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The abstracts from XXXVIII Annual Meeting have been revised and evaluated for the cientific committee.

C 01

VASCULAR EFFECTS OF ALDOSTERONE

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Aldosterone (Ald) acting on smooth muscle and endothelial cell induces vascular alterations through endocrine and/or paracrine mechanisms. These alterations affect vasoreactivity, oxidative status, endothelial function, and produces inflammation, stiffness and fibrosis.

Endothelial function and vascular reactivity: Ald contribute to endothelial dysfunction, which is characterized by reduced endothelium-dependent relaxations. This effect may be the result of a reduction in NO release and/or an increase in NO inactivation. An increase in NO metabolism is associated with an increase in superoxide anions, which scavenge NO to form peroxynitrite. Reactive oxygen species (ROS) production has been involved in the deleterious effects of Ald on vascular beds. Vascular NADPH oxidase activity and ROS production are increased by Ald treatment in several experimental situations. Endogenous Ald participates in the vascular alterations associated with hypertension in rats. We reported that treatment of SHR with eplerenone, enhanced Ach-induced relaxations. This effect of eplerenone was associated with increased aortic expression of eNOS mRNA expression and a reduction of NADPH oxidase mRNA expression, suggesting that endogenous Ald is related with endothelial dysfunction and a diminished NO availability by increasing oxidative stress in SHR. One of the earliest identified effects of Ald was its capacity to modulate vascular smooth muscle tone, through the modification of both vasoconstrictor and vasodilator responses. Two mechanisms have been associated with changes in vascular tone. A genomic mechanism, acting through protein synthesis, and a non-genomic mechanism that modulates intracellular Ca^{2+}, cAMP levels, Na+/H+ exchanger activity and phosphorilation of signalling molecules.

Inflammation: Studies from our laboratory suggested that endogenous Ald participates in the vascular inflammatory process associated with hypertension in the SHR. In this study treatment with eplerenone reduced enhanced vascular expression of cytokines through the modification of NFKb/IkB system. Ald-stimulated production of superoxide anions is a feasible mechanism able to activate NFkB, and the genes regulated by this transcription factor. Ald administration in rats increases coronary expression of COX-2, osteopontine and macrophage chemoattractant protein-1, supporting the notion that this mineralocorticoid is importantly involved in vascular proinflammatory process. This inflammatory response to Ald could be mediated in part by a fall in magnesium content and Ca^{2+} loading of peripheral blood mononuclear cell which was associated with an enhancement of H2O2 production.

Structural changes: Besides functional effects, chronic administration of Ald was able to induce structural vascular alterations, including hypertrophy, remodeling, stiffness and fibrosis. Most of the studies on Ald and development of fibrosis focused preferentially on the heart, with only a few recent reports have been focused vascular fibrosis. Vascular fibrosis involves the accumulation of extracellular matrix protein such as collagen, elastin, fibrillin, fibronectin and proteoglicans in the vascular media. The mechanisms involved in vascular fibrosis as mentioned for smooth muscle cell hypertrophy, seems to be mediated by increased ROS and endothelin-1 production, as well as salt loading.

C02

Role of aPKCζ and stem cell polarity signaling in tumors of the Central Nervous System

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Cell polarity is the characteristic that confers structural asymmetry within a cell. Polarity or structural asymmetry of compartments within a cell serves to coordinate the hierarchical and informational complexity of signal input, as well as direct vectorial, functional responses such as, polarized secretion, directional migration or asymmetric cell division. For example, cell polarity is crucial for keeping the developmental potential of stem cells at bay. Pathways that regulate cell polarity, when usurped or inappropriately activated, are very likely to lead to cancer and metastasis. However, the signaling pathways and cellular mechanisms that regulate the asymmetric localization of adherens junctions and cell polarity in neural progenitors remain elusive. We show that atypical protein kinase Czeta/lambda localizes at the apical membrane of proliferating neural stem cells in developing chick neural tube. The precise subcellular compartmentalization of this kinase activity provides an instructive signal for the apical assembly of adherens junctions in a PI3-kinase,Rac/Cdc42 signaling-dependent pathway and regulates neural stem cell polarity. Disrupting the endogenous compartmentalization of aPKCzeta activity led to the aberrant migration and increased proliferation of neural progenitors resulting in ectopic neuroblastic rosettes,structures reminiscent of Homer-Wright rosettes that are characteristic of primitive neuroectodermal tumors (PNETs) or ependymoblastomas. Thus, it is very likely that aberrant aPKCzeta signalling and disruption of polarity pathways can lead to tumorigenesis.

C 03

Molecular images as a tool in research. From Radiopharmacy to Radiopharmacology.

