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SCINDERIN, ITS MOLECULAR BIOLOGY AND ITS ROLE IN NEUROSECRETION AND LEUKEMIA.

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Scinderin (Sc), a Ca^{2+}-dependent F-actin severing protein was discovered in our lab. Sc gene (SCN) was also cloned in our lab. Sc has six domains with three actin-binding sites in domains 1, 2 and 5, two PIP_2 in domains 1 and 2 and two Ca^{2+} binding sites.The roles of Sc in chromaffin cells(CC) and in megakayoblastic leukemia (MKL) have been studied. CC cortical F-actin disassembly in response to stimulation allows the movement of secretory vesicles towards exocytotic sites . Recombinant Sc and Sc antisence oligonucleotides demonstrated that Sc controls cortical actin networks and exocytosis. Expression of Sc domains indicates that Sc acts as a molecular switch in the control of secretion. MKL cells show the absence of Sc expression, a protein present in normal megakaryocytes (MK) and platelets (Ptl). Sc expression in MKL (MEG-01) cells was followed by activation of specific transduction pathways leading to maturation, differentiation and apoptosis with release of Ptl-like particles. MKL cell ability to form tumors in nude mice was also inhibited by Sc re-expression. Sc promoter (Sc-Pro) has recently been characterized in our lab . Sc-Pro has several AP2 and 4 Dioxin Responsive Element (DRE) sites that recognize the Aryl Hydrocarbon Receptor (AhR).
Our experiments show: a) that in the Luciferase-Sc-Pro construct assay,Sc-Pro is stimulated by either ATRA (All-trans retinoic acid) or TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin; a ligand for the AhR), b) the presence of AhR in MEG-01 and CC cells, and c) that stimulation of MEG-01 cells by either ATRA or TCDD increased transcription and expression of Sc followed by maturation. In CC, Sc-Pro stimulation increases Sc expression and depolarization -induced cortical actin disassembly and exocytosis . It is concluded that in neurosecretory cells, Sc controls the availability at release sites of secretory vesicles and that in MKL cells, the lack of Sc expression seems to be responsible for their inability to enter into differentiation and maturation pathways characteristic of their normal counterparts.

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BIOLOGY OF THE TAM RECEPTORS - TYRO 3, AXL, AND MER

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Our laboratory has defined and analyzed an unusual set of three receptor protein-tyrosine kinases (PTKs) - those of the TAM family - which have appeared relatively late in metazoan evolution, and which are preferentially expressed in the mature immune, nervous, and reproductive systems (Lai and Lemke, 1991). These three receptors - Tyro 3, Axl, and Mer - together with their two ligands - Gas6 and Protein S - are remarkable in several respects. Genetic studies in our lab indicate that none of the TAM receptors plays an essential, or even detectable, role in the embryonic development of these organ systems. In this respect, these receptors are unique. Instead, our studies suggest that TAM receptors function only in established tissues. Mice carrying mutations in the genes encoding these receptors exhibit a spectrum of severe phenotypes in the nervous, immune, and reproductive systems (Lu *et al.*, 1999; Lu and Lemke, 2001; Lemke and Lu, 2003). But all of these phenotypes - which include photoreceptor death and retinal degeneration, lymphoproliferation, and the development of broad-spectrum autoimmunity - develop after birth, and appear to be either degenerative, or to reflect dysregulation of homeostasis within ensembles of interacting cells.

I will describe the basic features of this signaling system, and will present recent results on the role of Tyro 3, Axl, and Mer in the function of retinal pigment epithelial cells in the retina. I will also detail recent discoveries with respect to the role that these receptors play in regulating the activity of antigen-presenting cells - macrophages and dendritic cells - in the immune system.

Lai, C. and Lemke, G. (1991) An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. *Neuron* 6: 691-704.

Lu, Q. *et al.* (1999) Tyro 3 family receptors are essential regulators of mammalian spermatogenesis. *Nature* 398: 723-728.

Lu, Q. and Lemke, G. (2001) Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science* 293: 306-311.

Lemke, G. and Lu, Q. (2003) Macrophage regulation by Tyro 3 family receptors. *Curr. Opin. Immunol.* 15: 31-36

O1

PHARMACOKINETICS OF AMOXICILLIN IN ADULT LLAMAS (*Lama glama*)

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The purpose of this study was to characterized the pharmacokinetic of AMX after intravenous (IV), intramuscular (IM) and subcutaneous (SC) administration in adult llamas. Six female llamas (110,17 ± 25,17 Kg) recieved AMX (sodium salt, 20 mg/kg) by each route with two weeks washout period. Serial venous blood samples were taken at predeterminated times after drug administration. AMX plasma concentration were determined by microbiological assay, using *Bacillus subtilis* ATCC 6633 as test microorganism. Plasma disposition curves were analized using Topfit software. After IM and SC route AMX was totally avaible with an F value around 150% and 112%, respectively. C_{max} for IM administration was 40.38 ± 12.08 μg/ml. and T_{max} 0.30 ± 0.22 h. After SC administration Cmax was significant lower 12.51± 5.37 μg/ml and Tmax later 0.78 ± 0.42 h, but serum concentration stayed longer (MRTsc 3.21 ± 1.71 h) than for IM route (MRTim 1.37 ± 0.51 h). Total body clearence and volumen of distribution for the IV route were 9.07 ± 2.12 ml/h and 0.73 ± 0.19, respectively. Terminal half-life was 0.94 ± 0.13 h, 0.86 ± 0.28 h, 2.23 ± 1.18 h for the IV, IM, SC routes, respectively. Mean residence time for the IV route was 1.07 ± 0.30 h. These results show differences in some AMX pharmacokinetic

O2

PHARMACOKINETIC OF INTRAVENOUS CEPHALOTHIN IN CATS

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Introduction: Cephalothin (CFL) is a first generation cephalosporin used for surgical prophylaxis of infections. It has been widely used and studied in human beings as well as in some domestic animals. However, its pharmacokinetics behavior has not been characterized in cats. The aim of this study was to analyze the serum disposition of CFL after its intravenous (IV) administration to domestic cats.

Materials and Methods: 9 adult cats weighted 4.36 ± 0.98 kg received CFL 30 mg/kg by IV route. Blood samples were withdrawn at pre-determined times over a 6 h period. CFL serum concentrations were determined by microbiological assay using *Bacillus subtilis* (ATCC 6633) as test micro-organism. Plasma disposition curves were analyzed by non linear methods using WinNonlin software.

Results: After CFL IV administration, the maximum serum concentration ($C_{(0)}$) was 353.79±118.92 μg/ml with an area under concentration-time courve ($AUC_{(0-\infty)}$) of 187.83±37.45 μg.h/mL. CFL distribution was rapid ($t_{½(d)}$ 0.14±0.10 h) with a volume ($V_{(d(ss))}$) for this process of 0.19±0.03L/kg. Elimination half-life ($t_{½}$) was 1.07±0.23 h, with a mean residence time (MRT) of 1.19±0.20 h and a body clearance (Cl_B) of 0.17±0.03

O3.

Effect of stimulation of the subthalamic nucleus on extracellular concentration of striatal dopamine

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The subthalamic nucleus (STN) is considered a key structure in the physiology and physiopathology of the basal ganglia. Deep brain stimulation of the subthalamic nucleus at high frequency (HFS) is used in surgical treatment of Parkinson's disease. However, the mechanisms underlying the STN HFS in the therapeutic alleviation of motor symptoms are not known. This study analyzes this question from a neurochemical approach. The experiments were carried out in urethane rats. A microdialysis probe was implanted in the striatum and the STN was stimulated. Striatal extracellular concentration of DA and DOPAC were assayed by HPLC with electrochemical detection. Samples were collected every 20 min before (1h) during (20 min) and after (2h) STN HFS (130 Hz, 60 μs, 600 μA, during 20 min). Our preliminary results show that stimulation of the STN induced a significant increase of DA in the striatum (171.85 ± 16.7 % vs 93.89 ± 4 %, P = 0.012), with a maximal peak of 197.4 ± 12.71 %. (n = 2) and 40-60 min poststimulation (n=5). The changes in DOPAC were not consistent. Similar results were observed with microinjections of the $GABA_A$ antagonist bicuculine (25 ng / 0.3 μl) into the STN neurons. These results question the current view that HFS inhibits STN neurons and provide new neurochemical arguments to identify the mechanims underlying the STN HFS.

O4

ANTI-INFLAMMATORY ACTIVITY OF *Acacia visco* METHANOLIC EXTRACT AGAINST PHOSPHOLIPASE A_2 -INDUCED PAW EDEMA

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Previous studies showed anti-inflammatory effects on acute and chronic inflammation phases by *Acacia visco* methanolic extract (Biocell Vol 28, 2004). On the other hand, it is well known that phospholipase A_2 is a target implicated in the pro-inflammatory process and its activation is believed to be the rate-limiting step for the generation of the family of metabolites from arachidonic acid. The aim of this work was to evaluate the anti-inflammatory activity of *Acacia visco* methanolic extract from leaves (*Av*MeL) and bark (*Av*MeB) against phospholipase A_2-induced paw edema in rat. The experimental groups were injected with *Av*MeL or *Av*MeB 200mg/Kg (i.p.), inflammation control group received saline. One hour later all groups were injected (Hamilton syringe) with 10 μl bee venon *Apis mellifera* phospholipase A_2 (0.5 μg/μl) into the paw. Swelling measured with a micrometer at the times indicated (10, 20, 30 and 40 min) was calculated by subtracting the value at time zero from the readings taken after the injection (Calhoun W. J. et al, 1989). The edema peaked at 10-20 min., then declined. Higher edema inhibition was observed at 10 minutes by *Av*MeL (66%) and *Av*MeB (38%). According to these results, phospholipase A_2 inhibition could be implicated in anti-

O5

POSTNANTAL EXPRESSION OF p53 AND BAX IN THE CENTRAL EXTENDED AMYGDALA (AmexCe) OF RATS.

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Apoptosis helps to maintain cell number and tissue size through activation of intracellular systems like caspases, Bax, Bcl-2, etc. Induction of p53 protein expression is involved in apoptosis. Specifically, p53 was shown to bind directly to DNA consensus sequences located in the promoter of the BAX gen which promote cell death. We studied the expression of both P53 and BAX in the early postnatal period in Central Extended Amygdala (AmexCe), structure that modulate neurovegetative and behavioral function. Normal males Wistar rats (n:24) of postnatal age (PN) 1, 7, 15 and 20, (6 animals /age) were used. Brains were fixed and stained by inmunohistochemistry. Cells were counted through a microscope with a LEICA DC 200 camera and KS Lite v2.00 program and posterior statistic analysis, and expressed as cell/mm2. Results: Both, p53 and Bax were expressed in the studied period. 1)p53 positive neurons were found at PN1: (100) lightly increasingf at PN7: (120) and PN15 (130) rising notably at PN20 (254). Bax expression showed a similar profile, with 40 cells in PN1 and PN7, increasing lightly to 55 cells at PN 15 and to 147 at PN 20. There is a parallel expression pattern of these proteins, suggesting that in this postnatal period both, p53 and BAX, contributes to define the final neuronal population in AmexCe.

O6

EFFECT OF PROBIOTIC FOODS AS AN ADJUVENT TO TRIPLE THERAPY FOR ERADICATION OF *HELICOBACTER PYLORI* INFECTION IN CHILDREN

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Current recommendations for treatment of *Helicobacter pylori* infection include a proton pump inhibitor in combination with two antibiotics. The aim of our study was to evaluate the potential activity of probiotic foods as an adjuvent to antibiotic triple therapy for eradication of *H. pylori* infection in children. 63 *H. pylori* positive children, diagnosed by ^{13}C-Urea Breath Test (UBT) and endoscopy, were included in this study. Patients were randomised in 2 groups, receiving one week triple therapy plus either probiotic foods (treated group) or placebo (control), administered for three months. Post-treatment UBT controls were performed 1 and 3 months after the end of antibiotic treatment. We found no significant differences in eradication rates (ER) between treated group (ER=45.5%) and control (ER=40.0%). In addition, symptoms improvement after treatment did not differ statistically between groups. Our results showed that the studied doses and combination of probiotics failed to improve eradication rate and symptoms in children receiving antibiotic triple therapy for *H. pylori* infection.

O7

COMPARATIVE ANTHELMINTIC ACTIVITY OF ALBENDAZOLE SULPHOXIDE ENANTIOMERS AGAINST *HAEMONCHUS CONTORTUS*

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Albendazole (ABZ) is a broad spectrum benzimidazole anthelmintic widely used in human and veterinary medicine. ABZ sulphoxide (ABZSO) is the main anthelmintically active molecule of ABZ recovered from the bloodstream and tissues after ABZ administration to different animal species. ABZSO is a chiral molecule existing as the (+) and (-) enantiomeric forms. The (+)ABZSO predominates in plasma, tissues and target parasites obtained from ABZ-treated sheep. However, the relative contribution of each enantiomeric form to the overall anthelmintic activity of ABZSO remains unknown. The work reported here evaluates the comparative anthelmintic activity of ABZ, racemic ABZSO, (+) and (-) ABZSO against *Haemonchus contortus* using a jird model. ABZ susceptible *H. contortus* infective larvae (L_3) were pre-incubated with 20 nmol/ml of either ABZ, racemic ABZSO, (+) and (-)ABZSO or without drug over 48 h. After the incubation, the L_3 were exsheated and orally administered to immunosuppressed jirds. On day 13 postinfection, the jirds were killed, and the remaining parasites counted to determine the percentage of clearance (PC) for each molecule assayed. The PC were: 99.3% (ABZ), 93.8% (racemic ABZSO), 93.8% (+ABZSO) and 72.5% (-ABZSO). These results indicate that the (+)ABZSO isoform has a significantly higher nematodicidal activity than (-)ABZSO, which confirms the pharmacological relevance of the higher concentrations of (+)ABZSO previously measured in tissues and target parasites collected from ABZ-treated animals.

O8

TOXICITY OF NAPHTOIMIDAZOLES DERIVED FROM β-LAPACHONE WITH TRYPANOCIDAL ACTIVITY.

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Screening of naphtoimidazoles derived from β-lapachone showed that 3 of them: N1 (phenyl); N2 (3-indolyl), N3 (phenyl-p-CH_3), displayed effective trypanocidal activity on epimastigotes (IC50 µM/24hs, N1: 82.8 ± 7.4, N2: 36.0 ±1.9, N3: 30.7±3.6). The aim of this work was to evaluate toxicity of these compounds. In rat liver microsomes, N1 (50,10, 5 µM), N2 (50, 10 µM) and N3 (50,10,5,1, 0.5 µM), inhibited thiobarbituric acid-reactive substances (TBARS). In presence of cumene hydroperoxide, these compounds also inhibited TBARS products. O_2 microsomal uptake was not modified by these naphtoimidazoles. Activities of cytochrome P-450 catalyzed microsomal enzymes aminopyrine N-demethylase and 7-ethoxycoumarin O-deethylase were not affected by these naphtoimidazoles. N1, N2 and N3 did not trigger ascorbate oxidation as measured by O_2 uptake. When evaluating mitochondrial oxidative phosphorylation system , N1 (50 µM) and N3 (50, 25 µM), with malate-glutamate as substrate, stimulated O_2 uptake in state 3 and inhibited it in state 4; as a result, respiratory control index values decreased significantly. Our preliminary results of both trypanocidal activity and toxicity suggest

O9

EFFICACY OF TWO FLUBENDAZOLE FORMULATIONS OF AGAINST THE HYDATID DISEASE IN MICE.

