


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

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

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

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In vitro treatment with fluoxetine induces desensitization of 5-HT4 receptors in rat hippocampus

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P141

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


**References:**


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**P142**

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

MR García, R Gimenez-Lloret, P Camps, D Muñoz-Torrellas, Mª Victoria Clos Guillen, A Badia Sancho "Pharmacology, Therapeutics and Toxicology Department, Neuroscience Institute, Autonomous University of Barcelona (UAB), Barcelona, Catalonia, Spain; "Pharmacological Chemistry Laboratory (Unite associated to CSIC), Pharmacy Faculty and Biomedical Research Institute (IREB), Barcelona University (UB), Barcelona, Catalonia, Spain." Cognitive enhancing properties and putative adverse effects of huprineX (β-12-amino-3-CL-tobiiol-ethyl-6,7,10-11-tetralydro-7,11-methano cycloaiclglycinoline 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 PKC, 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 showed that the pharmacological treatment increased 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 in both phospho-PKC and phospho-α4-2/4 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 dementia caused by cholinergic dysfunction.

**References:**

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**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. It has been suggested that Nrf2, a nuclear factor that is involved in the transcriptional binding 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 and its phosphorylation were determined by Western blotting and immunoprecipitation. Nrf2 levels were manipulated in HEK cells using small interfering RNA (siRNA) against Nrf2 or Keap1 transcripts, and chemical treatments were used to determine the effect of Western blotting and a luciferase-reporter gene assay for Nrf2-mediated gene transcription. Oxidative stress was induced using 6-hydroxydopamine and cyto toxicity was determined using没ev producers. The increase in Nrf2 phosphorylation and its mediated gene transcription, siRNA against Nrf2 transcripts also showed knock down at the protein level and subsequent transcriptional activity. The results demonstrated a significant increase in Nrf2 protein and mRNA levels and these results showed that the Nrf2-Keap1 pathway could be a potential therapeutic target in preventing further neurodegeneration caused by oxidative stress in Parkinson’s disease.

**References:**

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**P144**

**Effects of sertraline on experimental models of psychosis in mice**

E Pani, E Bilge Eskekizer Osmangazi University, Eskisehir, Turkey

Recently, it has been reported that treating negative symptoms of schizophrenia with a combination of a typical antipsychotic and a selective serotonin reuptake inhibitor may have beneficial effects to treatment of negative symptoms (Chekowky et al. 2007). The present study was designed to study the effects of sertraline on neuroleptic-induced catalepsy: apomorphine-induced climbing behaviour and amphetamine-induced stereotypy in male Swiss mice. Male Swiss 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 (10 mg/kg, s.c.) 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:**


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**P145**

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

J Pincas-Bravo, A Rodriguez-Gastelumendi, I Ruiz, F Pilar-Cuellar, A Pauzin, P Departamento de Fisiología y Farmacología and Instituto de Biomedicina y Biotecnología de Cantabria IBTREC (Universidad de Cantabria-CSIC-IDICIN); CIBER-SAM. Instituto de Salud Carlos III, Santander, Cantabria, Spain

Bilateral olfactory bulbectomy (OB) is a validated animal model of depression showing behavioural, neurochemical and structural features similar to what has been 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, as a putative new target for antidepressant development. 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 reuptake inhibitor (SSRI) fluoxetine (110 mg/kg/day, 14 days; s.c). Male (n = 30) and female (n = 30) adult Sprague-Dawley rats 12 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 an avidin-peroxidase technique. The number of β-catenin immunopositive cells increased (15 ± 2%) in subgranular zone of hippocampal dentate gyrus (SGZ) of OB + vehicle group (P < 0.01 vs. sham-operated + vehicle) and OB + fluoxetine animals (P < 0.05 vs. OB + vehicle). These results suggest the involvement of this proliferative pathway in the mechanism of action of fluoxetine that might represent a putative new target for antidepressant development. Supported by Fundación Alicia Koplowitz, Fundación de Investigación Médica Mutua Madrileña and Ministerio de Educación y Ciencia (SAF-07/61862).

**References:**

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**P146**

**In vitro treatment with fluoxetine induces desensitization of 5-HT4 receptors in rat hippocampus**

B Vidal, A Martin, R Mostany, A Pazos, A Castro Departamento de Fisiología y Farmacología and Instituto de Biomedicina y Biotecnología de Cantabria IBTREC (Universidad de Cantabria-CSIC-IDICIN); CIBER-SAM. Instituto de Salud Carlos III, Santander, Cantabria, Spain

Fluoxetine, a 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 serotoninergic systems. The 5-HT4 receptors are G-protein coupled receptors which are involved in the control of several neurotransmitter systems such as GABAergic, dopaminergic, serotonergic and noradrenergic neurotransmission. 5-HT4 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 SSRIs fluoxetine (5 and 10 mg/kg/day p.o.) in both density and functionality of 5-HT3 receptors in rat hippocampus using receptor autoradiography ([125I]I-R13808) and electrophysiological recordings. Chronic fluoxetine decreased the density of 5-HT3 receptors in the CA1 field of hippocampus (18.3 ± 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 acetylcholine-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-HT3 receptors occurs after chronic fluoxetine, this effect being already evident at relatively low doses. They also suggest that the interaction with these receptors could be of relevance in the mediation of the clinical effects of the drug.

Supported Ministerio de Educación y Ciencia (SAF04-00941 and SAF07-61862) and Fundación Alicja Kowolowitz.

P148

Pyrimidine analogue (14-nitrophenyl): 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. We have prepared a 4-6 trimethyl (1H, 4H) pyrimidine – 2 thiol, a new pyrimidine analogue with structural resemblance to phenobarbital. 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. The test compound, for PTZ induced convulsions, 20 mg, 40 mg and 80 mg per kg body weight dose produced mean percentage protection of 48.54% (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%, 70% and 90% 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 observed 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.

P147

Pyrimidine analogue (14-nitrophenyl): 4-6 trimethyl (1H, 4H) pyrimidine – 2 thiol: A potential new antiepileptic

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P149

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 3-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 extracted from the pellet using a guanidinium-HCl (5 M) solution. The levels of Abeta peptides were analyzed by using commercial 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 aporetic events. In this context, we investigate mildronate (MIL), a 3-(2,2,3-trimethylhydroxiane) proprionate dihydrate), which previously was found to act as anti-inflammatory agent in azothymidine (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; histopathologic 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 ischemic nerve and myelin tissue were examined histologically and immunohistochemically (anti-myelin 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 glycosis, focal apoptosis and caspase-3 activity. ii) In rats' sciatic 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 mitochonrdia-protecting action, indicating its beneficial effectivity also in other mitochondrial pathologies (e.g. Parkinson’s disease diabetes).

Neuroprotective peptide V155 attenuated aromatase expression in cholesterol-deprived astrocytes

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CCAAT-enhancer binding protein (C/EBP) is one of the transcription factors and has a role in regulation of inflammation (Poll, 1998). It is known that C/EBP activates variety of genes that are related with acute phase of inflammation and immunity. 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 (100–120 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 hippocampal 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.

References:

Age related changes in glial fibrillary 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 fibrillary 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 (PN10 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.

Neuropeptide V153 attenuated aromatase expression in cholesterol-deprived astrocytes

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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