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The rapidly emerging biomedical research discipline of Molecular Imaging (MI) enables the visualization, characterization and quantification of biologic process taking place at the cellular and sub-cellular levels within the intact living organism. The overall goal of MI is to interrogate biologic process in the cell of a living subject to report on and reveal their molecular abnormalities that form the basis of disease. This is in contrast to classical diagnostic imaging where documented findings are the result of the end effects of these molecular alterations, usually in the form of macroscopic and well-established gross pathology. MI includes the field of Nuclear Medicine (SPECT and PET) and other strategies that do not depend on radioactivity to produce imaging signals (optical, bioluminescence and Magnetic Resonance). The emergence of MI strategies has made possible the achievement of several important biomedical research goals that open the door to advancement of study in molecular medicine. These various accomplishments include: (1) development of non invasive "*in vivo"* imaging methods to reflect gene expression and more complex events such as protein-protein interactions; (2) ability to monitor multiple molecular events near simultaneously; (3) capacity to follow cell trafficking and cell targeting; (4) optimization of drug and gene therapy; (5) capability of imaging drug effects at a molecular and cellular level; (6) assessment of disease progression at a molecular pathologic level; (7) advancement of the possibility of achieving all the above mentioned goals rapidly, reproducibly and quantitatively, in support of monitoring a time-dependent manner the experimental, developmental, environmental and therapeutic influences on gene products in a single living subject. Although many laboratory based proof-of-principle and validation studies have been conducted using MI approaches, a great deal more experimental research will be necessary to translate MI into routine clinical practice.

MI assays offer four significant advantages over conventional techniques used in *"in vitro"* and cell culture biologic research. These advantages include assessment of whole animal phenomena, repeatability, functionality and quantification.

Radiopharmacy is actually a well established discipline that supports the clinical Nuclear Medicine imaging. It grows on the basis of Radiopharmacology and other disciplines that helped it to develop and convert labeled compounds in Radiopharmaceuticals that are routinely used in clinical Nuclear Medicine. The aim of this field is to study the functionality of tissues and organs in a living organism. In the last years this discipline was the first area to grow towards the MI due to the advances in labeled probes, equipment and principally, with the intervention of professionals of different areas, moving again to the basic Radiopharmacology.

In this conference it will be presented some basics in molecular sciences, with emphasis in Radiopharmacology, and the fundamentals of molecular imaging in clinical and experimental pharmacology including how imaging can be used, to assess specific molecular targets with the belief that in near future, specific imaging of such targets will allow earlier detection and characterization of disease, earlier and direct molecular assessment of treatment effects, and a more fundamental understanding of the disease processes.

M 01

Chronic treatment with high doses of corticosterone decreases cytoskeletal proteins in rat hippocampus

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Hypercortisolism is a common trait of Cushing's disease and depression. These two disorders also share hippocampal volume decrease and cognitive symptoms as for example memory impairment (Starkman et al., 2001; Campbell & MacQueen, 2003).
When hypercortisolism is induced by exogenous administration of glucocorticoids or by exposure to chronic stress in experimental animals, alterations in the hippocampus have also been found. In the above mentioned cases, the atrophy of apical dendrites of pyramidal neurons has been reported by means of Golgi staining (Wolley et al., 1990; Magariños & McEwen, 1995; McKittrick et al., 2000; Sousa et al., 2000; Bisagno *et al*., 2000).
Given that the hippocampus is one of the brain regions with higher glucocorticoid receptors density, and considering that its normal function is critically implicated in memory processes, it has become widely accepted that the effect of high doses of glucocorticoids may be responsible for the alterations in hippocampal trophism, and consequently for the hippocampal-dependent memory impairment.
Bearing in mind that understanding the mechanisms of these pathological conditions could be helpful to improve pharmacological treatments of Cushing's disease and depression, the objectives of our work were to improve the subcellular insight into the hippocampal trophic changes reported by means of Golgi staining in several hypercortisolemic models and to study the links between high glucocorticoid plasma levels, hippocampal dendritic atrophy and cognitive impairment.
In an experimental model of hypercortisolism we analysed the effects of chronic treatments (21 days) with two doses of corticosterone (100 and 200 mg) on different structural, functional and behavioural parameters in two hippocampal (CA3 and DG) and in one control area (Globus Pallidus). The following cytoskeletal proteins were measured by immunohistochemistry: microtubule-associated protein 2 and the high, medium and low subunits of intermediate neurofilaments (NF-H; NF-M and NF-L, respectively). Glutamate release was measured in an ex-vivo experiment by HPLC. Finally, the inhibitory avoidance test was conducted in order to evaluate animal's performance.
Our results demonstrated that both doses induce alterations in the intermediate neurofilaments studied in the hippocampus. However, only the higher one changed MAP-2 immunoreactive structures, a condition that also correlates with a diminution in the K^+-induced glutamate release profile and with a poor performance in the behavioural test applied.
All these results, altogether with the fact that in the control area MAP-2 reduction could not be demonstrated, and that all the cited changes seemed not to be related to neuron loss suggest that the pathways linking glucocorticoid receptors and cytoskeletal proteins could be an interesting target to be explored in the search for treatments to illnesses associated with hypothalamic-pituitary-adrenal axis dysfunction.

M 02

Role of aPKCζ and stem cell polarity signaling in tumors of the Central Nervous System

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Cell polarity is the characteristic that confers structural asymmetry within a cell. Polarity or structural asymmetry of compartments within a cell serves to coordinate the hierarchical and informational complexity of signal input, as well as direct vectorial, functional responses such as, polarized secretion, directional migration or asymmetric cell division. For example, cell polarity is crucial for keeping the developmental potential of stem cells at bay. Pathways that regulate cell polarity, when usurped or inappropriately activated, are very likely to lead to cancer and metastasis. However, the signaling pathways and cellular mechanisms that regulate the asymmetric localization of adherens junctions and cell polarity in neural progenitors remain elusive. We show that atypical protein kinase Czeta/lambda localizes at the apical membrane of proliferating neural stem cells in developing chick neural tube. The precise subcellular compartmentalization of this kinase activity provides an instructive signal for the apical assembly of adherens junctions in a PI3-kinase,Rac/Cdc42 signaling-dependent pathway and regulates neural stem cell polarity. Disrupting the endogenous compartmentalization of aPKCzeta activity led to the aberrant migration and increased proliferation of neural progenitors resulting in ectopic neuroblastic rosettes,structures reminiscent of Homer-Wright rosettes that are characteristic of primitive neuroectodermal tumors (PNETs) or ependymoblastomas. Thus, it is very likely that aberrant aPKCzeta signalling and disruption of polarity pathways can lead to tumorigenesis.