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Flubendazole (FLBZ) has been shown efficacy against protoscoleces of *Echinococcus granulosus in vitro*. The aim of this work was to evaluate the clinical efficacy of two FLBZ formulations of against hydatid cyst developed in mice. BalbC mice were intraperitoneally (ip) infected with *E. granulosus* protoscoleces (1500/animal). Nine month after infection, the mice were divided in three groups (n= 10): 1) control, untreated; 2) orally treated with a FLBZ-cyclodextrin solution; 3) orally treated with a FLBZ-carboxy-metylcellulose suspension. Groups 2 and 3 were dosed at 5 mg/kg every 12h for 25 days. After treatment, animals were euthanised and the recovered hydatid cysts were weighed and subjected to an ultrastructure study (TEM, SEM). Significantly lower weight was observed in the cyst recovered from Group 2, compared with the Group 1 (control) and Group 3. The higher efficacy achieved with the FLBZ cyclodextrin solution, may be related to a greater FLBZ bioavailability, and higher concentrations achieved at the cyst localization site.

O10

MECHANISMS UNDERLYING SEXUAL DIFFERENCES IN THE RELAXANT EFFECT OF ANANDAMIDE IN RAT MESENTERIC BED.

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The vasorelaxant effects of the endocannabinoid anandamide (AEA) are greater in mesenteric beds from female than from male Sprague-Dawley rats (Peroni RN *et al.*, *European Journal of Pharmacology* 493:151-60, 2004). The aim of the present work was to study the mechanisms involved in the sexual differences in the AEA-induced vasorelaxations in rat mesenteric beds isolated from Sprague-Dawley rats.
Endothelial removal (0.1% w/v saponin 45 seg) potentiated the reduction of vasoconstrictor responses to 10 nmol noradrenaline caused by AEA (0.01-10 μM) in mesenteric beds isolated fronm male ($p<0.001$) but not from female rats. The cyclooxygenase inhibitor indomethacin (10 μM) increased AEA relaxations in both sexes ($p<0.05$). On the other hand, AEA effects were almost abolished by the nitric oxide synthase (NOS) inhibitor L-NAME (100 μM, $p<0.01$) in males and females even in tissues where the endothelium was previously removed. In addition, relaxant responses induced by AEA were reduced in the presence of the selective neuronal NOS inhibitor N^{ω}-propyl-L-arginine (5 μM) in both sexes ($p<0.05$). On the other hand, the irreversible endothelin ET-1 receptor antagonist BQ610 0.1 μM decreased noradrenaline contractions only in males ($p<0.05$) and this effect was abolished by endothelial removal. In conclusion, although prostanoids and neuronal nitric oxide could be involved in the vasorelaxant effect of anandamide in mesenteric beds of Sprague-Dawley rats, only endothelin is likely to be linked to the lower

O11

AUTORADIOGRAPHIC STUDY OF μ-OPIOID RECEPTORS IN PREPUBERTAL MICE OF EITHER SEX DURING MORPHINE WITHDRAWAL SYNDROME AND ITS PREVENTION WITH BACLOFEN.

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We have previously shown that the $GABA_B$ agonist baclofen (BAC) attenuates the expression of naloxone (NAL)-precipitated morphine (MOR) withdrawal in male as well as female mice. In order to extend these observations, our aim was to analyze μ-opioid binding sites in various brain areas in mice of either sex during MOR withdrawal and its prevention with BAC. Prepubertal Swiss mice were rendered dependent by i.p. injection of MOR (2 mg/kg) twice daily for 9 days. On the 10th day, dependent mice received NAL (6 mg/kg, i.p.) 60 min after the last dose of MOR, and another pool of dependent mice received BAC (2 mg/kg, i.p.) previous to NAL. Mice were sacrificed, brains were collected and different areas were dissected to perform autoradiographic studies with [^{3}H]-DAMGO. The μ-opioid labeling significantly increased in caudate putamen (CPu), nucleus accumbens core (NAcC), mediodorsal thalamic nucleus (MDTh), basal amygdala and ventral tegmental area of MOR withdrawn males vs control groups. Conversely, opiate receptor labeling was not modified in any of the areas studied of females. BAC reestablished μ-opioid receptor levels modified by MOR withdrawal only in CPu, NAcC and MDTh of males. The sexual dimorphism observed herein confirms the greater sensitivity of males in response to MOR. Our results also suggest that the effect of BAC in preventing the expression of MOR withdrawal signs

O12

INFLUENCE OF IRON DEFICIENCY IN THE RADIOPHARMACEUTICAL BEHAVIOR OF RED BLOOD CELLS LABELED WITH ^{99M}TC (^{99M}TC-RBC)

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Red blood cells (RBCs) labeled with ^{99m}Tc are commonly used in the evaluation of cardiac function, gastrointestinal tract bleeding, red blood cell volume or splenic sequestration. Generally stannous ion is used as reducing agent. A proposed mechanism is that once the stannous ion (Sn) and the pertechnetate (^{99m}Tc) reach the interior of the RBC, the radionuclide is mainly house in the □-chain of hemoglobin. The aim of this study was to determine if hemoglobin content reduction, an indicator of iron deficiency anemia, could affect the efficiency of RBC labeling and the biological distribution of this radiopharmaceutical. We studied 30 rats fed for 3 weeks after weaning with diets with iron contents of 6.5 ppm (group A), 18 ppm (group B) and 100 ppm (control). For all groups, the labeling yields were always higher than 97%; the percentage of radioactivity was mostly founded in blood with almost negligible radioactivity the rest of the studied organs. We can conclude that the decrease in hemoglobin content, an indicator of iron deficiency anemia, does not interfere

O13

BRACHYTHERAPY OF SQUAMOUS CELL CARCINOMA IN CATS USING A ^{32}P PATCH.

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Brachytherapy has been a big challenge in nuclear medicine in order to apply this therapeutic modality to cancer treatment. The aim of this work was to evaluate a silicon patch coated with Pirocarbotrat™ for topical application in skin cancer lesions. We selected four adult cats with squamous cell carcinoma (SCC). Measurements of the lesions were taken to specially designed the patches for their application on the lesion surface. Dosimetric calculations were done in each case taking into account the time of exposure and the activity contained in the patch. Clinical evaluation showed that in one case tumor disappeared and in the other three cases, lesion reductions were about 50% of their original size. Peritumoral fibrosis and central necrosis appeared in the treated site. The shared feature in all the four cases was the great local inflammatory response at the site where patches were applied. All these responses are in accordance with those expected after radiation therapy and they are also indicative of the effectiveness of the treatment. However, the histopathological results of the follow-up biopsies, showed that total remission was not achieved. This clinical experience allows us to confirm treatment efficacy of the ^{32}P patch for skin cancer but signalling the importance of the planning dose for future experiences in order to achieve

O14

EFFECT OF LINDANE ON WATER PERMEABILITY IN THE ISOLATED URINARY BLADDER OF THE BUFO ARENARUM TOAD

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The permeability to water (Jw) in tight epithelia exposed to an osmotic gradient is very low in the absence of ADH, and increases considerably (hydrosmotic response) following exposure to the peptide via a process activated by cAMP and insertion of aquaporins (water channels) in the apical membrane. The possible participation of intercellular communication in the process has not been adequately explored. We have recently shown that some gap junction inhibitors (octanol, carbenoxolone), while devoid of effect per se, bring about changes in the bladder´s Jw response to hydrosmotic agents. Thus, the Jw response to oxytocin, which mimics the effect of ADH in the toad skin, is significantly reduced, whereas neither the response to nystatin (which increases Jw by "perforating" the membrane, without participation of the physiological activation process) nor to exposure of the apical border of the skin to hypertonic solutions (which permeates the paracellular pathway by opening the tight junctions) are affected. Another gap junction blocker, lindane, shares most of these effects. In contrast with the other blockers tested, however, the increase in Jw brought about by apical exposure to a hypertonic solution is increased by exposure to the compound. Our results add further evidence that gap

O15

ANGIOTENSIN CONVERTING ENZYME (ACE) AND NEUTRAL ENDOPEPTIDASE (NEP) IN HUMAN UMBILICAL ARTERY (HUA)

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The objective of the present study was to evaluate kininase activity exerted by ACE and NEP as a function of incubation time in an isolated HUA model. HUA rings were mounted under isometric tension in Krebs solution at 37ºC and bubbled with 95% O_2/ 5% CO_2. After 120 or 300 min, concentration-response curves (CRCs) to BK were obtained. Some of the rings were exposed to phosphoramidon 10 μM and captopril 1 μM for 30 min before the exposure to BK. Results are expressed as mean ± SEM. Statistical analysis was performed by ANOVA followed by Tukey´s post-test. $P<0.05$ was considered as a statistically significantive difference. After a 2 h incubation period, BK elicited a concentration dependent contraction of HUA (pEC_{50} 8.32 ± 0.07; n=7). Neither captopril nor phosphoramidon modified BK induced responses at this timepoint. BK potency after 5 h incubation period (pEC_{50} 7.76 ± 0.07; n=17) was significantly lower than the one observed at 2 h. Additionally, captopril (pCE_{50} 8.34 ± 0.07, n=10) and phosphoramidon (pCE_{50} 8.61 ± 0.10, n=10) produced a significant potentiation of BK induced responses after 5 h. Simultaneous treatment with both inhibitors significantly potentiated BK induced responses at this timepoint. These results suggest that kininase activity exerted by ACE and NEP are increased in a time dependent fashion in isolated HUA.

O16

EFFECT OF ANG II ANTAGONIST ON THE RECEPTOR LOCALIZATION IN HINDBRAIN DURING DEVELOPMENT

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Ang II receptors are differentially expressed during the development and this fact has been related to a potential role of these receptors in development and organogenesis. We studied the localization of Ang II receptors during hindbrain development in offspring of pregnant rats treated with Ang II and antagonist. Wistar rats were treated during the last week of pregnancy with: saline (control), Ang II, losartan (AT_1 antagonist) and PD123139 (AT_2 antagonist). Pups were analized at two different ages, PND0 and PND8. New-born rats hindbrains were obtained, snap frozen in isopentane and kept at –70 °C until processed. The study was performed at different levels of the brainstem and cerebellum. Since cerebellum development is mainly postnatal in rodents, a few structures were identified in PND0 animals, where AT_2 receptors were present in the inferior colicullus (CIC), genu facial nucleus (7), and inferior olive (IO). In PND8 animals, AT_2 subtype was present the same nucleus and cerebellum areas and cerebellar peduncles. AT_1 receptors were present in Sp5O nucleus and the cerebellar cortex. An increase in binding intensity was observed in treated animals, as compared to control animals. The present results suggest that treatment of

O17

HOMING AND MOVILIZATION OF HEMATOPOIETIC PROGENITOR CELLS POST- PACLITAXEL TREATMENT .

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HCAM, a homing receptor for hematopoietic progenitors and MMP-2 a matrix metalloproteinase required for their movilization, are critical proteins involved in hematopoiesis.

We investigate HCAM and MMP-2 expressions in murine bone marrow (BM) and spleen after a single dose of Paclitaxel (Px) treatment (29 mg/Kg i.p) along 10 days of study.Experimental data of HCAM and MMP-2 expressions (inmunohistochemistry) were corretaled with absolute cellularites, apoptotic percentages (TUNEL), total BM splenic and peripheral blood hematopoietic colonies (semisolid cultures) and differential hematopoietic percentages in each tissue (light microscopy).

HCAM is normally expressed in BM but not in spleen, whereas MMP-2 failed to be significantly expressed in any of these hematopoietic tissues. During post-Px hemopoietic recovery, HCAM descrease significantly until the 7^{th} day while it was only noticed in spleen by the 3^{rd} day. However, MMP-2 is overexpressed between the 1^{st} and 3^{rd} days in BM. In the period of maximum apoptosis and minimal cellularity, the expressions of HCAM and MMP-2 exhibit an inverse relationship. We also noticed changes in the kind and frequency of hematopoietic colonies along the study.

These results suggest that changes in MMP-2 and HCAM expressions in BM allow movilization and homing of hematopoietic progenitors in the splenic tissue during post-Px recovery.

Key words: Paclitaxel- HCAM- MMP-2- Apoptosis-

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O18

VANADYL SULPHATE MODIFIES GLYCEMIA AND VASCULAR PROSTANOID PRODUCTION IN DIABETIC RATS.

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Vanadium salts have been suggested as a possible agent for treating diabetes mellitus (DM). The aim of this work was to study the effects of vanadyl sulphate (V) on glycemia and prostanoid (PR) release in aorta (A) and mesenteric vascular bed (MVB) of diabetic rats. DM was induced by a single injection of streptozotocin (STZ), 55 mg/kg. Thirty days after STZ, the animals were sacrificed and A and MVB excised. Tissues were incubated for 60 min at 37ºC and PR released were measured by HPLC. V treatment (125 mg/l in drinking water) did not modify any parameter in the non-diabetic control group, but reduced glycemia and body weight in diabetics. Regarding PR release in diabetics, we found no differences in A between V-treated and untreated animals. In MVB, DM reduced release of prostaglandin $(PG)E_2$ and of prostacyclin (PGI_2), both vasodilators, and thromboxane A_2, a vasoconstrictor. In this preparation, V treatment increased PGE_2 and PGI_2, with no alterations in the other metabolites measured, both vasoconstrictors. In conclusion, 30 days of STZ DM modified PR release in the MVB in favour of vasoconstrictors, meanwhile oral V treatment partially restored such imbalance.

1

NEURONAL FRONTAL CORTEX AND HIPPOCAMPUS GLUTAMATE UPTAKE IN PREHEPATIC PORTAL HYPERTENSIVE RATS.

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Portal hypertension constitutes a major complication of human and animal cirrhosis that frequently leads to central nervous system (CNS) dysfunction. This pathology creates difficulties to splanchnic circulation across liver parenquimal vasculature, to reach cava vein. A second important syndrome in this pathology is hepatic encephalopathy (sub clinical or ouvert). Here, we examined the influence on prehepatic portal (PH) rats on synaptic activity of glutamate (Glu) transporters in neuronal frontal cortex (CF) and hippocampus (Hic). PH was produced by performing a calibrated stenosis of portal vein as described by Lores-Arnais (2005). The animals were sacrified by decapitation 14 days after portal vein stenosis. Synaptosomes from CF and Hic were freshly prepared for determination of the time course for Glu uptake in Krebs Ringer buffer at 30ºC for different periods up to 30 min. It was documented a statistical significance decrease more Hic than CF in PH rats against sham. We conclude that the HP hipertensive rats decrease the uptake of Glu in CF and Hic suggesting that it represent toxic levels of Glu in rat brain.

2

EFFECTS OF HYDROXYDECANOATE (5-HD) ON PRECONDITIONED PERFUSED RAT HEARTS EXPOSED TO ISCHEMIA-REPERFUSION.