M 03

STRESS AND MOLECULAR MECHANISMS OF CRH SIGNALING TROUGH CRHR1 IN SPECIFIC AREAS OF THE CENTRAL NERVOUS SYSTEM

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CRH is the key mediator of the neuroendocrine, autonomic and behavioral responses to stress. Dysregulation of CRH/CRHR1 function in limbic structures and corticotrophs has been shown to be involved in the initiation of pathological stages, anxiety/depression and Cushing disease. We demonstrated that ERK1/2 activation mediates CRH effects in corticotrophs and in the central nervous system. In mice, icv CRH administration in vivo, acting through CRHR1, activates ERK1/2 in specific limbic areas (hippocampus and basolateral amygdala) related to external environment information processing and behavioral aspects to stress. Other regions related to central CRH system but involved in the processing of the ascending visceral information and neuroendocrine-autonomic response to stress, did not show CRH-mediated ERK1/2 activation. We have extended this analysis to a conditional mouse mutant (CRHOE) in which CRH overexpression is restricted to limbic brain. pERK1/2 levels were assessed in wild type (WT) and CRHOE in basal and after restriction stress. In absence of stress, pERK1/2 levels in the hippocampus and basolateral amygdala, as well as corticosterone values, were similar in WT and CRHOE, indicating that CRH overexpression in limbic structures does not alter the HPA axis. pERK1/2 signal in the amygdala was decreased in CRHOE under stress. There was no correlation between elevated corticosterone levels caused by stress in both groups and ERK1/2 activation, indicating that glucocorticoids are not responsible for the MAPK regulation in these areas. Binding analysis indicated that decreased levels of pERK1/2 cannot be explained by down-regulation of CRHR1, and suggest that conditions of chronic exposure to CRH would activate inhibitory mechanisms of ERK1/2. We are exploring the signaling network that mediates MAPK activation by CRH in hippocampal cell lines using molecular and pharmacological tools combined with proteomics. We generated stable cell lines expressing CRHR1 that respond to CRH as evaluated with appropriate reporters. Having determined ERK1/2 activation by CRH in this cell line, we started the search for proteins that interact with the intermediate kinase B-Raf that belongs to the canonical MAPK signaling cascade involving MEK1/2, and ERK1/2 in neuronal cells. We performed non-denaturing immunoprecipitations (IPs) in cell lysates from non-stimulated for CRH-stimulated cells. In immunoprecipitates with anti-B-Raf antibodies resolved by SDS/PAGE, a protein band was differentially detected, subjected to mass spec, and identified as vimentin. We investigated if the 14-3-3 proteins, functional signaling modulators expressed in brain, were associated to B-Raf by IPs with anti 14-3-3 antibodies in cell lysates. Our results indicate that 14-3-3 proteins, B-Raf and vimentin associate in a protein complex in CRH-stimulated cell lines of hippocampal origin. Determining the specificity of CRH signaling is crucial to identify new therapeutic targets.

M 04

Effect of stress on neural circuits involved in the emotional modulation

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Numerous findings indicated that experiencing relevant threatening situations leads to subsequent exaggerated emotional responses and anhedonia. Also, the emergence of a negative emotional state -characterized by distress, disphoria, anhedonia, anxiety and other exaggerated emotional reactions- is a common behavioral output following the abrupt abstinence from diverse drugs of abuse, providing a powerful motivational factor to relapse after discontinuation of drug administration. It is known that the amygdala plays a major role in the generation of such negative affective states. In this report we demonstrated that discontinuation from chronic ethanol (ETOH) and from benzodiazepine (BDZ) administration facilitated the formation of a new fear memory. Similarly, previous exposure to an uncontrollable stressor increased fear memory. In addition, the infusion of bicuculline into the basolateral amygdala complex (BLA), but not into the central amygdaloid nucleus, induced the same behavioral effect. Using extracelullar recording methods, we demostrated that in withdrawn or stressed animals show increased neuronal excitability and facilitated LTP in the BLA. Pre-treatment with midazolam prevented both the facilitating influence of stress on fear memory and synaptic excitability in the BLA. These data suggest that facilitation of fear conditioning could be causally related to increased neuronal excitability attributable to depressed GABAergic inhibition in the BLA. To test this hypothesis, inhibitory postsynaptic potentials (IPSPs) were studied in the BLA´s pyramidal neurons, using whole cell patch-clamp. In control animals, a small picrotoxin-sensitive IPSP was evoked by sub threshold stimulation of external capsule. When an action potential (AP) was evoked by supra threshold stimuli, the IPSPs were considerably larger. On the other hand, in DZM or ETOH withdrawn and in stressed rats IPSPs were significantly reduced. Firing of an AP by a depolarizing pulse applied through the patch pipette consistently evoked a glutamatergic antagonists-sensitive inhibitory postsynaptic current (IPSCs) of control animals. In contrast, in slices from BDZ or ETOH withdrawn or stressed animals, IPSCs were greatly decreased. It is concluded that a history of severe stress or withdrawal to hypnotic-sedative drugs results in the suppression of feed-back inhibition in BLA projection neurons, which represents an essential mechanism underlying the emergence of a negative emotional state, including exaggerated fear and anxiety.

1

Role of dopamine striatal receptors on the spontaneous activity of the neurons of thalamic reticular nucleus (TRN) Cipolone S, Lomastro J, Decono Ambesi M, Fillipini B, Pazo, JH. Facultad de Medicina, Departamento de Fisiología, Laboratorio de Neurofisiología, UBA.

In previous work from our laboratory we have shown that electrical and chemical stimulation of globus pallidus (GP) and substantia nigra reticulata (SNr) modify the spontaneous activity of the neurons in the TRN. This nucleus is the unique with inhibitory action on the thalamus, which allows a direct control of the thalamo-cortical and cortico-thalamic connections. The objetive of the present study was to clarify the role of the striatal DA receptors in modulating TRN neuronal activity. The experiments were performed in rat anesthetized with uretane (1.2 g/kg, i.p.). The spontaneous activity of the neurons of TRN was recorded with glass microelectrodes. The striatal DA receptors were stimulated with a microinjection of apomorphine (7μg / 0.5 μl). Thirty five neurons were recorded into the TRN. The 70% responded to activation of DA receptors and 30% did not respond. The 35% responded with excitation and the 35% with inhibition of their spontaneous activity. When the strionigral pathway was damaged the unique response obtained by stimulation of the DA receptors was inhibitory. The increased neuronal activity of the TRN could be attributed to simultaneous inhibition of the GP and SNr after activation of DA receptors and the inhibition to activation of the indirect pathway. The last could be attributed to the action of the direct pathway.

2

DISPOSITION OF SUPROFEN ENANTIOMERS IN CATS WITH TOXIC HEPATIC DISEASE

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Suprofen (SPF) is a non-steroidal anti-inflammatory drug (NSAID) who belongs to the 2-arylpropionic acids subclass. As result of its chiral characteristics, this compound have shown a marked enantioselective behaviour (i.e, chiral inversion of (R) to (S) enantiomer), with a high degree of interspecies variation. In addition, 2-arylpropionic acids are mainly eliminated by glucuronidation. Previous data have shown that SPF is either poorly or not inverted in all species studied. Our data about rac- SPF disposition in the cat are accordingly with that (Castro *et al*, 2001). The plasma, bile and urine disposition of racemic SPF was investigated to evaluate eventual modifications on SPF racemic disposition after the induction of toxic hepatic disease with CCL_4. In CCL_4 treated animals the AUCs of R-(-) and S-(+) SPF were greater than in healthy animals, the clearance smaller and the half-life shorter. No glucuronides were found in either bile or urine. The total amount of unmodified SPF excreted into bile was smaller in treated animals. In this matrix R-(-) SPF was twofold that of the concentration S-(+) enantiomer. Urine showed an inverse disposition. S-(+) concentration was greater than R-(-) and almost double of the same enantiomer in normal animals. The appearance of enantioselective processes at the bile and urine elimination of unchanged SPF in treated animals should not be underestimate taking into account the potentially clinical and toxicological consequences that it could represent.

3

"Calcium levels in brain areas of neonate rats exposed to 2,4-dichlorophenoxyacetic acid through mother`s milk"

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In the last years environmental pollution by pesticides has been increasing due to their extensive usage in agriculture, one of these is the phenoxyherbicide 2,4-D that have been related with a range of adverse health effects. In previous works, our laboratory had demonstrated that 2,4-D induced selective oxidative stress, mitochondrial alterations and cell death, in brain areas of neonate rats and cell cultures exposed to 2,4-D. The aims of the present work was to determine if the effects previously described involve an alteration of brain Calcium homeostasis. Therefore, we decided to study the calcium levels in brain, liver and serum, as well as in different brain areas in 2,4-D-exposed pups through their mother's milk. Ca^{2+} level decreased in all the brain areas studied, cerebellum (33%), prefrontal cortex (43%), striatum (65%), hypothalamus (46%), hippocampus (47%) and midbrain (34 %). No changes were observed in the other tissues. In conclusion the data indicates that 2,4-D induces a Ca^{2+} alteration that could be related with the oxidative stress and cell death.

4

Modification of 99mTc-MDP biodistribution in zinc deficiency

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Introduction: Previous studies demonstrated that Zn deficiency in animals affects both femur weight and Zn content. Aim: To determine if Zn deficiency modifies the 99mTc-MDP biodistribution. Materials and Methods: 20 female and 20 male Sprague Dawley rats were separated in 4 groups (n=10). The control groups (female: G1, male: G2) were fed during 3 weeks with commercial diet (Zn: 100 ppm) and the deficient groups (female: G3, male: G4) with Zn deficient diet (Zn: 3 ppm). After a 3-week treatment, the biodistribution of 99mTc-MDP was evaluated in all the groups. For this purpose, 500 μCi of 99mTc-MDP were intravenously injected to each animal and after 2 hours liver, spleen, gastrointestinal tract, kidneys, heart, lungs, femur and blood were extracted and their weight and activity was determined. The results were expressed as percentage of injected activity (Ainy%) and percentage of injected activity/gram (Ainy/gr%). Results: The results show that Zn deficiency modifies the 99mTc-MDP biodistribution in deficient groups (G3, G4). In deficient groups, a statistical increase in the Ainy/gr% of femur was observed when compared to control groups (G1: 1.14 ± 0.21% and G3: 2.09 ± 0.94%; $p<0.05$, G2: 1.26 ± 0.53% and G4: 2.53 ± 0.88%; $p<0.05$). These differences are sex independent. Conclusion: The biodistribution of 99mTc-MDP is modified in rats with induced Zn deficiency. The future perspective will be oriented to study the clinical significance of Zn deficiency when performing diagnostic studies with 99mTc-MDP.