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The investigation aimed to assess the role of the mitochondrial ATP sensitive potassium channel (K-ATP) in the protective effects of ischemic preconditioning (PC) on ischemic- reperfused rat hearts. It was used Langendorff-perfused hearts exposed to 25 min ischemia (I) and 30 min reperfusion (RP). PC was achieved by a 3 min I followed by 5 min RP. It was measured the isovolumically heart pressure and the heart rate and calculated the product (RPP). The mitochondrial permeability was measured trapping ^{3}H-2-deoxiglucose (DG) as DG-6P, the cellular viability using para-phenyltetrazolium and glycogen, after extraction, using an enzymatic method. The hearts were loaded with DG during 30 min. 100 µM 5-HD, a selective inhibitor of K-ATP, was added 5 min before starting the I. The 5-HD abolished the effects of PC on the heart function (RPP-15 min ; control (C):49±7, PC:81±1, PC-5-HD 37±1, on the end diastolic pressure at 5 min RP, C:39±1, PC:4±2, PC-5-HD:46±1 and on the end I glycogen (µg/100mg dw; C:78±2, PC:184±31, PC-5-HD:91±19). 5-HD did not affect the effect of PC on the mitochondrial permeability (trapping of DG as 10^5 x dpm/units of citrate synthetase/total dpm/gh; C:96±14, PC:16±12,PC-5HD 10±8 and on the cellular viabilty (as % of risk area; C 21±6, PC:60±8,PC-5-HD:58±1). These data suggest that K-ATP is involved in

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CELL DISTRIBUTION OF THE SECRETION PRODUCT OF RAT PARS TUBERALIS IS AFFECTED BY THE ADMINISTRATION OF ALBENDAZOLE

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Specific groups of cells from the *Pars tuberalis (PT)* secrete a product of unknown function. Microtubules participate actively in cell secretion; their integrity is crucial for the secretory activity of the cell. It was shown that albendazole (ABZ), an anthelmintic drug, depolymerizes microtubules (MT) of the helminth and hence, kills the parasite. ABZ in rats also affects the microtubule network. The main purpose of this work was to evaluate putative modifications induced by ABZ in the release of the secretory product of *Pars tuberalis* of adenohypophysis of rats. Six groups of adult Wistar rats were used (one control group and five experimental). To the experimental rats (n=3 in each group) ABZ was administered orally as follows: 0.5, 1.0, 1.5, 2.0 and 2.5 g/Kg of body weight; animals were killed after 48 h after administration. PT secretory product was located in cells by immunocytochemical techniques; a polyclonal antibody against the product was raised in rabbit. Results indicated that while in brain cells of control animals the location of the secretory product was mainly paranuclear, in experimental animals administered with increasing doses of ABZ the product distributed in a dose dependent way: with the lower dose (0.5 g/Kg) no detectable difference was observed with controls; with increasing doses (1.0; 1.5 and 2.0) the secreted product was distributed evenly in the cytoplasm; finally, with the 2.5 g/Kg dose it could not be

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TREATMENT WITH ACE INHIBITORS DURING PREGNANCY AFFECTS ANGIOTENSIN II RECEPTOR EXPRESSION IN OFFPRING´S HINDBRAINS.

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Recently, a new role for Angiotensin II (Ang II) receptors in growth and cellular proliferation was proposed. A differential expression of Ang II receptors has been observed in brain areas. Localization of Ang II receptors in hindbrain was studied by autoradiography in offspring of pregnant rats treated with ACE inhibitors. Treatment was performed during the last week of pregnancy with vehicle (control) or ACE inhibitors (Enalapril and Captopril). Pups at two different ages, PND0 and PND8, and different levels of the brainstem and cerebellum were analyzed. For the two developmental stages, PND0 and PND8, AT_1 binding was very low at all analyzed levels and lower in treated than in control animals. In PND0 animals, binding corresponds mainly to AT_2 subtype and is associated to brainstem nuclei, as Inferior Olive (IO) and facial nucleus (7). We did not detect binding associated with cerebellum. PND8 animals show binding associated with superior (SC) and inferior colicullus (IC) and facial nucleus. Unlike PND0, in PND8 AT_2 binding was present in cerebellar areas. At PND8, AT_2 binding was lower in treated than in control animals. AT_1 and AT_2 binding was observed in cerebellar complementary areas. These results agree with previous studies performed in PND15 animals. In conclusion our findings suggest that treatment of pregnant rats during late gestation, affect offspring's AT_1 and AT_2 receptors expression

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MODULLATION OF EXTRACELLULAR MATRIX METABOLISM IN HUMAN ARTICULAR CHONDROCYTES BY THE SODIUM DICLO- FENAC AND GLUCOSAMINE

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Osteoarthritis (OA) is characterized by a degeneration of articular cartilage. As an irreversible step in OA occurs when collagen is degraded, it was thought that the major enzyme accounting for collagen type II degradation was collagenase (MMP-1). Nitric oxide (NO) is a free radical that contributes to inflammatory and arthritic tissue destruction. The aim of this study was to investigate the effects in vitro of sodium diclofenac ,and glucosamine on the production of collagenase-1 (MMP-1) and levels of NO, by human articular chondrocytes. Chondrocytes were cultured in the absence or presence of 1-10 µg/ml of DICLO and GLUCO. NO-[2]/NO-[3] concentrations were determined using the Griess assay, ELISA was used to quantify MMP-1.

MMP-1 in the absence of NSAIDs was 1970± 665 ng/ml, in presence of DICLO was 1140 ±155 ng/ml had no significant effect. MMP-1 in presence of GLUCO was 950 ± 89 ng/ml ($p<0.05$). NO in absence of NAIDs was 47.3 µM. DICLO and GLUCO had no significant effect on NO production

Our studies demonstrate :1- differences between DICLO and GLUCO with respect to their ability to modulate the proteases. 2- had no significant effect on NO production. These drugs do not slow down the progression of OA.

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PENTOXIFYLLINE PROTECTS MURINE PERITONEAL MACROPHAGES FROM LIPOPOLYSACCHARIDE-INDUCED APOPTOSIS

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Lipopolysaccharide (LPS) induces macrophage (MØ) secretion of TNFα. Pentoxifylline (PXF) is a non-specific inhibitor of phosphodiesterase, an enzyme involved in TNFα synthesis and release. In this study we evaluated whether PXF downregulates LPS-induced TNFα's effects on macrophages bioactivity.

Murine MØ were isolated by peritoneal lavage 3 days after 3% thioglycolate injection. Cells were washed with ClNa (0,9%) and resuspended in culture medium. MØ ($1x10^6$ cells/ml) were cultured at 37ºC with LPS (100 ng/ml) with or without different concentrations of PXF (0,01; 0,1 and 1 mM/ml) for 30' and 60' against controls. Viability, apoptosis and necrosis were evaluated by light microscopy. Data are expressed as the mean ± SEM of 3 separate experiments performed in duplicates. When LPS and LPS+PXF cultures were compared, we found that at 30' PXF failed to cause variations on MØ survival. At 60' PXF caused a non-significative increase in MØ viability (maximum with 1mM/ml: 68% ± 7,1) compared to LPS cultures. Apoptotic percentages decreased in LPS+PXF (all concentrations) in a significant way ($p<0,01$). Necrotic patterns were not modified in none of the culture assays.

These results suggest that PXF protects MØ from LPS TNFα-mediated injury, allowing survival. Its use in clinical scenarios in which MØ are primed may be of clinical relevance.

Key words: macrophage, lipopolisaccahride, pentoxifylline.
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SEROTONIN-INDEPENDENT EFFECT OF FLUOXETINE AND CLOMIPRAMINE ON T-CELL PROLIFERATION.

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Drugs that target the serotoninergic system are the most commonly prescribed therapeutic agents and are used for treatment of a wide range of behavioral and neurological disorders. These drug effects on immune responses were described related to their capacity to inhibit serotonin reuptake. In order to ascertain if these drugs are able to modulate the proliferative response by a direct mechanism we analyzed fluoxetine and clomipramine effects on mitogen-induced T-cell proliferation. We observed that serotonin was able to stimulate the suboptimal Con A-induced proliferation and to inhibit the optimal one at all concentrations tested. The serotonin stimulatory, but not the inhibitory effect was suppressed by a HT-2 antagonist ketanserine. Low doses of fluoxetine were able to stimulate proliferation induced by sub-mitogenic concentrations of Con A but inhibit the optimal lectin induced proliferation. Low and high doses of clomipramine stimulated sub-optimal and inhibited optimal proliferation. Serotonin decreased inhibitory and increased stimulatory effect of fluoxetine. However, serotonin only impaired the clomipramine stimulatory effect on proliferation. Also, ketanserine impaired clomipramine, but not fluoxetine, stimulatory effect. These results indicate that fluoxetine and clomipramine are able to regulate T-cell proliferation by different serotonin-dependent and independent mechanisms underlining a complex cross-talk between this drugs and serotonin pathways.

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INHIBITORY EFFECT OF CEDRON *(Alloysia citriodora)* ON RAT DUODENAL MOTILITY IN VITRO

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In our country the leaves of the plant "cedrón" *(Alloysia citriodora* Palau, Verbenaceae) are widely used in folk medicine to treat gastrointestinal disorders, and as a dietary supplement in the way of an aromatic infusion. Nevertheless, there were not bibliographic reports about experimental studies of its pharmacological properties. Then, in this work, we evaluated the effects of a liophylized from an acqueous extract of "cedrón" (identified as herbarium number LPE 1039, and prepared as a decoction of 60 g of dried leaves in 200 ml) at concentrations from 0.01 to 6 mg/ml on the contractility of isolated duodenal muscles from rats. Dose-response curves (DRC) of acetylcholine (Ach) were done, obtaining a pD_2 of 5.77 ± 0.13 (n=12). Liophylized of "cedrón" produced a non-competitive inhibition of the Ach-DRC, with a fifty%-inhibitory-concentration (IC50) of 0.69 ± 0.17 mg lioph. by ml (n=9). For studying the origin of such inhibition, we have done dose-response curves of Ca (pD_2 of 2.69 ± 0.25, n= 4) in a depolarizing medium. These Ca-DRC were also inhibited in a non-competitive way by the liophylized of "cedrón" (IC50 of 2.75 ± 0.49 mg lioph/ml). The present results suggest that: **a)** Cedrón reduced the spasmogenic effect of Ach, which can explain its folk use as antiespasmodic, **b)** that effect was not related to blockade of Ca-influx but to another interference with contractility.

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SEDATIVE EFFECT OF CEDRON *(Alloysia citriodora)* ON MICE IN THE OPEN FIELD AND HPLC FINGERPRINT

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"Cedrón" *(Alloysia citriodora* Palau, Verbenaceae) is widely used in folk medicine. We evaluated whether it has activity on the spontaneous behavior of mice, and its chromatographic profile. A liophylized was obtained from a a 30% acqueous extract of dried leaves (herbarium LPE 1039). Doses of 0.15, 1 and 10 mg lioph/ Kg were injected via IP on mice and evaluated in an open-field with 15 squares of 10 cm^2. The number of crossed lines (CL) and rearings (Re) were respectively evaluated during 5 min, in a whole period of 160 min. "Cedrón" reduced CL after 40 min from 114.3±16.6 (n=11) to 70.2±15.8, to 48.2±11.0* and to 10.8±2.9* (n=6) at 0.15, 1 and 10 mg lioph/Kg, respectively. It also reduced Re from 75.3±4.1 to 31.0±10.1*, 22.7 ±4.7* and 3±0.5* at 0.15, 1 and 10 mg lioph/Kg, respectively. Both effects were kept until 160 min for the higher dose. Diazepam at 10 mg/Kg potentiated the effect of "cedrón" 1 mg/Kg. Chromatographic profiles by HPLC were obtained with RP18 column with two mobile-phases: 2-propanol/tetrahydrofurane (THF)/water (5:15:85) and water/THF/ 2-propanol/acetonitrile (88: 8:1.6:2.4) with 0.05% phosphoric acid, UV-detection at 336 nm. Both systems well separated the peaks, and were identified vitexine and isovitexine. The present results suggest that: **a) "**Cedrón" reduced the spontaneous locomotion and exploratory behavior at doses (0.75 to 50 mg of leaves/Kg) which are

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MELATONIN IS MORE EFFECTIVE THAN VITAMIN E IN RESTORING IMPAIRED AORTIC RING RELAXATION IN RATS AFTER SUBTOTAL PANCREATECTOMY.

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In a previous study we showed that a decreased acetylcholine-induced relaxation (Ach-IR) followed subtotal pancreatectomy (Ppx) in rats. The effect was amplified by pre-incubation in a high glucose solution -HG- (44mM/l), a situation that results in oxidative stress mainly through superoxide anion accumulation. Melatonin (MEL) added to the medium, significantly increased Ach-IR. Based on these results, we hereby compare the effect of vitamin E – alfa tocopherol- (VE) vs MEL on Ach-IR of rats turned intolerant to carbohydrates. Rings of thoracic aorta were placed in organ chambers, and isometric tension was recorded. Dose response curves to Phenylephrine (PhE) and Ach were performed with and without VE and MEL (10^{-5} M). The effect of incubating aortic rings in a Krebs solution with HG was also evaluated. Rings pre-treated with HG showed a diminished relaxation to Ach as compared to rings incubated with HG+VE or HG+MEL. MEL added to the media was more effective than VE in improving Ach-IR (10^{-7}-10^{-5}): 10^{-7} 41.48±6.9 vs 56.12±4.38 (P< 0.01); 10^{-6} 17.06±2.59 vs 31.42±2.57 (P< 0.01); 10^{-5} 8.32±1.34 vs 20.13±2.06 (P< 0.05). Conclusions: These results show that MEL is more effective than VE in increasing the Ach-IR of rings incubated with HG. The difference between these two antioxidants may rely on the ability of MEL to diffuse readily into intracellular

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MUCIN RELEASE BY PILOCARPINE IN RAT SUBMANDIBULAR GLAND

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Among the organic constituents of saliva mucin is considered the major protective component within the oral cavity and the esophagus. Mucin is released by the submandibular and sublinglual glands. The aim of the present study was to evaluate the mechanism underlying mucin release by cholinergic stimulation, in rat submandibular gland. Results showed that carbachol and pilocarpine induced mucin release in a dose-dependent manner. Carbachol increased mucin release by 15% with 10^{-8} M until 92 % with 10^{-4} M while pilocarpine increased a 17 % with 10^{-8} M and achieved a 160 % of increment with 10^{-4} M. The effect of the agonists was antagonized by atropine. The selective muscarinic receptors antagonists 4-DAMP (M_3), pirenzepine (M_1) and tropicamide (M_4) induced a right shift of pilocarpine dose-response curve increasing the CE_{50} of pilocarpine. AF-DX 116, antagonist of M_2 muscarinic receptor subtype, had no effect on pilocarpine-induce mucin release. Inhibition of cycloxygenase by indomethacin ($5x10^{-6}$ M) and aspirin ($5x10^{-4}$ M) resulted in an inhibition of pilocarpine-induce mucin release. The inhibitory effect of indomethacin and aspirin was prevented by PGE_2, 10^{-8} M. It is concluded that pilocarpine induced mucin relase through the activation of M_1, M_3 and M_4 muscarinic receptors subtypes and the exocytosis mechanism involved PGs release.

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CLOZAPINE ACUTE ADMINISTRATION MODIFIES NEUROTENSIN EFFECT ON NA^+, K^+ -ATPASE ACTIVITY.

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Synaptosomal membrane Na^+, K^+-ATPase is inhibited by neurotensin (NT), an effect which involves its high affinity receptor (NTS1). Herein, we studied NT effect on synaptosomal membrane Na^+, K^+-ATPase of rats pretreated with the atypical antipsychotic clozapine. Different doses of clozapìne were administered i.p. to rats, which were decapited at 30 min or 18 hs. Cerebral cortex was removed and subjected to differential and sucrose gradient centrifugation to obtain synaptosomal membrane fractions. In the presence of 3.5×10^{-6} M NT, Na^+, K^+-ATPase activity decreased 44 % in control membranes. Thirty min after injection of 3, 10 and 30 mg / kg clozapine NT failed to inhibit enzyme activity. At variance, 18 hs after administration of 3 mg / kg or 5.6 mg / kg clozapine, NT decreased Na^+, K^+-ATPase activity 40 or 20%, respectively. At doses of 18 and 30 mg / kg clozapine, NT inhibitory effect was totally prevented. These results support the hypothesis of an interplay among NTS1 receptor, dopaminergic D_2 receptors and Na^+, K^+-ATPase activity at central synapses

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POSTNATAL ACUTE STRESS-INDUCED CHANGES IN NEURONAL CEREBRAL CORTEX OF L-SERINE AND GABA UPTAKE.