5

Study of the mechanism of the antiproliferative action of limonene, isolated from *Tilia x viridis*, upon a lymphoma cell line: participation of nitric oxide.

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The monoterpenes are non nutricional dietary compounds present in essential oil of vegetable species. In previous studies, we demonstrated that limonene exerts antiproliferative action upon a murine lymphoma cell line (BW 5147). Nitric oxide has been implicated in control of cell proliferation. The aim of the present work was to analyze the participation of nitric oxide in the antiproliferative action of limonene, isolated from *Tilia x viridis*, analyzing the participation of total nitrites specially NO in cell cycle and apoptosis, by flow cytometry. Results (average ± SEM): proliferation on tumoral cells dpm (24 hs) Basal 22315± 1500; L10µg/ml: 19000 ± 1800; L10µg/ml+ L-NAME: 22800 ± 1900. Total nitrites 4 hs SI: L10µg/ml: 12.7 ± 1.5; L20 µg/ml: 16 ± 1.2 L 40 µg/ml: 8 ± 0.5. Cell cycle: **% of cells in G0/G1** (4 hs): basal: 10.68 ± 1.5; L 10 µg/ml: 23.7 ± 2.0; L20 µg/ml: 20.35 ± 2.2. **% of cells in G2/M (4 hs)**: basal: 32.93 ± 2.5; L10µg/ml: 23.7 ± 2.0; L10 µg/ml + L-NAME: 34.24 ± 2.5**. % of subdiploid cells**: basal: 15 ± 1.5; L10µg/ml: 25 ± 2.0; L10µg/ml + L-NAME: 18 ± 1.2. The limonene presented antiproliferative action on lymphoma cell line, inducing cell arrest in G_0/G_1 and apoptosis The nitric oxide is an intracellular signal involved in these actions.

6

BLOCKADE OF SMOOTH MUSCLE M_3 RECEPTORS OF RAT ILEUM BY TROPANE-TYPE ALKALOIDS PRESENT IN *Schizanthus litoralis, grahamii, pinnatus* and *hookeri*.

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The cholinergic antagonistic effect of tropane type alkaloids, extracted from aerial parts of *Schizanthus litoralis* (Sl), *grahamii* (Sg), *pinnatus* (Sp) and *hookeri* (Sh), was evaluated *in vitro* in ileum of rats. Segments of ileum of male Sprague-Dawley rats, weighting between 200 and 250 g, were collected, incubated for 40 minutes in Tyrode at 30°C, with 95% of O_2 and 5% of CO_2. The effect of the alkaloids of Sl, Sg, Sp and Sh extracts on the maximal contractile response in rat ileum induced by increasing doses of the cholinergic agonists carbacol or acetylcholine, was evaluated. The results showed that maximal contractions of the ileum, induced by cumulative carbacol or acetylcholine doses, were diminished when applying doses of $1x10^{-4}$, $1x10^{-3}$, $1x10^{-2}$ mg/ml of the tropane type alkaloids, extracted from Sl, Sg, Sp and Sh. The EC_{50} of carbacol or acetylcholine significantly diminished, compared with control carbacol or acetylcholine, when applying tropane-type alkaloids. The observed results allow to concluding that the alkaloids have affinity for the M_3 receptors of rat ileum, determinig a lower contractile response due to a cholinergic competitive antagonism to acetylcholine or carbacol.

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7

Population distribution of limphocytic pgp activity according to the sex.

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The aim of this work was to construct a frequency histogram with a sample of PGP activity in lymphocytes and to study the performance of a binormal distribution adjustment for the whole group and, a male, and a female only group (age between 20 and 60 years).

The limphocytic PGP activity (FR) was measured by the extrusion of Rodamina 123 followed for flow cytometry in the presence and absence of verapamil, a PGP inhibitor.

♦ **For the whole group (n=61):** the histogram was adjusted by a mixed distribution of a proportion p= 0.70 of a normal distribution with mean μ_1=2.11 and standard deviation σ_1=0.43, and a proportion (1-p)=0.30 of a normal with mean μ_2=3.29 and standard deviation σ_2= 0.26.

That is FR= 0.70 x N (2.11; 0.43) + 0.30 x N (3.29; 0.26)

♦ **For the female group (n=36) it was obtained:**

FR= 0.82 x N (2.09; 0.48) + 0.18 x N (3.37; 0.12)

♦ **For the male group (n=25) it was obtained:**

FR= 0.56 x N (2.18; 0.35) + 0.44 x N (3.25; 0.31)

In all the cases the performance of the adjustment was checked by the Kolmogorov-Smirnov test. **Conclusion**: there were no statistical differences shown between the whole group and each single sex group in relation with the distribution.

8

Effect of a prenylated flavonoid on the activities of membrane-bound enzymes and on mitochondrial membrane potential.