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During early development, a number of reports demonstrated that the postnatal stress modified the neuronal responses to aminoacidergic transmitters, this fact, appears to be associated with neurobiological alterations. In this work, we investigated the influence of synaptic activity on L-Serine (Ser) and GABA uptake responses in postnatal acute cold stress conditions. Experiments were performed with cerebral cortex (CC) of rats in different postnatal day of the birth until young adulthood. Neonates were stressed by cold stress for 1 hour at 4ºC. Unhandled neonates, left undisturbed in their home cages, served as control. Upon termination of cold stress exposure, neonates were killed by decapitation. The brain was removed from the cranial cavity; CC was dissected. Neonatal CC synaptosomes were freshly prepared for determination of incorporation of radiolabeled amino acids in Krebs Ringer buffer at 30°C. We observed that the acute cold stress increased the time course both Ser and GABA uptake at 7, 13, 21 postnatal days while decreased at 5 postnatal day and in adult stage compared with control group. The values of Vmax and Km either L-serine or GABA uptake decreased in the stressed animals respective with control groups. These results suggested a possible relation between acute cold stress and the mechanism associated with neuronal activities of both Ser

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STRIATAL MODULATION OF THE RETICULAR THALAMIC NUCLEUS.

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The thalamic reticular nucleus (TRN) consists of a collection of GABA-containing neurons that receives colateral from corticothalamic and thalamocortical projections as well as from the basal ganglia. The external globus pallidus (GP) and the substantia nigra pars reticulata project to TRN. The neurons of the TRN innervate almost all thalamic relay nuclei. The aim of this study was to analyze the influence of striatal dopamine receptors on TRN neurons. The experiments were preformed in rats anesthetized with urethane. The activity of the TRN was recorded with microelectrodes and the striatal DA receptors were activated by microinjections of 7ug / 0.5 μl of apomorphine. TRN neurons were identified by their spontaneous bursting discharge. Our preliminary results shown that activation of the DA receptors induced 66% (range 63 to 92%, n = 25) of inhibition of the spontaneous activity of TRN neurons (one way ANOVA and H-S, $P < 0.001$). The lesion of the strionigral pathway, in 2 experiments, left unchanged the inhibitory action of the striatum. From the above results, we conclude that the striatum could modulated the thalamocortical activity through the RTN, action probably not mediated by the direct pathway

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PERINATAL PROTEIN MALNUTRITION ENHANCES REWARDING PROPERTIES OF MORPHINE IN ADULT RATS

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The rewarding properties of morphine were assessed in adult rats submitted to a protein malnutrition schedule at perinatal age (D-rats), as compared with well-nourished animals (C-rats) using the Conditioned Place Preference (CPP) paradigm. Dose-response curves to morphine (0.75, 1.5, 3, 6 and 12 mg/kg i.p.) revealed in D-rats a conditioning effect with doses of 1.5 and 3 mg/kg whereas doses of 6 and 12 mg/kg did not show any conditioning place preference. In C-rats, morphine elicited place preference with doses of 3 and 6 mg/kg, whereas 12 mg/kg did not show conditioning effect. Furthermore, when the animals where pretreated twice a day for three days with increasing doses of morphine (5, 10 and 20 mg/kg, s.c.) only D-rats showed sensitization to the conditioning effect of a low dosage of morphine (0.75 mg/kg i.p.). Related to the higher rewarding effects, sensitized D-rats showed a selective and significant increase in FosB expression in nucleus accumbens (core and shell), basolateral amygdala and medial prefrontal cortex, brain areas related to the rewarding neuronal circuits. These results suggest that a deficient nutritional status during early life may induce in adult subjects an increased responsiveness to behavioral effects of morphine and/or enhanced reinforcement during abstinence

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EFFECT OF DEHYDROLEUCODINE (DHL) ON THE FIRST EMBRYONARY STAGES OF *BUFO ARENARUM* **(ANURA: BUFONIDAE)**

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Artemisia douglasiana Besser plant, popularly known as "matico", is used in popular medicine as a cytoprotective agent for gastric ulcers, for external treatment of skin injuries and for dermal ulcers. The active principle of this plant is dehydroleucodine (DhL), a sesquiterpene lactone of the guaianolide type. The literature described toxic effects in vitro mammalian cell culture test of DhL. Amphibian embryos and larvae are excellent models for the studies of development and toxicity tests of chemical compounds. In order to study the toxic effect of DhL in non-mammalian species we selected embryos from B. arenarum obtained by in-vitro fertilization. Groups of 20 blastula-stage embryos, developing normally, were selected under stereoscopic microscope and placed in 15 cm diameter glass petridishes by triplicate with : 1mM, 3mM, 5mM and 7mM DhL dissolved into 1% (v/v) DMSO followed by dilution in Ringer solution, control groups were also achieved. The assay was conducted for 96 h with solution renewal every 24 h. Embryo jellied coat was removed with a 2 % (w/v) cysteine solution at pH 8.1. The number of dead and surviving malformed embryos were recorded for each dish. Embriolethality was 100 % for 7, 5 and 3 mM DhL at 72, and 96 h respectively, at 1 mM there was a delay in the rate of developing at stages 12-13. Control groups developed normally, dead embryos showed altered pigmentation and blastopore failed to close. DhL

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NEUROTOXICITY OF KAINIC ACID IN THE MEDIAL NUCLEUS OF EXTENDED AMYGDALA (MEXA) IN FEMALE RATS. ROLE OF TESTOSTERONE.

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Sex hormones contributes to modulate brain function through the lifespan. Moreover, it has suggested that Estradiol prevents neuronal loss in the CNS. However, there are less consistent data on the neuroprotective effects of Testosterone (T). Here we have assessed this role of T in the MExA of female rats after the administration of an epileptogenic dose of Kainic Acid.

Twenty Wistar female rats were used, 4 at Diestrus (D), 4 at proestrous (P) and 12 were ovariectomized (OVX). 21 days after surgery, animals received a single injection of T (OVX+T) or dihydrotestosterone (OVX+DHT). Three days after, all groups received a single IP injection of KA (8 mg/kg). Control animals of each group (D, P, and OVX) were injected with saline.

Twenty-four hours after the KA all animals were fixed, the brains sectioned and stained for neuronal death with the Amino-Cu-Ag technique. Neurons were counted using a Scion Program. Data were analysed with ANOVA followed by the Fisher post hoc test.

Results: 1) D rats showed more neuronal death than P. 2) OVX increased neural death as compared with D and P. 3) In OVX rats lesions are similar to D. 4) T replacement in OVX attenuated KA- induced neuron loss. 5) DHT did not protect neurons against kainate excitotoxicity.

These results indicate that the neuroprotection seen in OVX+T was mediated trough aromatization to estradiol by

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EFFECT OF β PINENE, ISOLATED FROM *TILIA CORDATA* MILL. FLOWERS, ON NORMAL AND TUMORAL LYMPHOCYTES PROLIFERATION.

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Tilia cordata is a plant used in popular medicine for ansiety and as immunostimulant. In a previous work, we demonstrated that, a dichloromethanic extract obtained from *T. cordata* flowers presented a selective antiproliferative action on a murine lymphoma cell line (BW 5147). The terpenes limonene and beta pinene (β p) were identify by gas chromatography-mass. The aim of the present work was: a) to analyze the effect of isolated β p on BW 5147 cells and on normal murine lymphocytes proliferation, through tritiated tymidine uptake, b) to determine cytostatic or cytotoxic effects by tripan blue exclusión method, d) to investigate the induction of apoptosis by nuclear Hoechst dye assay and e) to analyze the effect on total nitrites levels by the Griess method. Results (media ± SEM): tumoral cells (CI_{50} µg/ml, 24 hs: 79,4 ± 5,0; 48 hs: 15 ± 1,2; 72 hs: 6 ± 0,5). Normal cells: Stimulation index (SI) (β p 0,01 µg/ml: 1,4± 0,08 ; β p0, 1: 1,65 ± 0,07 µg/ml; β p 1 µg/ml: 3,4 ± 0,3; β p 10 µg/ml: 3,2 ± 0,2; β p 100 µg/ml : 2,4 ± 0,18; β p 1000 µg/ml : 2,3 ± 0,2). Total nitrites 24 hs SI: (β p 0,01 µg/ml: 2,2± 0,2 ; β p0, 1: 2,07 ± 0,18 µg/ml; β p 1 µg/ml: 1,06 ± 0,08 ; β p 10 µg/ml: 0,7 ± 0,05 ; β p 100 µg/ml : 0,70 ± 0,06). βp presented cytostatic and cytotoxic effect these effects

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MATE DECOCTIONS EFFECT ON THE ACTIVITY OF INTESTINAL PGP

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Many of herbal constituents, in particular flavonoids, have been were reported to modulate P-glycoprotein (Pgp). Pgp is a efflux pump. Pgp interacts with a broad range of substances, and limits oral drug absorption. We have analyzed the influence of mate decoctions on intestinal Pgp activity, considering the wide use in our society of the same one, and possible importance that it would have in the kinetic variability that it is observed in different drugs that are substrates of this transporter. We have taken as a model to begin this study, the isolated and everted rat intestine sac. It was validated with two of its recognized substrates (rhodamine123 5µM and ^{3}H-digoxin 0.2µCi- 50uM) and one inhibitor (verapamil 100µM). To this end, the isolated tissues were incubated with the respective substrates and the efflux kinetics analyzed during 1 hour. The lineal transport was verified for both substrates, measured in a spectrophofluorimeter and liquid scintillation counter, respectively. Verapamil 100µM inhibited the Rhodamine123 transport by 38 % ($p<0.001$) and also antagonized the ^{3}H-digoxin efflux by 44.3 % ($p<0.001$). When evaluating the effect of the decoctions of mate (2%P/V), we observed that it presents an inhibitory effect on the Pgp activity by 44 % ($p<0.001$). On the other hand chlorogenic acid (100µM), which is one of the components which appears in the greatest concentration in the extract, not produced any

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OXIDATIVE STRESS AND NITRIC OXIDE SYNTHASE ACTIVITY ARE INCREASED IN RATS WITH SUBTOTAL PANCREATECTOMY.

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In a previous study we have shown that a decreased acetylcholine-induced relaxation (Ach-IR) is obtained in aortic rings of rats with subtotal pancreatectomy (Ppx). This effect is amplified by pre-incubation in a high glucose solution -HG- (44mM/l) which induces superoxide anion accumulation. When nitric oxide (NO) combines with equimolar concentrations of superoxide, peroxynitrite (ONOO-) is formed. This compound is a powerful oxidant and cause cellular toxicity. The aim of this study was to evaluate the presence of oxidative stress and the activity of nitric oxide synthase (NOS) in Ppx rats. Fasting blood glucose determinations and oral glucose tolerance tests (OGTT) were performed. Glucose was measured and lipid peroxides in plasma were estimated colorimetrically by evaluating thiobarbituric acid reactive substances (TBARS). NOS activity was estimated in aortic tissue by monitoring the formation of L-[14C]citrulline from L-[14C]arginine. OGTT was altered in Ppx rats 60 min after glucose load (control: 5.62 ± 0.16 vs Ppx: 9.64 ± 0,28 mM/l, $P< 0.001$). TBARS and NOS were higher in Ppx than in controls (Ppx: 1.90±0.09 vs control 1.60±0,06 nM/l, $P< 0.05$) and (Ppx: 720.8 ±30.3 vs control 577.9±34.4 pM/g/min, $P< 0.01$). Conclusions: These results shows that in rats with Ppx TBARS and NOS activity are increased. The decreased Ach-IR obtained in aortic rings incubated in a HG medium could be related to an increased oxidative stress. Although an elevation of NOS activity was observed, a decreased bioability of NO, probably due to ONOO- formation, cannot be ruled out.

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NITRIC OXIDE AS MEDIATOR OF *BACCHARIS POLIFOLIA* GRISEB. GASTROPROTECTION IN RATS

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Baccharis polifolia Griseb, known as "quincha mali", is commonly used for its digestive properties. Gastroprotective activity and mechanism of action by *Baccharis polifolia* Griseb. extract (*Bp*E) were investigated. Role of nitric oxide (NO) in the gastroprotection induced by *Bp*E was evaluated.

Methods and Results: Twenty four hours before the experiments, Wistar rats were fasted. Absolute ethanol (EtOH) was employed as ulcerogenic agent (Method of Robert *et al.*, 1979). *Bp*E reduced ethanol-induced gastric mucosal damage ($p<0.001$ vs. control of absolute ethanol). L-NNA, NO synthase inhibitor, antagonised gastroprotective activity of *Bp*E ($p<0.001$ vs. *Bp*E + EtOH) . The last effect was reversed by L-Arg ($p<0.01$ vs. L-NNA + *Bp*E + EtOH; ANOVA and posterior comparison by Tukey-Kramer).

Conclusion: *Baccharis polifolia* Griseb prevents the formation of gastric lesions induced by absolute ethanol at a dose of 250 mg/kg. These facts support the use in traditional medicine of *Baccharis polifolia* to treat digestive disorders.

We conclude that the protection by *Baccharis polifolia* against ethanol-induced gastric mucosal injury is due, at least in part, to NO activity.

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HEPATOPROTECTIVE ACTIVITY OF A*RTEMISIA DOUGLASIANA* BESSER. STUDY OF ACUTE TOXICITY

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Artemisia douglasiana Besser (*Ad*), popularly known as "matico", have been used in folk medicine for gastrointestinal disorders. The aim of the present work was to study the hepatoprotective activity, using the model of experimental liver damage induced by carbon tetrachloride (Cl_4C) in rats and the acute toxicity in mice. Infusion (10%) was prepared. Hepatoprotective activity: serum aspartate (AST) and alanine aminotransferase (ALT) were determinated. The extract of *Ad* produced marked reduction of both, AST and ALT ($p<0.001$, ANOVA-Tukey), relative to the control group. Acute toxicity: mice were fasted for 4 hours and given oral increasing doses of dry extract, redissolved in water. It was administered to five (one group served as control) groups of 6 mice each (3 male and 3 female). The doses studied were 5-2000 mg/kg body weight and animals were observed for 14 consecutive days to register mortality or other toxic symptoms. The effects on the behavioural response have been investigated using an actograph. None of the animals treated with extract showed any visible symptoms of toxicity at dose as high as 2000 mg/kg. There were no signs on symptoms of restlessness, respiratory distress, diarrhea, convulsions, coma. The relative wet weight of lungs, heart, liver, spleen and kidneys were not significant vs. control group. *Ad* did not induce change on the spontaneous activity in mice. In conclusion, under the

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***EX-VIVO* EXPERIMENTAL MODEL FOR ASSESSING ENROFLOXACIN BIOTRANSFORMATION IN ENDOMETRIAL TISSUE OF HEALTH RATS**

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Enrofloxacin (EFX) is a broad spectrum antimicrobial (ATM) marketed in Veterinary Medicine for the treatment of several diseases. Biotransformation of EFX is mainly performed in liver undergoing de-ethylation to ciprofloxacin (CFX) (main active metabolite). Extra-hepatic biotransformation processes of this molecule are still unknown in several species. The main goal of this work was to evaluate the EFX endometrial biotransformation ability in rats. Uterine horns (n = 6) of three adult virgin rats were used in this study. After uterine horn extraction, a solution of EFX (10μg/mL) in glucose based broth Dulbecco, pH 7,4 were placed and incubated by immersion by 1 y 2 h at 37ºC. Liver was also incubated and used as positive control being it the maximum body biotransformation pattern. After chemical extraction, both tissues were analysed by HPLC with fluorescence detection (excitation 294nm y emission 500 nm). First outcomes obtained in this trial, showed that rat endometrial tissue is able for metabolising EFX to CFX in a rate roughly 2%, compared with the liver biotransformation pattern rate (5%). These results may contribute to the understanding of endometritis treatment and further studies are required for clarification.

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MILK EXCRETION OF ANTIPARASITIC DRUGS IN DAIRY SHEEP: RESIDUES IN MILK-DERIVED PRODUCT

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Ivermectin (IVM), eprinomectin (EPM) and moxidectin (MXD) are broad-spectrum endectocide antiparasitic drugs extensively used in food-producing animals. The patterns of IVM, EPM and MXD excretion in milk were comparatively characterized following their subcutaneous (IVM, MXD) (200 $\mu g.kg^{-1}$) and topical administration (EPM) (500 $\mu g.kg^{-1}$) to lactating dairy sheep. A pool of milk collected from all the animals in each experimental group was used for cheese elaboration. IVM, EPM and MXD concentrations were measured in milk and dairy products using an HPLC-based methodology with fluorescence detection. Residual concentrations of these compounds were recovered in milk up to 30 (IVM), 15 (EPM) and 35 (MXD) days post-treatment. During milk processing a high proportion of parent drug was found in the curd. IVM, EPM and MXD concentrations in the elaborated cheese tended to increase during the ripening period, reaching the highest residual level at 40 days cheese maturation. The scientific evidences shown here, indicate that IVM, EPM and MXD residues in cheese are between 3 and 5-fold higher than those measured in the milk used for its elaboration. The impact of these residual drug concentrations in milk-derived product on human safety are under evaluation.

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RELATIVE BIOAVAILABILITY OF AN ORAL SOLUTION OF INDINAVIR: DEVELOPMENT OF PEDIATRIC FORMULATIONS OF ANTIRETROVIRAL DRUGS.

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Bioavailability studies are indispensable since pharmaceutical forms for administration to special populations, such as pediatric patients, are lacking. The aim of the present work was to compare the bioavailability of an oral solution of indinavir (INDs) with a capsule formulation (INDc) in adult volunteers and to develop pediatric formulations based on microencapsulated, taste-masked IND.

Plasma concentrations of 6 volunteers were determined at 6 time points. Pharmacokinetic parameters were calculated using TOPFIT. Assessment of relative bioavailability (n=6) showed a Cmax of 3,3±1,2 µg/ml and 3,8±1,0 µg/ml after INDs and INDc administration, respectively. Tmax was significantly lower for the solution (0,5h) than for capsules (1,3±0,2h). No difference in AUC was found between both formulations (AUCs/AUCc=1,14).

In addition, new IND formulations were prepared by a double emulsion-solvent evaporation technique. Their bioavailability will be assessed in further studies.

This study demonstrated a faster absorption after INDs intake, achieving similar plasmatic levels as compared to INDc administration.

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PHARMACOKINETIC BEHAVIOUR OF A LONG-ACTING CEPHALEXIN FORMULATION IN COWS

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Objective: to determine the pharmacokinetic behaviour of a long-acting cephalexin formulation after i.m. administration in cows.

Material and methods: six Aberdeen Angus healthy adult cows, weighing 519.5 ± 33.26 kg received an i.m. dose of 7.5 mg/kg of a long-acting cephalexin formulation. Blood samples were taken at pre-established times. Drug plasma concentration was determined by a microbiological method using *Micrococcus luteus* ATCC 9341 as test microorganism. Plasma disposition curves were analyzed by a non compartmental model using PcNonlin software.

Results: cephalexin reached a Cmax of 5.6 ± 0.79 µg/ml at 2.08 ± 0.97 h. It showed an $AUC_{(0-\infty)}$ of 29.09 ± 8.71 µg.h/ml and an MRT of 4.12 ± 1.07 h. $T_{>CIM90}$ was 9.5 h (26.39% of the administration interval) for *S. aureus* and 3.5 h (9.72% of the administration interval) for coagulase-negative staphylococci.

Discusion: Previous studies have determined that cephalosporin efficacy is maximum when plasma levels remain upon the MIC of target microorganisms for at least 50-60% of the administration interval. Therefore, our results suggest that, at the recommended dosage (7.5 mg/kg every 36 h), this formulation does not fulfill the efficacy parameters established for a successful therapy

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ERYTHROMYCIN PHARMACOKINETICS IN PREGNANT GOATS AND PLACENTAL TRANSFER.

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The objectives of this study were to study the pharmacokinetics of erythromycin (ERY) administered by the intramuscular (i.m.) and intravenous (i.v.) routes to pregnant goats, and to determine the placental transfer of the drug. Six female pregnant goats with 106-125 days of pregnancy received an i.v. and i.m. dose of 10mg/kg and 15mg/kg, respectively, of ERY with a two weeks wash-out period. Blood samples were withdrawn at pre-determined times. In the same animals with 149 days of gestation, 10mg/kg of i.v. ERY was administered previous cesarean surgery was performed and blood samples from the mother, the umbilical vein, and aminotic fluid were taken. Drug concentration was determined by a microbiological method using M*icrococcus luteus* ATCC 9341 as test microorganism. Plasma disposition curves were analyzed by a non linear methods applying PcNonlin software. After i.v. administration ERY distribution was wide (V_{ss}4.45±2.41 l/kg), and elimination half life was 1.42 ± 0.51 h. After i.m. route ERY was totally available with a F value around 100%, T_{max} was 0.45 ± 0.83 h and C_{max} 1.03 ± 0.57µg/ml. Low ERY concentrations (0.33±0.20µg/ml) were detected only in two of the six foetuses. No drug concentrations were detected in amniotic fluid. These results suggest that ERY could be advisable for use in pregnant goats due its wide distribution, good availability and low access to fetal blood.

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FUNCTIONAL RELEVANCE OF ENDOTHELIAL ANGIOTENSIN-CONVERTING ENZYME (ACE) IN THE BIOLOGICAL INACTIVATION OF DES-ARG^{10}-KD (DAKD) IN HUMAN UMBILICAL VEIN (HUV).

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Introduction and goals: ACE is a metallopeptidase that degrades the endogenous BK B_1 receptor agonist DAKD in isolated HUV. ACE is present in the plasmatic membrane of endothelial cells. The aim of the present study was to evaluate the functional relevance of endothelial ACE in the biological modulation of the DAKD responses in HUV. **Methods and Results:** HUV rings were mounted under isometric tension in Krebs solution at 37ºC. After 300 min, concentration-response curves (CRCs) were obtained to DAKD (pCE_{50}: 8.92±0.06; n=6). The presence of Captopril 1µM, a selective ACE inhibitor, enhaced contractile responses elicited by DAKD (pCE_{50}: 9.28±0.02; p<0.05). Endothelium removal induced a significant leftward shift of CRCs to DAKD (pCE_{50}: 9.41±0.06; p<0.05). However, this potentiation wa not different from the response elicited by DAKD in presence of Captopril 1 µM in HUV intact rings. Captopril 1 µM failed to affect CCRs to DAKD in deendothelized rings (pCE_{50}: 9.26±0.04). No differences were observed in maximal responses. The state of endothelium was confirmed by histological studies. **Conclusion:** The present results indicate that ACE's enzymatic activity localized in endothelial HUV cells is functionally relevant in modulating DAKD vasoconstrictor responses in HUV rings

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TP RECEPTOR EXPRESSION IN HUMAN UMBILICAL VEIN (HUV). Errasti, A, Cesio, C, Pelorosso F, Souza, G, Daray, F, Rothlin, R. III Cátedra de Farmacología. Facultad de Medicina. UBA. Paraguay 2155, piso 9, 1121. farmaco3@fmed.uba.ar.

Introduction: TXA_2 mimetic U-46619 and prostaglandin-like compounds as 8-iso-PGE_2 and 8-iso-$PGF_{2\alpha}$ promote a potent and efficacious constriction of HUV. These effects are blocked by TP receptor antagonists suggesting that TP receptors are involved in this vessel (*Daray y col., Br J Pharmacol, 2003, 139; 1409-1416* and *Eur. J. Pharmacol., 2004, 19; 499:189-95*). Therefore, the aim was to analyze the expression of functionally established TP receptor in HUV by RT-PCR and Western blot. **Methods and results:** Total RNA (HUV) and proteins (HUV and platelets) were extracted employing Trizol and RIPA lysis buffer, respectively. RNA was quantified at 260/280nm and proteins were measured with Bradford at 595 nm. PCR products were electrophoresed on 2% agarose gels with ethidium bromide and photographed under UV. Endonuclease digestion was used to confirm product identity. Proteins were electrophoresed on 10% SDS-PAGE and electrotransferred onto nitrocellulose membranes which were blocked in TTBS buffer with 5% milk; then incubated overnight with anti human TP rabbit polyclonal antibodies. Membranes were revealed with alkaline phosphatase-conjugated goat anti-rabbit IgG. Immunoreactive bands were detected by chemiluminescence and compared with those obtained in human platelet. **Conclusion:** The results indicate that whole HUV express aTPα variant at mRNA level and a protein of similar molecular weight that one observed in human platelet, a rich source of TPα receptors.

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ANXYOLITIC EFFECTS OF THE NEUROSTEROID PREGNANOLONE: INFLUENCE OF GENDER AND HORMONAL BACKGROUND.

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It has been reported that neurosteroids exposure may influence both the pharmacological properties of the $GABA_A$ receptor as well as the manifestation of anxiety in both sexes. To test this hypothesis this study compared the behavioral effects of pregnanolone regarding different hormonal status and gender. Adult Sprague-Dawley male rats, intact and castrated, and females at 15th day of pregnancy were used (n = 8 animal/group). Pregnanolone 6 µM and Krebs (KRB) solution were inyected by intraventricular brain injection. Anxiety (total arm spent exploring the open arm: TOA) and locomotion activity (number of total arm entries: TLA) were tested on an elevated plus-maze. Our results showed that TOA was significantly shorter in treated pregnant females ($p < 0.05$, Students *t* test). On the other hand, castrated males treated with testosterone did not show any change in their anxiety levels. However, when treated with estrogen the animals were clearly more anxious that their corresponding controls. Interestingly, pregnanolone was able of reverting this anxiolytic effect. From our results we conclude that: 1) progesterone was probably responsible of priming the pregnanolone effect in pregnant females; 2) the priming effect was sex-dependent since it was not present in

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"FITTING OF A WEIBULL DISTRIBUCION TO DATA OF A CONTROLLED RELEASE FORMULATION"

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The aim is to find a mathematical model that describes the percentage of drug dissolved in time t, for a controlled release formulation, in a in vitro study. The trial was performed using as active principle Paracetamol in alginate microparticles which placed in gelatin capsules covered with a sheet of alginate.

The experimental decision was carried out taking three glasses of 900 ml each one, the ones that contained a simil gastric intestinal fluid in which was varying the pH since 1.5 until 7.2, during the trial.

The model presented is associated to the probability distribution of Waybill, and is appropriated to characterize the drug release in systems formed by polymeric matrixes.

To perform the fitting of the percentage of drug released M (t) in time t, based on the Weibul's distribution , is a matter to find a function $M(t) = 100.(1 - e^{-at^b})$, where t is the time since the active principle begins to be freed, *a* is a scale parameter and *b* is a shape parameter . The coefficients *a* and b of the fitting model were calculated applying the Gauss Newton's numerical method.

The values obtained made a good fitting to the data, what represent an alternative process to compare dissolution profiles.

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MORPHOLOGIC ALTERATIONS OF MALE REPRODUCTIVE TRACT IN MICE FED WITH A SOYBEAN DIET SUPPLEMENT .

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Isoflavones have been shown to affect androgens levels at interacting with estrogen receptors and multiple other molecular targets. The consequences of dietary soy isoflavones were examined in reproductive tract of male mice NMRI descendents of mothers fed with a soybean diet supplement. In this study, the effects of exposure to dietary soy for sixty mice pregnant were examined during organogenesis period of gestation. Adult male mice were obtained from a multi-generational assay where the mothers was fed diets containing soy protein supplemented with increasing amounts of LACTOVISOY®, a commercially available isoflavone supplement; a control group was maintained on a soy-free diet. The diets were designed to approximate human consumption levels and ranged from 1 - 1.65 mg / Kg. The results were analyzed by Fisher's exact test. An significant increase in the frequency of not descended testicle in right unilateral form was determined ($p<0.01$). Histological examination revealed a significant decrease in the spermatogonium and spermatozite I cells ($p<0.01$). These results suggest that in adult male mice, soy induces decrease in number of germinal epithelium cells. These findings open new routes to assay the hormonal profiles and the reproductive capacity with soy based diets. Supported by grant 016-ME-

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EFFECTS OF ZIPRAZIDONE ON AGGRESSIVE BEHAVIOR IN AN ANIMAL MODEL. *TORRECILLA, M; *CASASSA, F; *FACHINELLI, CC; **RODRIGUEZ ECHANDIA, E. *Lab. Psic. Exp. y Comp. UDA-CRICYT. **UNEFCO FCMédicas, UNCuyo.mtorrecilla@lab.cricyt.edu.ar

Objective: the action of Ziprazidone, an atypical antipsychotic proposed for the treatment of acute agitation, on the behavior of dominants, intermediates and submissive male pigeons in a conflictive situation for food competition was investigated.

Methodology: fifty pigeons maintained at 80% of their weight were divided in pairs of similar level of dominance for food competition trials. Twenty three types of behaviors were recorded by means of structured observation. In ranking sessions of five minutes of interactions, pigeons were ranked as dominants (n=14), intermediates (n=26) and submissives (n=10) as a function of their total time of aggression. In control sessions each pair received 1 ml of saline sixteen min before the interactions. In the experimental sessions ziprazidone (3 mg/1 ml) was administered to one of the animals; the other received 1 ml of saline.

Results: Dominant pigeons exhibited a significant difference in aggressive behavior ("T" test $p<0.05$). These was lowered by ziprazidone. Persuing which range 15% of total time in the controls disappeared in the experimental pigeons. The same occurs with hooking and pecking, the strongest aggressive behavior components in pigeons. In intermediate pigeons we found equivalent results. The present study shows that ziprazidone, a 5-HT and D2 dopamine receptors antagonist and inhibitor of 5-HT and NA reuptake was

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SPLANCHNIC O_2 CONSUMPTION DURING INFUSION OF AMMONIUM INTO THE MESENTERIC VEIN IN SHEEP.