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The prenylated flavonoid 2´-4´-dihidroxy-5´-(1´´´-dimethylallyl)-6-prenylpinocembrin (6PP), obtained from Dalea elegans, shows antimicrobial activity. Previous results of 6PP showed dose-dependent anti-radical, and antioxidant activities. 6PP, also inhibited and/or uncoupled mitochondrial respiration and inhibited F_0F_1 ATPase activity (Elingold et al, SAIC 2006). We study the effect of 6PP on succinate deshidrogenase (SDH) and NADH-oxidase activities and mitochondrial membrane potential (MMP). 6PP promoted significantly inhibition of SDH and NADH oxidase activity in a concentration-dependent manner (IC_{50} of 25 μM and 19 μM respectively). The effect of 6PP on MMP was investigated in coupled mitochondria and was measured by rhodamine 123 fluorescence. Using malate-glutamate as substrate, 6PP (50 μM) significantly decreased 32% MMP after 25 min incubation, while 6PP (100 μM) significantly decreased by 47.5 and 56% MMP for 10 or 25 min incubation, respectively With succinate as substrate, 6PP (50 and 100 μM) decreased MMP by 33 and 48.4% respectively after 10 min incubation. On the other hand, 6PP (25, 50 and 100 μM) decreased MMP for 25 min incubation (34.4, 55 and 56%, respectively). In summary, we have partially characterized the activity of the prenylated flavonoid 6PP, demonstrating its toxic effects on isolated rat liver mitochondria.

9

IN VITRO EFFECTS OF ESSENTIAL OILS OF *ROSMARINUS OFFICINALIS* ON *ECHINOCOCCUS GRANULOSUS* PROTOSCOLECES

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Human cystic echinoccocosis is a zoonosis caused by the metacestode *Echinococcus granulosus*. At the present the chemotherapy with aromatic plants is becoming an alternative form of treatment. The aim of the present work was to determine the *in vitro* protoscolicidal effect of essential oils (EO) of rosmary (*Rosmarinus officinalis*) against *E.g.* Protoscoleces (PSC) were incubated with EO in water and propyleneglycol (PG) at concentrations of 10, 5, 1µg/ml. PSC incubated with culture medium and culture medium containing PG were used as controls. Vitality was assessed every 6 days using the methylene blue exclusion technique and samples were taken for electron microscopy. Treatment finished at day 80. Vitality decreased to 9.89 ± 3.5 % after 54 days Vitality results coincide with the tissue damage observed at the ultrastructural level. Morphological changes included contraction of the soma region, formation of blebs on the tegument, rostellar disorganization, loss of hooks and destruction of microtriches. Thus EO of rosmary can hopefully be considered in the future for more evaluations with other EO and synthetics drugs as benzimidazole carbamate derivatives to evaluate the possible anthelmintic synergic mechanisms on PSC.

10

PHARMACOKINETICS OF INTRAMUSCULAR AND ORAL TRIFLURALIN ADMINISTRATION IN MICE BLOOD AND HEART

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Trifluralin (TFL) (α,α,α-2,6-dinitro-N-N-dipropyl-p-toluidine) presents anti-*Trypanosoma cruzi* activity and potential therapeutic effect for the treatment of Chagas disease. This study was undertaken to assess oral and IM, TFL pharmacokinetics. We used 108 adult male CF1 mice that received a single oral or IM, TFL (50 mg/kg in peanut oil). Mice tissues and blood samples were obtained at 0.08, 0.17, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 12 hr after TFL administration. TFL was determined by HPLC and analyzed by non-compartmental models. After IM, the drug was rapidly absorbed (Ka= 3.4 h^{-1}); maximum blood concentration (C_{max}) of 28.2 µg/mL was obtained at 0.5 hr (t_{max}), and the drug was eliminated with half-life ($t_{1/2}$) of 1.2 hr. After oral TFL administration, Ka was 1.2 h, C_{max} of 7.8 µg/ml was obtained at 2 hr, and $t_{½}$ was 1.2 hr. Following oral and IM injection, half-life in heart tissue was (2.7 h), C_{max} of 0.2 and 0.6 µg/ml was obtained at 2.0 and 1.0 hr, respectively, and the penetration ratio into heart tissue was 6.3 and 4.0 %, respectively. Finger-print demonstrated that TFL was not degraded by light. TFL and metabolites were higher in liver and feces. Perirrenal, subcutaneous adipose tissue, cardiac and skeletal muscle exhibited important TFL concentrations. We conclude that TFL could be a new alternative drug for the treatment of Chagas disease.

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11

EFFICACY OF NOVEL ATIMICROBIAL PEPTIDE AGAINST MASTITIC DAIRY CATTLE.

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Mastitis in dairy cattle is induced by several bacteria strains, producing high shortage on milk production and several economic losses. Failure on traditional antimicrobial (ATM) therapy has been observed by high ATM multi-resistant bacterial strains. The efficacy of a novel environmental isolated *Enterococcus faecalis* - ATM peptide CECT7121 was assayed as new alternative on mastitis therapy. Bacteria strains recovered from mastitic cattle were (15) *S. aureus*, (10) *Streptococcus dysgalactiae*, (7) *Streptococcus uberis*, (1) *Streptococcus agalactiae.* The bactericidal activity of ATM CECT7121 was assessed by using *"in vitro"* killing curves.