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Metabolism of ammonium (NH_4^+) absorbed from the gut increases liver O_2 consumption, thus reducing energy available for body tissues. Short lasting episodes of high NH_4^+ absorption, frequently associated with the intake of diets rich in non-protein N or rapidly rumen degradable protein, were simulated in 5 wethers (42±3.4 kg BW), fitted with chronic indwelling catheters in aorta and splanchnic veins, via infusion of 340 µmol $NH_4^+HCO_3^-$/min into the mesenteric vein for 3 h, over 7 d. On the last day, portal and hepatic blood flows, and arterial, portal and hepatic concentrations of NH_4^+ and O_2 were measured during the last 90 min of the NH_4^+ infusion to calculate net mass transfers of NH_4^+ and O_2 across portal-drained viscera (PDV), liver and splanchnic tissues. Measurements were repeated on the following day, after withdrawal of the NH_4^+ infusion (control period). NH_4^+ infusion increased PDV NH_4^+ absorption (232 *vs.* 669 µmol/min; sed, 36; P=0.001), liver NH_4^+ uptake (276 *vs.* 698 µmol/min; sed, 64; P=0.007) and O_2 consumption by the liver (1169 *vs.* 1347 µmol/min; sed, 26,6; P=0.007), the PDV (1082 *vs.* 1355µmol/min; sed, 75; P=0.04) and the splanchnic tissues (2509 *vs.* 2926 µmol/min; sed, 88; P=0.006). Liver O_2 consumption equated 0.42 mol per mol of extra NH_4^+ removed, a value somewhat larger than the standard 0.3 mol O_2/ mol N predicted by the stoichometry of the ornithine cycle. Overall, incremental splanchnic energy expenditure was 455 kJ / mol NH_4^+ removed by the liver (based on 460 kJ/

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POPULATION PHARMACOKINETICS OF INDINAVIR IN PEDIATRIC HIV-INFECTED PATIENTS.

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Nineteen ambulatory patients receiving indinavir/ritonavir (r) were included, in which two plasma levels of indinavir were determined by HPLC (trough and peak). Demographic, anthropometric, clinical and immunological data was collected. Two polymorphisms of P-glycoprotein (P-GP) encoding gene (C3435T in exon 26 and C1236T in exon 12) that could alter indinavir bioavailability were studied.

11 of 19 patients yielded subtherapeutic levels (< 0.15 µg/mL). In 8 of them, dosage was increased to 400/100 mg/m^2 indinavir/r/12hs, but two of these patients remain achieving subtherapeutic levels. The disposition of indinavir was best described by a single compartment model with first order absorption and elimination using NONMEM. Population pharmacokinetic parameters such as clearance (θ_1=27.7 l/h),and distribution volume/weight(θ_2=1.97 l/kg)) were estimated. Patients who are homozygous for the mutation in both exons showed higher plasma levels of indinavir than the heterozygous ones. The results could suggest a relation between different P-GP polymorphisms, and the variability observed in indinavir plasma levels although the differences were not statistically significant.

OPCION PREMIO

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ALTERATION OF T LYMPHOCYTE ACTIVITY BY ZINC DEFICIT INVOLVES DIFFERENTIAL MODULATION OF PKC ISOENZYMES.

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Zinc (Zn) is essential to the structure of numerous signaling proteins that share cysteine-rich domains (zinc-finger structures) as a common motif and is known to be essential for all highly proliferating cells, especially those from the immune system. The aim of this study was to analyze the direct effect of Zn deficiency on normal T lymphocyte cultures and to ascertain the role that protein kinase C (PKC), an enzyme containing cysteine-rich domains, and its isoenzyme profile play in these actions. For this purpose addition of the intra-(TPEN) or extracellular (DTPA) specific zinc chelators in murine mitogen-induced normal T cell proliferation was studied. Both TPEN and DTPA exerted dose-response inhibition of normal T cell proliferation and viability, that was reversed by previous incubation of the chelator with adequate Zn concentrations. Zn chelators significantly diminished PKC activity in normal T lymphocytes. When analyzing PKC isoenzyme expression by western blot, a decrease in conventional □ and novel □PKC was observed in TPEN-treated normal T cells respect to control, that was reverted by Zn preincubation of the chelator. Moreover an increment in atypical PKC □ isoform was also found. As both PKC □ and □ are essential signalling molecules for normal T lymphocyte activity, diminished expression of these isoform would be related with inhibiton of mitogen-induced proliferation of normal T lymphocytes. Increment in PKC □

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MENTHOL EFFECT ON TRANSDERMAL RELEASE OF QUERCETIN

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Transdermal administration of many drugs is generally a problem owing to stratum corneum barrier. For this reason penetration enhancers, which ussually disrupt the highly ordered membrane structure, are added to formulations. Ideally, these enhancers are pharmacologically inert and have an inmediate but reversible effect on the stratum corneum. Due to important biological applications (reduction of arterial pressure and endotelial disfunction, anti-inflammatory activity, etc) of quercetin (Q), dietary flavonoid widely distributes in nature, in this work transdermal drug permeation of Q in Carbopol Gel (CG) and the influence of menthol as enhancer through abdominal pig skin was studied. Experiments were carried out using Franz vertical diffusion cells. Skin was pretrated with phosphate saline solution (PBS) pH 7.4 and it was used as receptor phase. At predetermined intervals 100 µl of receptor phase were removed and replaced with an equal volume of it. The quantity of drug released was determined by UV-VIS spectrophotometry at 255 nm. All permeation studies were performed by triplicate. Results of experiences of Q in CG with different percentages of menthol demonstrated that aproximately 2.5% was the best. Permeation parameters calculated were: J_m=4.81 x 10^{-7} g.cm^{-2}.s^{-1}, P=2.13 x 10^{-5} cm.s^{-1}, D=8.52 x 10^{-5} cm^{-2}.s^{-1}. Menthol affects skin permeation increasing quercetin solubility and altering stratum corneum barrier properties.

OPCION PREMIO

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ANTIBACTERIAL ACTIVITY OF XANTHATIN AGAINST *HELICOBACTER PYLORI*

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Xanthatin, was isolated from *Xanthium cavanillesii* Schouw, known as "abrojo". The aim of this study was investigate the antibacterial activity of xanthatin against *Helicobacter pylori* cultures from standard strains and clinical isolates. Two standard strains and ten clinical isolates from the Molecular Biology and Microbiology Laboratory stock (UNIFAG-USF) were used. All cultures were incubated in microaerophilic atmosphere at 37ºC for 48 h. and was evaluated by agar dilution method. Xanthatin showed antibacterial activity against all strains of *Helicobacter pylori* tested at a concentration of 1 mg/ml. In other study, twenty four hours before the experiment, Wistar rats were fasted. Absolute ethanol was employed as ulcerogenic agent (method of Robert *et al*., 1979). Infusion 5% of *Xanthium cavanillesii* was prepared. The extract (500 and 1000 mg/kg) and xanthatin reduced ethanol-induced gastric mucosal damage in rats (ANOVA and posterior comparison by Tukey-Kramer: $p<0.001$ vs. etanol control).

The results presented indicate that xanthatin and the extract of *Xanthium cavanillesii* prevent the formation of gastric mucosal lesions induced by absolute ethanol in rats, and xanthatin has significant antimicrobial properties against *H. pylori*. Xanthatin could represent an useful tool in relieving digestive disorders.

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HISTOLOGICAL CHANGES IN RAT LIVER TISSUE INDUCED BY *PHILODRYAS PATAGONIENSIS* COLUBRID SNAKE VENOM

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Little is known about the systemic effects caused by *Philodryas patagoniensis* colubrid snake venom. For that reason, in this work we studied the histological changes in rat liver tissue after i.v. administration of this venom. Thus, it was administered through a polyethylene catheter introduced into the iliac vein of rats anesthetized with chloral hydrate and heparinized. Four rats were used for each dose: 0.23, 0.45 and 0.90 mg of venom. Aliquots of blood were withdrawn at different time intervals for enzymatic determination of alanine aminotransferase, aspartate aminotransferase and creatine kinase levels. After 2 hr the animals were sacrificed, and samples of liver were taken to microscopic examination. Histological observations showed hydropic degeneration. Serum alanine aminotransferase and aspartate aminotransferase increased levels were demonstrated. Our results indicate that *P. patagoniensis* venom causes histological changes to liver tissue after i.v. administration. These changes are initiated at early stages of envenomation and may be associated with a behavioral or functional abnormality of the liver during envenomation. It is hoped that these results may provide new insights into

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P-GLYCOPROTEIN INVOLVEMENT ON IVERMECTIN disposition kinetics: INFLUENCE OF GENDER AND ADMINISTRATION ROUTE

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Ivermectin (IVM) is a substrate of the drug transporter P-glycoprotein (P-gp). The goals of the studies reported here were: a) to characterize a gender influence on the P-gp-mediated intestinal secretion of IVM (Phase I), and b) to evaluate the effect of the IVM administration route on the IVM/P-gp interaction in sheep (Phase II). Wistar (30 male, 30 female) rats received IVM (200 µg/kg) alone or co-administered with itraconazole (ITZ) (5 mg) (a P-gp inhibitor agent). Rats were sacrificed (between 6 and 72 h pt). Blood, gastrointestinal tissues and lumen contents were collected (Phase I). In Phase II, twenty-four (24) female sheep were divided in four experimental groups, which received IVM (50 µg/kg) by intravenous (IV) and intraruminal (IR) routes either alone or co-administered with ITZ. Plasma was collected up to 15 days and IVM concentrations measured by HPLC. ITZ induced a marked enhancement on IVM plasma Cmax and gastrointestinal tissues concentrations, which resulted higher in male (112 to 307 %) than in female (19-102 %) rats. The route of administration affected the IVM-P-gp interaction. ITZ did not change the IVM plasma disposition after the IV treatment. However, a markedly higher IVM systemic availability was observed in the presence of ITZ after the IR administration of the antiparasitic drug compared to the treatment without the P-gp-modulator agent.

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LOW MOLECULAR WEIGHT HEPARIN INTERACTS WITH THE C1Q SUBUNIT OF THE COMPLEMENT SYSTEM.

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Vascular injury induces a protrombotic-proinflammatory programme. Low molecular weight heparins (LMWHs) are heterogeneous glycosaminoglycans (GAGs) that exert antithrombin and anti-X_a inhibition. We study the relationship between structure and biological activities for different commercial LMWHs, and particularly their interaction with the first protein complex of the human complement system (C1) and its subunit C1q. LMWHs employed were from Sigma, Syntex and Sandoz laboratories. Chemical analysis: sulfate content and molecular mass determination by polyacrilamide gel were performed. Biological activity *in vitro* studies: anti factor X_a, APTT and haemolytic complement assay were carried out. C1 and C1q isolation were performed as were described by Bing and Tenner, respectively. Interaction assays between LMWHs and isolated proteins were run under low ionic strength (25 mM), pH~6.3 and in the presence of calcium ions (2mM). LMWHs studied showed similar chemical characteristics (4,5-6.0 kDa, sulfate content 24.86±1.70μg%) and similar anticoagulant and anticomplementary activity (anti X_a 107 U/mg, IC_{50} 24 μg /ml, respectively). The specific interaction between LMWHs and C1 and C1q recruited a percentage of the GAGs that increased four times its anticoagulant activity (with C1 37.06% and with C1q 8.03%). Complete C1 complex is requiered for specific interaction, nevertheless a very small fraction of LMWH could interact with C1q. These

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INDUCTION OF ERYTHROPOIETIN-HYPERSECRETORY STATE BY ACTIVATION OF THE ANDROGENIC RECEP-TOR.

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Chronic administration of testosterone (T) induces an erythropoietin hypersecretory state (EPO-HS), as derived from abnormally higher EPO response to hypoxia in mice with transfusion-induced polycythemia. The present study was designed to test the hypothesis that the state is induced through activation of the androgenic receptor. CF#1 mice that were orchidectomized when aged 30 d received 3 weekly sc injections of 2.0 mg of T, 5α or 5ß-dyhidrotestosterone (DHT) for 2.5 wk. They received one ip injection of 1.2 ml of heterologous packed red cell on the 4th day after treatment and were exposed 10 h later to air maintained at 0.5 atm. for 15 h in an altitude chamber. EPO concentration in plasma collected at the end of the hypoxic exposure was determined by immunoassay (Medac, Hamburg, Germany). Polycythemia totally abolished the EPO response to hypoxia. It was significantly higher in mice treated with T or 5α-DHT. 5ß-DHT elicited no response. In mice similarly treated with T but studied at different times thereafter, it was found that the EPO-HS persisted for as long as 81 d. Data confirmed the experimental hypothesis and indicate that induction by testosterone of an erythropoietin-hypersecretory state requires the activation of the androgenic receptor.

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NEUTRALIZATION OF THE HEMOLYTIC ACTIVITY OF THE CROTALUS DURISSUS TERRIFICUS VENOM BY F(ab`$_2$) ANTI-CROTALIC PLA$_2$

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We examined the ability of F(ab´)$_2$ from IgG antibodies obtained in rabbits against PLA$_2$, to neutralize whole venom from *Crotalus durissus terrificus*.

PLA$_2$ was isolated from the whole venom by gel filtration chromatography (Sephadex G-75). Specific anti-sera was obtained by subcutaneous and intramuscular inoculation of rabbits with PLA$_2$ (700 μg.ml^{-1}) and Freund adjuvant. IgG antibodies were purified from rabbits anti-sera by FPLC and digested with pepsine to produce F(ab´)$_2$ anti PLA$_2$. Pepsine digestion was developed in a antibodies/enzyme ratio of 50:1 for 18 h at 37 ºC.

In order to evaluate the ability of the F(ab´)$_2$ anti-PLA$_2$ to neutralize the activity of the venom, Ouchterlony test, kinetic inhibition test and indirect hemolytic activity test were carried out. The neutralizing capacity of this anti-venom was comparable to that of commercial anti-serum raised against the whole venom.

These results strongly suggest that F(ab´)$_2$ against PLA may be considered as an anti-venom that would not produce adverse reactions and/or the inclusion of it as a supplement in polyclonal anti-venoms.

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DECREASED RELAXATION IN ASPARTAME INCUBATED AORTIC RINGS OF RATS IS POSSIBLY MEDIATED THROUGH A REDUCTION OF NITRIC OXIDE PRODUCTION.

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In a previous study we have shown that an impaired vascular reactivity is obtained in aortic rings of rats incubated with Aspartame (As). The aim of the present study was to evaluate the mechanisms involved. Rings of thoracic aorta were mounted on stainless steel hooks and suspended in tissue baths. Tension development was measured by isometric force transducers connected to an amplifier. At the end of the equilibration period, the maximal force generated by adding a depolarizing solution of KCl was determined. After washing, two rings were used as control and two were incubated in the presence of As (10^{-5} M and 10^{-6} M), and then cumulative dose-response curves to phenylephrine (Phe) and acetylcholine (Ach) were performed. As 10^{-5} increased Phe contraction and As 10^{-5} and 10^{-6} M diminished Ach relaxation. In rings with denuded endothelium incubated with AS+ and without (C), precontracted with Phe, concentration-response curves to sodium nitroprusside (SNP) were obtained (10^{-10}-10^{-5}M). No significant differences were observed in both groups during contraction and relaxation (SNP 10^{-6}: As+: 96.1±0.71 vs C: 94.97±0.97 % NS), Similar results were obtained in rings pre-treated with N-nitro-L-arginine. Conclusions: These results support the hypothesis that in rings incubated with As the decreased relaxation to Ach may be due to a reduction in nitric oxide production.

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ERYTHROID DIFFERENTIATION OF BONE MARROW CELLS INJURED BY TAXOL CORRELATES WITH BCL-X_L INDUCTION.

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The long form of B-lymphoma-x (Bcl-x_L), an outer mitochondrial protein, has been proposed to mediate the antiapoptotic action of erythropoietin (Epo) on erythroid progenitor cells.