ATM peptide CECT7121 inhibited the growth of whole assayed strains. Inhibition diameters for *S. agalactiae, S. uberis, S. dysgalactiae* and *S. aureus*, were ranged from 10,1 ± 0,1 mm (*S. aureus* MR2102) to 16,7 ± 0,7 mm (*S. dysgalactiae* MR2064) showing to be highly sensitive. Whole bacterial strains (99%) died within the 2 h assayed after killing curve performing. Further studies by developing of new pharmaceutical formulations are being challenged at our laboratory.

12

Effect of endogenous male and female sex hormones on the release and function of prostanoids. Martorell A, Sagredo A, Aras-López R, Balfagón G and Ferrer M. Departamento de Fisiología, Facultad de Medicina, UAM. C/Arzobispo Morcillo 4, 28029-Madrid. Spain. mercedes.ferrer@uam.es

The beneficial effect of estrogens on cardiovascular function has been reported. However, low testosterone levels are associated with the development of cardiovascular diseases. It is known that prostanoids modulate vascular tone. Therefore, the aim of this study was to analyze the effect of endogenous male and female sex hormones on the release and vasomotor effect of prostaglandin (PG) I_2, $PGF_{2\alpha}$, PGE_2, and thromboxane (TXA_2). For this purpose, aortic segments from male (control and orchidectomized) and female (control, in oestrous phase, and ovariectomized) Sprague Dawley rats were used to analyze: (i) the release of PGI_2, $PGF_{2\alpha}$, PGE_2, and TXA_2, and (ii) the vasomotor response to exogenous PGI_2, $PGF_{2\alpha}$, PGE_2, and TXA_2. Orchidectomy and ovariectomy increased the release of PGI_2, $PGF_{2\alpha}$, PGE_2, and TXA_2. Orchidectomy decreased the vasodilator response induced by PGI_2, did not modify the contractile response elicited by TXA_2 and $PGF_{2\alpha}$, and increased that induced by PGE_2. Ovariectomy increased the contractile response induced by $PGF_{2\alpha}$, PGE_2, and TXA_2, while decreased the vasodilator response induced by PGI_2. These results show that orchidectomy and ovariectomy regulate the production and vasomotor effect of prostanoids, indicating that both endogenous androgens and estrogens are cardioprotective in males and females, respectively.

Supported by FIS (PI051767), Medical Research Foundation (MMA-06) and DGCYT (SAF-06-07888).

Energetics of the ischemia-reperfusion in neonatal rat hearts under cardioplegia-high K: effects of KB-R7943, caffeine and clonazepam on Ca^{+2} homeostasis.

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Ischemia-reperfusion (I-R) in hearts from neonatal rats (10-12 days old) was energetically studied after treatment with CPG (a high-K-low Ca cardioplegia). Perfused hearts were pretreated with CPG-Ca 0.5 mM or Krebs-C before exposing to 15 min I- 45 min R while measuring contractile force (F) and heat release (H_t). During R, F recovered more in isometric (90 ± 5%) than isotonic (61 ± 10%) in both CPG- and C-hearts. H_t (in $mW.g^{-1}$) fell from 10.3 ± 0.6 to 5 ± 0.4 by CPG and 1.3 ± 0.5 by I, and recovered to 12 ± 1 by R (121% of pre-I). In isotonics F recovered more (*) when CPG -Ca 0.5 mM was added with: 10 mM caffeine (91 ± 7%), 2 mM Ca (83 ± 6%), and 5µM KB-R7943 (inhibitor of reverse SL-NCX and mitochondrial Ca-uniporter, MtCaU, 87 ± 9%). The (+) inotropism of KBR was not modified by 10 µM clonazepam (Clz, inhibitor of Mt-NCX). H_t increased more than F during R, except after CPG-Ca 2 mM (the higher muscle economy). The pretreatment with 10 mM caffeine+20 µM KB-R7943 (a NCX non-selective blocker) in CPG-Ca 2 mM reduced F recovery from 95.3 ± 7.7% to 41.4 ± 10%* at 5 min R and increased H_t in CPG and I (6.5±1.7 $mW.g^{-1}$). Results suggest that in CPG-Ca 0.5 mM and I-R of neonatal hearts: caffeine did not decrease SR store but increased Ca^{+2} influx, KBR increased a Ca^{+2} uptaked by MtCaU, Clz showed that Mt-NCX does not play a role, and the SL-NCX removed diastolic Ca^{+2}. Homeostasis of Ca in I-R of neonates was very different to adults. *$p<0.05$ *X-408 UNLP-05/07; CONICET PIP 6024/05; UBA O023.*

14

Xanthatin, new antiulcer agent derived from *Xanthium cavanillesii*. Acute toxicity

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Xanthatin is a natural product isolated from *Xanthium cavanillesii* Schouw, known as "abrojo". Previously we reported that xanthatin (X) prevents damage induced by several ulcerogenic agents (Favier *et al*., J. Ethnopharmacol. 2005, 100:260-267). X has significant antimicrobial properties against *H. pylori*. Moreover, we described the mechanism of gastroprotection of xanthatin in a previous study (Favier *et al*., Biocell 30(1), 2006)

The aim of the present study was to determine the acute toxicity of X and the infusion of *Xanthium cavanillesii* in mice.