We investigate in a time course study (1-10 days) Bcl-x_L induction on murine bone marrow (BM) cells after a single dose of Taxol (Tx) treatment (29 mg/Kg i.p). These expressions were compared to those evaluated after *"ex vivo"* Epo rh stimulation (BM cultures with 2 UI/ml Epo rh for 2 h, 37ºC, 5%CO_2). Bcl-x_L expressions (westernblotting) were correlated with total erythroid cells ($x10^6$/ femur) and hemoglobin-synthesizing erythroblasts (%Fe^{59} uptake).

On the 1st day post-Tx, BM erythroid cells fell 4 times compared to control ($p<0.001$), remained decreased until the 7th day ($p<0.05$) and returned to normality by day 10 post-Tx.

^{59}Fe incorporation on hemoglobin-synthesizing erythroblasts post-Tx treatment revealed less isotopic uptake than control between 1 to 5 days ($p<0.01$). However, % ^{59}Fe uptake returned to normality from the 7th day until the end of the experience. Epo rh *"ex vivo"* treatment of BM cells caused overexpression of the apoptotic supressor protein, Bcl-x_L, between 7 to 10 days ($p < 0.01$) whereas it remained under control values from 1 to 3 days.

These results suggest that Bcl-x_L does not mediate the antiapoptotic effect of Epo rh, but it prevents ineffective erythropoiesis due to apoptosis in late-stage, hemoglobin synthesizing erythroblasts.

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MITOCHONDRIAL mNaXCa IN CARDIAC ISCHEMIA-REPERFUSION: CALORIMETRY OF CLONAZEPAM

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During ischemia-reperfusion (ISQ-REP) Ca homeostasis and contraction are affected. We found that clonazepam (CLO), which inhibits the mNaXCa, reduced ischemic contracture (ΔLVEDP), pressure development (P) and heat released (Ha) of beats during REP, both in control (C) and in 25 mM K-0.5 mM Ca-cardioplegic (CPG) hearts (ISHR, 2004). We now studied whether it was due to a lower Ca contribution from mitochondria (Mit) to sarcoplasmic reticulum (SR). Perfused rat hearts were stimulated at 1 Hz (30°C) in a calorimeter until steady P and H (heat, mW/g). Either C, CPG, C+CLO or CPG+CLO were perfused during 20 min, followed by 45 min of global ISQ, 45 min REP with Krebs-10 mM caffeine-36 mM Na (K-caf-Na) and 15 min Krebs-C (REV). REP increased ΔLVEDP in +73.3±5.6 mm Hg (at all conditions) and ΔH by (in mW/g, $p<0.05$): 8.3±0.8 (C-CLO) > 6.5±0.4 (C) > 4.5±0.5 (CPG)= 5.1±1.5 (CPG-CLO). ΔLVEDP decreased during REP at constant H, suggesting Ca-removal, while CPG and CLO decreased it more. From ΔH_{REP} it can be calculated a Ca flux (112-206 nmol Ca/g.s) compatible with those from Mit or SR. REV reduced ΔLVEDP with ΔH: +2.4±0.8 mW/g equivalent to a Ca efflux via sarcolemmal NaXCa (30 nmol Ca/g.s) when restoring $[Na]_o$. Results suggest: **a)** During ISQ-REP Mit gives Ca to myofilaments and to SR via the mNaXCa, **b)** Mit could remove Ca by consuming energy; **c)** CPG

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P-GLYCOPROTEIN, A MULTIDRUG EFFLUX PUMP, ACTIVITY IN HUMAN LYMPHOID CELL.

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The efflux transporters of the intestinal cellular membrane present a special interest since they are involved in processes of drugs absorption and bioavailability. The P-glycoprotein (Pgp) is distributed in different tissues from the organism, in addition to the intestine, among them the lymphoid cell. In this communication, the obtained preliminary results of the analysis of 10 volunteers of both sexes (between 20 and 60 years), are reported. Their state of health was evaluated by means of a clinical protocol that contains inclusion criteria. For the analysis of the Pgp activity, the isolation of mononuclear cells of peripheral blood samples is made, by the method of Ficoll-Paque. The isolated cells are incubated with rhodamina 123 (50uM) by 30min. Successive washings are made and then they are incubated for 3 hours in rhodamine-free media at 37º. Like negative control, the cells are incubated at 4ºC. The analysis is made by means of flow cytometry (FAC-SCAN Ortho-Cytoron). The lymphoid population distribution of rhodamine 123 indicates, that in normal conditions two populations with high (M1) and low concentration of fluorescent dye appears. In control condition % of M1 are of 39 +/- 6 %. At 4º are: 75 +/- 6% and in the presence of a Pgp inhibitor, verapamil (100uM): 77+/- 7%. Then we can conclude that the Pgp is the most important efflux

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FUNCTIONAL INVOLVEMENT OF M_1 AND M_3 MUSCARINIC RECEPTOR SUBTYPES IN ACETYLCHOLINE (ACH)-INDUCED VASOCONSTRICTION IN HUMAN UMBILICAL VEIN (HUV).

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Introduction: The present study attempted to characterize pharmacologically the muscarinic receptor subtypes mediating contraction of HUV. **Methods and results:** HUV rings were placed under isometric tension in Krebs solution at 37ºC. After 2.5 h, concentration response curves (CRC) to ACh or McN-A-343 (M_1 receptor selective agonist) were obtained in the presence or absence of different antagonist (applied 60 min before CRC). CRC to ACh were antagonized by several compounds: atropine (non-selective muscarinic receptors antagonist; pK_B 9.75), pirenzepine (M_1 receptors antagonist; calculated pA_2 7.58), methoctramine (M_2 receptors antagonist; pK_B 6.78) and pFHHSiD (M_3 receptors antagonist; calculated pA_2 7.94).PD102807 (M_4 receptors antagonist) was ineffective against ACh. Simultaneous exposure to pirenzepine and pFHHSiD produced a greater inhibition of Ach-CRC than obtained in conditions of individual antagonism. McN-A-343 produced a similar maximal response but a less potent one than ACh. The pA_2 estimated for pirenzepine against McN-A-343 was 8.54. **Conclusion**: The data obtained in this study demonstrates the role of M_1 muscarinic receptor subtypes and suggest the involvement of M_3 muscarinic receptor subtypes in ACh-induced vasoconstriction in HUV.

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EVALUATION OF ANGIOTENSIN CONVERTING ENZYME (ACE) EXPRESSION IN DEVELOPMENT LUNG TISSUE OF THE RAT.

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The lung is the central organ in extrinsic respiration and has several important non-respiratory functions. Renin-angiotensin system in the lung could mediate changes in vascular tone and permeability, fibroblast activity, and epithelial cell survival. Thus, variations in the renin-angiotensin system could be crucial in etiology of respiratory diseases. Furthermore, patients with respiratory syndrome show increased pulmonary ACE activity. Although the tisular ACE (sACE) has been described in the lung its physiological role have not yet been clearly established. The aim of the present work was to investigate ACE tissue expression in lung during development in rat. ACE expression in lung from Wistar rats was determined at different postnatal stages: PND1, PND8, PND15, PND30, PND60. The ACE expression was semi-quantified by multiplex RT-PCR. mARN was obtained from lung tissue and we set up a procedure for co-amplification of both ACE and GAPDH sequences. To set up the multiplex amplification, two sets of primers were used during the RT step. Very low expression level was observed at early stages of development while a high expression level was observed in stage PND60. This protocol allows us a semi-quantification of the expression level, thus a higher expression level was observed in adult (PND60) rat lung. We conclude that changes in sACE expression in rat lung

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INHIBITION OF *TRYPANOSOMA CRUZI* GROWTH IN VITRO BY CINNAMALDEHYDE.

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All the drugs recommended for the treatment of Chagas disease have serious limitations. New drugs are urgently needed. In previous SAFE meetings we presented the activity of methanolic extract of *Cinnamomum cassia* on *T cruzi* epimastigotes. In this work we assessed the activity of the main compound of *C cassia* bark methanolic extract: 3-phenyl-2-propenal (cinnamaldehyde). Concentrations ranging 3.8-380 µM were assayed on two stages of *T cruzi* clone Bra C_{15} C_2: A) epimastigotes cultured in F-29 medium at 27ºC. B) extracellular amastigotes in modified F-29 medium at 27ºC. Allopurinol was used as positive control. On the epimastigote stage cinnamaldehyde showed IC_{50}= 4.84 µg/ml (36.6 µM) and *C cassia* Me(OH) extract IC_{50}=3.9 µg/ml. On the amastigote stage cinnamaldehyde showed $IC_{50} < 5$ µg/ml. Our inhibition values are similar for those reported for benznidazole (the reference drug for Chagas disease) IC_{50}= 1.6-8.4 µg/ml. These results allow us to suggest that cinnamaldehyde could be a potential drug for treatment of Chagas disease.

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MOLECULAR CHARACTERIZATION OF TUBULIN OBTAINED FROM *FASCIOLA HEPATICA* SUSCEPTIBLE AND RESISTANT TO TRICLABENDAZOLE

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Benzimidazole anthelmintics (BZD) alter the dynamic tubulin-microtubules equilibrium, inducing irreversible changes on different basic cell functions and death of target parasites. The broad safety margin of these drugs in mammals is based on a selective and greater affinity for parasite tubulin compared to host mammalian tubulin Triclabendazole (TCBZ) is an halogenated BZD used to control the fluke *Fasciola hepatica*. TCBZ intensive use has resulted in the development of resistant liver flukes. Using immunochemistry with specific monoclonal antibodies against β-tubulin, the aim of this work was determinate certain molecular features of tubulin obtained from TCBZ-susceptible (S) and resistant (R) *Fasciola hepatica* in comparison with rat brain tubulin The techniques used in this study were electrophoresis (PAGE) and immunochemistry with specific monoclonal antibodies against β-tubulin fractions. Some quantitative differences were observed. Tubulin identification was accomplished in mammalian and S *F. hepatica* samples. However, the used methodological approaches were unable to detect tubulin in *F. hepatica* resistant (R) to TCBZ. These preliminary results may represent a further step to understand the mechanisms of resistant to TCBZ in liver flukes.

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INVOLVEMENT OF THE CD95(FAS/APO-1) RECEPTOR SYSTEM ON HEMATOPOIETIC APOPTOSIS INDUCED BY TAXOL.

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CD 95 (Fas/APO-1) system regulates several physiological and pathological processes of cell death. The aim of this work was to evaluate CD95 expression (immunoblotting) in a time-course study on hematopoietic recovery (0–10 days) using a murine model following a single dose of Taxol (Tx, 29 mg/kg i.p) in bone marrow (BM) cells with or without “ex vivo” human recombinant erythropoietin stimulation (Epo rh 1 UI/ml). Variations of CD95 expression were correlated with BM cellularities and apoptotic indexes (TUNEL assay). We noticed, on the 1st day post Tx, the maximal apoptotic index (24 ± 0.81 % $p<0.01$) and the minimal BM cellularity (28 ± 4.2 % under control $p<0.001$). Apoptosis returned to normal values (3.08 ± 0.61%) by the 3rd day, while BM cellularities decreased until the 4th day and started to recovery from day 5 post Tx.

Up regulation of the cell death receptor expression (CD95/Fas) was significantly noticed between the 1st and 2nd days ($p<0.01$ over control values). However, this expression decreased from the 3rd until the end of the experience. Evenmore, CD95/Fas patterns did not change with Epo rh *“ex vivo”* stimulation.

These results suggest that Tx changed CD95 receptor expression during hematopoietic recovery. These variations are directly correlated to hematopoietic cells proliferation. Moreover, Epo rh failed to cause changes in the pattern of CD 95 expression, suggesting that once apoptosis has been triggered, the addition of this hormone did not modify the course of apoptotic hematopoietic

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IN VIVO HEMATOPOIETIC RECOVERY AFTER ETOPOSIDE TREATMENT.

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Etoposide (ET) a topoisomerase II inhibitor widely used in cancer therapy, is suspected of inducing severe alterations on hemato-poietic progenitors.

We used an in vivo murine model to investigate the effects of a single dose of ET (40 mg/kg, i.p.) on the hematopoietic recovery.

We determined in a time course study (0-20 days) hematological peripheric (Hb, Hct, reticulocytes, WBC, RBC counts) and bone marrow parameters (BM cellularities, viability, apoptosis and mitosis). Changes in erythropoiesis and myelopoiesis were evaluated from BM and peripheral blood experimental outcomes.

Data show that ET caused a reduction of BM viability ($p< 0.05$) cellularity ($p< 0.01$) and mitosis ($p<0.01$) from 2 days while apoptosis increased ($p < 0.01$) at 2, 5 and 15 days. Erythroid and myeloid BM cells showed a decrease after 2 days of ET injection ($p < 0.01$). Since myeloid absolute cell counts returned to normal values by the 5th day and erythroid cells restitution were noticed by the 20th day post ET, drug injury seemed to be stronger on erythroid than on myeloid lineage. Moreover, all peripheral blood parameters decreased at 2 days post-ET.

These results suggest ET caused an acute and deep injury on BM hematopoiesis by the 2nd day. In addition, myeloid and erythroid lineages showed different temporal patterns of

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ROLE OF THE SEX IN MORPHINE PROPERTIES: RELATIONSHIP TO PLASMA MORPHINE AND NALOXONE LEVELS. [1,2]Diaz S, [1,2]Hermida P, [1]Joannas L, [1]García C, [3]Villaamil E, [3]Ridolfi A, [3]Olivera M, [1,2]Rubio M, [1,2]Balerio G. [1]ININFA (CONICET), [2]Cát. de Farmacología, [3]Cát. de Toxicología, FFyB (UBA) Junín 956 5º piso, Bs. As. sildiaz@ffyb.uba.ar

Sex differences have been observed for morphine (MOR) analgesia as well as for MOR withdrawal syndrome precipitated by naloxone (NAL). Our purpose was to evaluate the involvement of the sex in the analgesic response to chronic MOR and NAL-precipitated withdrawal and whether these possible sex differences might be due to differences in MOR and NAL plasma levels. Swiss mice were rendered dependent by i.p. injection of MOR (2 mg/kg), twice daily for 9 days. On the 10th day dependent mice received NAL (6 mg/kg, i.p.) after MOR. The analgesic response and withdrawal signs were determined by the hot plate and the open field, respectively. In addition, MOR and NAL plasma levels were measured by GC and HPLC, respectively at different times. No sex differences were found for the analgesic response to chronic MOR, whereas the expression of MOR withdrawal signs was more marked in males Pharmacokinetic analysis showed a slight difference between male and female MOR concentration curves, whereas no sex differences were observed between NAL disposition curves. The analysis of kinetic and dynamic results also indicates a delay between the time-course of MOR plasma levels and the time-course of the analgesic effect in either sex. In conclusion, although males and females respond differentially to NAL-precipitated withdrawal, a pharmacokinetic factor would not appear to

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NEUROTOXICITY OF KAINIC ACID IN THE MEDIAL NUCLEUS OF EXTENDED AMYGDALA (MEXA) IN MALE RATS. DIFFERENCES IN THE ROLE OF TESTOSTERONE AND DIHYDROTESTOSTERONE.

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Testosterone (T) promotes neuroprotection indirectly via enzymatic conversion to estradiol by aromatase. If androgens can protect neurons from toxic-epileptogenic insults in adult animals remains unclear. We investigated this issue by modulating androgenic status in male rats prior to challenge with Kainic Acid (KA). Adult male rats (n=4 per group) were maintained in the following conditions: a) gonadectomized (GX) to deplete endogenous androgens, b) GX + replacement with dihydrotestosterone (GH-DHT), c) GX + replacement with T, (GH-T), d) normal control animals (NC). E) Control animals of each group were injected with saline. Three days after supplementation of hormones, all groups received a single IP injection of KA (8 mg/kg). Twenty-four hours after the KA injections the brains were fixed, sectioned and stained for neuronal death with the Amino-Cu-Ag technique. Neurons were counted using a Scion Program (NIH). Data were analysed with one way ANOVA followed by the Fisher post hoc test.

Results: when compared GX vs NC, we observed more neuronal death in the GX groups ($p<0.001$). T showed a partial neuroprotective effects against neurodegeneration. However, DHT replacement afforded no neuroprotection. Thus, this findings suggest that the effect of T on neuronal protection depends on the balance of its conversion to estradiol by the enzime aromatase.

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STUDY OF THE HYPOTHALAMIC CARDIOVASCULAR EFFETS OF ANGIOTENSIN-(1-7) IN SINOARTIC DENERVATED RATS.

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The aim of the work was to study the cardiovascular actions of the intrahypothalamic injection of angiotensin-(1-7) (Ang-(1-7)) and its effects on the pressor response to angiotensin II (Ang II) in sinoaortic denervated (SAD) rats and animals with simulated operation (SO). A carotid artery was cannulated for mean arterial pressure (MAP) measurement and a needle was inserted in the anterior hypothalamus for administration of peptides. Intrahypothalamic injection of Ang II (50 ng) induced a significantly greater presor response in SAD rats (□MAP= 13±2 mmHg, n=5, $p<0,05$ vs SO rats) with respect to OS group (□MAP= 7±1 mmHg, n=5). Administration of Ang-(1-7) did not induce changes of MAP in both experimental groups. The coadministration of Ang-(1-7) with Ang II diminished the pressor effect of Ang II in SAD rats (□MAP= 4±2 mmHg, n=5, $p<0,05$ vs Ang II administration), but it did not modify the same one in SO animals (□MAP= 8±2 mmHg, n=5).

Our results demonstrate a greater pressor response to Ang II in SAD rats comparing to control animals, indicating a sobreactivity of hypothalamic angiotensinergic receptors. Concomitant administration of Ang-(1-7) with Ang II reduced the pressor effect of Ang II SAD animals, suggesting that the hypothalamic renin-angiotensin system could limit Ang II sobreactivity by means of a greater Ang-(1-7) production.

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EFFECT OF THE BODY CONDITION SCORING ON PHARMACOKINETICS OF STREPTOMYCIN IN LACTATING GOATS.

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The aim of the present work was to assess comparatively the pharmacokinetic behaviour of streptomycin in goats with different body conditions. Twelve lactating healthy goats in production and with normal and diminished body conditions were used in two trials (T1 and T2, respectively). The animals received a monodose of streptomycin (10 mg/kg b.w.) by intravenous route. Blood samples were drawn before and after streptomycin administration. Results: The body condition scoring (BCS) were: BCS (T1)= 2.98 ± 0.18) and T2= 2.66 ± 0.2 ($p< 0.05$). The time of elimination ($t_{½}$ (T1)= 2.4 ± 0.6 and (T1)= 3.3 ± 0.9 h), the area under the curve $AUC_{(0-10)}$ (T1)= 88.2 ± 29.6 and (T2)= 130.3 ± 72.5 mg/ml/h) as well as the clearance (CL (T1)= 118.5 ± 52.4 and (T1)= 96.1 ± 20.6 (ml/h)/kg) were significantly different ($p<0.05$). In conclusion, the body condition scoring of a herd should be considered at the time of performing the streptomycin dosage regimens.

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INESCAPABLE STRESS INDUCES CYTOSKELETON DAMAGE THROUGH INCREMENT IN LIPID PEROXIDATION AND GSK-3□ ACTIVATION.

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Inescapable stress (IS) induces a decrement in the light neurofilament subunit (NFL) in the hippocampus, probably linked to the dendritic atrophy observed in experimental models of depression. NFL reduction could be due to either increased oxidative stress or the hyperphosphorylation of cytoskeleton proteins by glycogen synthase kinase 3□ (GSK-3□), leading to proteolysis. We explored these mechanisms in rats exposed to IS.

Adult rats were exposed to 60 inescapable foot shocks (0.6 mA, 15 sec). Controls did not receive IS. One hour or 4 days later, lipid peroxidation and total glutathione (GSH) were employed as parameters of oxidative stress. Cytoskeleton hyperphosphorylation was estimated by Western blot analysis of □-catenin level, a substrate of GSK-3□. One hour after the IS an increment in lipid peroxidation (22%, $p < 0.05$ vs control) and a decrement in the □-catenin level (49%, $p < 0.05$ vs control) were observed, but no modification in total GSH or GSK-3□ levels were found. Four days after the stress only □-catenin levels remained decreased (19%, $p < 0.05$ vs control).

Our results provide preliminary evidences supporting the hypothesis that oxidative stress and GSK-3□ activation could be involved in the cytoskeleton damage observed after IS.

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ROLE OF TXA_2 IN 8-ISO-PGE_2 INDUCED CONSTRICTION IN HUMAN UMBILICAL VEIN (HUV).

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Introduction: Isoprostanes are a group of prostaglandin-like compounds produced *in vivo* by free radical-catalyzed peroxidation of arachidonic acid. 8-iso-PGE_2 is one of the most important isoprostanes and we have demonstrated that this compound induced constriction in HUV (*Eur. J. Pharmacol., 2004, 19; 499:189-95*). It has been demonstrated that endothelin and cyclooxigenase metabolites are involved in isoprostanes effects; therefore, in the present study we attempt to characterize if they are involved in 8-iso-PGE_2 induced contraction in HUV.

Methods and results: HUV rings were mounted under isometric tension in Krebs solution at 37ºC. After 2 h of equilibration period, concentration response curves (CRC) to 8-iso-PGE_2 were obtained. Pretreatment with the endothelin-converting enzyme inhibitor, phosphoramidon 10μM, not modified the CRC to 8-iso-PGE_2. However, pretreatment with indomethacin (COX-1 inhibitor), NS-398 (COX-2 inhibitor) and furegrelate (TXA_2 synthase inhibitor) induced a concentration-dependent rightward displacement of CRC to 8-iso-PGE_2.

Conclusion: The present results suggest that 8-iso-PGE_2 induced vasoconstriction in HUV is partially TXA_2 dependent

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PHENOTYPE DETERMINATION OF THIOPURINE METHYLTRANSFERASE IN ERYTHROCYTES BY HPLC

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Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of thiopurine drugs, which are used in cancer chemotherapy and as immunosuppressive agents. TPMT activity is controlled by a common genetic polymorphism that contributes to interindividual variability in drug response to thiopurine drugs. Because of the clinical significance of the TPMT genetic polymorphism, determination of the TPMT activity in red blood cells may contribute to individualize thiopurine treatment. The aim of this work was to develop a simple reverse-phase HPLC method, avoiding liquid-liquid extraction step, and allowing the direct injection of the sample after precipitation with 70% perchloric acid. The formation of 6-methylmercaptopurine (6MMP) was assessed after the incubation of 6-mercaptopurine (6MP) in red blood lysates. The method was linear between 25 ng/ml and 450 ng/ml ($r=0.989$). The TPMT activity determined in pediatric patients with acute limphoblastic leukemia (LLA) was 8.5-39.9 nmol/h/ ml PRBC with a mean of 19.12 nmol/h/ ml PRBC.

The procedure described in the present work avoids laborious liquid-liquid or solid-phase extraction and could be implemented easily for routine phenotypic analysis of TPMT in patients scheduled for thiopurine therapy

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FORMOCRESOL AND FERRIC SULPHATE ON MURINE MACROPHAGES BIOACTIVITY.

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Formocresol (FC) and Ferric sulphate (FS) are medicinal drugs used in pulp therapy in temporary teeth. The inflammation of the pulp tissue in primary and in permanent teeth promotes macrophagic activation, releasing proinflammatory cytokines. The aim of this study was to measure the bioactivity of murine peritoneal macrophages (MPM) at different concentrations of FC (1:10, 1:100, 1:1000) and FS (1:100, 1:1000,1:10000).

MPM suspensions (1x 10^6 cells/ ml) were obtained 3 days post-tioglicolate injection by peritoneal washings with ClNa. MPM cultures were incubated with FC (Buckley's formulation) and FS, against controls, for 15' and 30' at 5% CO_2 (37ºC). Results were obtained as the mean of four single samples by group.

The adherence indexes for FC were significant at 15' ($p<0.01$) in all concentrations. Adherence index with FS was significant only at 15' ($p<0.05$) with 1:100 dilution. All dilutions of FC caused statistical significant values for apoptotic indexes. FS induced the maximum apoptotic indexes with 1:1000 dilutions. Cellular viabilities and necrotic indexes were affected with 1:10 FC treatment for 30' while FS did not cause variations in these parameters.

This study suggests that both, FC and FS enhance MPM adherence. Moreover, FS caused lower necrotic effect than FC, suggesting that FS has less toxicity for macrophagic populations.

Key words: Formocresol- Ferric sulphate- Macrophage-

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ADRENERGIC REGULATION OF LYMPHOCYTE PROLIFERATION

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An important physiological mechanism that influences immune regulation involves the sympathetic nervous system. The predominant and more studied adrenergic (A) receptor on T and B cells is the α_2-A, however little is known about the β-A stimulation. In this study we examined the effect of β-A agonism on the regulation of lymphocyte activation. Lymph node or spleen cells from BALB/c mice were stimulated by mitogens and lymphocyte activation was monitored by measuring [^{3}H]-thymidine incorporation. The α_2-A agonist, clonidine, stimulated the activation of both, lymph node cells by concanavalin A – a T-cell specific mitogen - and spleen cells by LPS – a T independent B-cell mitogen-. The α_1-A agonist, cirazoline, did not stimulate lymphocyte activation. The natural agonist, noradrenaline (NA), shows a biphasic effect on T cell proliferation, stimulating and inhibiting at low and high concentrations respectively. NA also stimulates B cell proliferation. When determining the specificity of signal transduction pathway, α_2-A stimulation did not inhibit adenylyl cyclase activity, but results in the activation of protein kinase C (PKC). Using RT-PCR technique, we investigated the expression of α_2-A receptor subtypes. A fragment of the α_{2B}-A receptor was amplified in T and B cells. These findings describe that β-A agonists are able to modulate lymphocyte proliferation through α_{2B}-A receptor mediated-activation of PKC.

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CHRONOKINETIC STUDY OF CEPHALEXIN IN DOGS

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The purpose of this study was to identify if time of day administration modified cephalexin relevant pharmacokinetic parameters administered to dogs. Six healthy adult Beagle dogs were given a single 25 mg/kg dose of cephalexin as a 5% oral suspension at 10.00 and 22.00 h after an 8 h fast, with a 2 week washout period. Blood samples were taken at predetermined times. Cephalexin plasma concentration was determined by a microbiological method with *Micrococcus luteus* 9341 ATCC as test microorganism. Pharmacokinetic analysis was performed by a non compartmental model using PcNonlin software. Statistical analysis was performed using the Wilcoxon matched pairs test. Results (mean ± SD) showed that elimination half life after the 10.00 h administration (1.79 ± 0.26 h) was shorter ($p<0.05$) than the calculated after the 22.00 h administration (2.69 ± 0.96 h). No statistically significant differences were detected for other parameters such as area under the curve, mean residence time, and time to reach the peak concentration. We conclude that time of day administration affects cephalexin elimination when administered orally to adult dogs.

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ENDOMETRIAL TISSUE BIOTRANSFORMATION OF ENROFLOXACIN IN SUSCEPTIBLE ENDOMETRITIS MARES.

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Mares´ endometritis, is the third clinical relevant disease. Therapeutic for endometritis usually fails since the understanding of the pharmacokinetic and pharmacodynamic processes in uterus are not fully established. Enrofloxacin (EFX) is approved for the treatment of endometritis in mares. *In vivo* studies revealed that high concentration of EFX and its active metabolite ciprofloxacin (CFX) reach the infected endometrium. This study is addressed on assessing the extra hepatic biotransformation of EFX in mares´ endometrial tissue.

Seven mares endometrial biopsies (0,289 g ± 0,44) were taken and incubated at 37ºC with EFX (10mg/mL) by 1h (n=3) and 2h (n=4) in a dextrose (45mg/10mL) based broth carbooxigenated Dulbecco.

After incubation whole samples were analysed by HPLC by fluorescent detection. Acquired amount of EFX by the endometrial tissue were comparable between 1 and 2h post-incubation. EFX underwent *in vitro* metabolism to its active metabolite CFX in a rate of 1,8%. Studies performed *in vivo* reported a 5% rate of metabolism of EFX in liver. This endometrial metabolic activity may be pivotal for improving the conventional therapeutic regimens on endometritis. More studies assessing EFX metabolism on cell culture monolayer are being performed at our laboratory.

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CITOTOXIC ACTIVITY OF AMERICAN PLANT EXTRACTS.

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As part of our work in the study of the bioactivities of American native plants, we assessed the cytotoxic activities of dichloromethane (D) and methanol (M) extracts obtained from five plants: *Grindelia chiloensis*, *Cecropia pachystachya, Senecio bergii*, *Ilex brasiliensis and Ilex paraguariensis* on polymorphonuclear human cells. Their aerial parts were successively extracted with D and M. Two different assays were used to assess cell integrity and cytotoxicity of the extracts: (1) monitoring the uptake of the vital mitochondrial dye, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) by cell mitochondria and (2) determining the exclusion of the cationic dye propidium iodide (PI) by intact membrane of living cells. Due to their membrane damage, dead cells are quickly brightly stained with PI and the fluorescence is analyzed by flow cytometry. Both test were carried out at the extract concentration of 100µg/ml. In the MTT test *G. chiloensis*, *C. pachystachya, S. bergii*, showed citotoxic activities (46.5, 55.5 and 68.0 % of cell viability respectively). The methanolic extracts did not show any citotoxity activity. The PI test confirmed the citotoxic effect of the same three extracts, showing *G. chiloensis* the highest activity. Other assays are needed for establish the mechanims of this kind of effect.

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ANTIOXIDANT ACTIVITY OF AMERICAN NATIVE PLANT EXTRACTS.

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Naturally ocurring antioxidant compounds can reduce the harmful activities of free radicals and apparently to protect the structural integrity of cells and tissues. .In the present work we assessed the total antioxidant capacity (TAC) of methanolic extracts obtained from eigth native plants of America : *Blepharocalyx tweedie, Cecropia pachystachya, Senecio bergii, Minthostachys mollis Grindelia chiloensis, Mentha x rotundifolia, Ilex brasiliensis y Bauhinia forficata* using different experimental models : a) scavenging of DPPH and ABTS $^{\bullet+}$ radicals, b) the reduction of Fe $^{3+}$ in the FRAP assay and c) inhibition of the lipid peroxidation of rat brain homogenates measured as tiobarbituric acid reactives substances (TBARS). Total phenol content of extracts were determined by the Folin Ciocalteau reagent.

All extracts were able to reduce the Fe $^{3+}$ in the FRAP assay with ranges between 280 – 940 eq ascorbic acid / mg dry extract and there was a very good correlation between the phenol content and the reducing activity (R^2 = 0,942- $p < 0.01$). *C. pachystachya e Ilex brasiliensis* exhibited the highest TAC in the DPPH and ABTS $^{\bullet+}$ tests with CI_{50} values below 10 µg/ml.When tested in brain homogenates at a concentration of 100µg/ml, only *C. pachystachya* inhibited the TBARS production with percentages of inhibition greater than 50 (77.9 %).

Our results show that the *C. pachystachya* metanolic extract is an important source for the isolation of compounds with a great total antioxidant activity.

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