Acute toxicity was produced according to the method of acute toxicity, Guidance No. 423, OECD. *Xanthium cavanillesii* infusion or xanthatin were administered to five lots of six Rockland mice (3 males and 3 females), *p. o*. The control group received vehicle and the test groups received infusion or X (5, 50, 300 and 2000 mg/kg). The animals were observed carefully every 2 days to record toxic manifestations, and also to measure body mass. After the 14 days experimental period mice were sacrificed. The organs were observed macroscopically and the relative weights were determined. No toxic symptoms or death occurred in each infusion or X group. The relative weights of kidney, liver, lung, spleen and heart were not statistically different from control (ANOVA). Xanthatin and the infusion of *Xanthium cavanillesii* did not present acute toxic effects.

15

Consumption of antidepressants in Pavón Arriba.

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Objectives: to evaluate the consumption of antidepressant drugs (ADs) in Pavón Arriba, a town with about 2000 inhabitants, located in the south of Santa Fe, during the first six months of 2007; and to contribute to the rational use of them through a pharmacoeconomic analysis (PA). Methods: It was study the consumption of ADs through the calculation of defined daily doses per 1000 inhabitants per day (DIDs), collecting all the medical prescriptions from the pharmacies of this town. It was performed the PA by cost-minimization method among different commercial brands for each used drug and among different drugs from the main group utilized. Results: Consumption (DIDs) for 98% of ADs: paroxetine: 2,55; fluoxetine (F): 2,13; escitalopram: 0,83; citalopram: 0,69; sertraline: 0,53; amitriptyline: 0,74 ($p<0,001$ consumption of selective serotonin reuptake inhibitors (SSRIs) vs. tricyclic ADs). The expenditures of ADs reached $5926 in the time of this study. Choosing the lowest price as reference to each drug (price($)/DDD) among different commercial brands and presentations dispensed in this town, it would be saved 6% of the cost. If among the SSRIs (84% of expenditure of ADs), it is selected F as reference (reference price($)/DDD, lower than other in SSRIs), it would be saved, only among SSRIs, 37% of the cost. Conclusion: It is prevalent the consumption of IRSS among ADs. The PA, which was performed using only the drugs suggested by medical professionals in their prescriptions, permits thinking it is necessary and possible to decrease the expenditure of ADs.

16

Ethynylestradiol (EE) modulates the expression of the basolateral transporter Mrp3 in rat liver. Ruiz ML, Villanueva SSM, Luquita MG, Arias A, Ochoa JE, Mottino AD y Catania VA. IFISE (CONICET)-Fac. de Cs. Bioq. y Farm. (UNR). Suipacha 570. (2000) Rosario. E-mail: vcatania@fbioyf.unr.edu.ar

In previous studies we observed that repeated administration of the cholestatic estrogen EE to rats (5 mg/Kg, 5 consecutive days) reduces the expression of the canalicular transporter multidrug resistance-associated protein 2 (Mrp2), whereas basolateral Mrp3 is increased. Mrp3 induction is linked to Mrp2 downregulation, accumulation of Mrp common substrates into the hepatocyte (induction by substrate) being postulated as a potential mechanism. **Aim**: To evaluate if induction of Mrp3 protein by EE may result alternatively from direct modulation of *Mrp3* gene by EE. **Methodology**: Adult male Wistar rats (n=3) received a single dose of EE (5 mg/kg, s.c.) or its vehicle (propyleneglycol, controls (C)). Six h later, bile flow and biliary excretion of bile salts and glutathione were determined and liver samples collected. In separated experiments, hepatocytes isolated from normal rats were cultured for 4 h in the presence of EE (1 or 10 µM, or propyleneglycol). Total RNA was extracted from liver samples and from cultured hepatocytes. Mrp3 mRNA was determined by quantitative RT-PCR. **Results**: bile flow (C=1.43±0.22 *vs* EE=1.23±0.12 µl/min/g liver) and biliary excretion rate of bile salts (C=51±10 *vs* EE=48±4 µmol/min/g liver) and glutathione (C=2.43±0.63 *vs* EE=1.30±0.35 µmol/min/g liver) were not affected by the single dose of EE, indicating absence of cholestasis. Mrp3 mRNA significantly increased in EE rat liver (300%, $p<0.05$) as well as in cultured hepatocytes exposed to 10 µM EE (400%, $p<0.05$). **Conclusion**: *in vivo* Mrp3 mRNA induction occurred in spite of absence of cholestatic symptoms (which rules out accumulation of Mrp substrates). Induction of mRNA also occurred in cultured hepatocytes, shortly after incorporation of EE. Taken together, these results suggest a direct modulation of *Mrp3* gene by EE.

17

Rosuvastatin therapy and levels of inflammatory markers in patients with acute myocardial infarction

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The inflammation plays a fundamental role in pathogenesis and complication of the atherosclerosis. The determination of C-reactive protein (CPR) plasma levels provides an excellent reflection of the underlying inflammatory process, since it is positively correlated with other markers such as pro-inflammatory cytokines. Even in absence of the atheroma plaque rupture, the Tumor necrosis factor alpha (TNF-α) power the procoagulating properties of the cells contributing to thrombotic complications. Aims: in patients with acute myocardial infarction treated with Rosuvastatina. 1- To determine the variation of the inflammatory markers: TNF-α and CPR values. 2- To quantified LDL-cholesterol. Material and Methods: plasma samples were obtained from 8 patients admitted at the cardiac coronary unit with acute myocardial infarction and during the following 4 weeks of treatment with 10 mg of Rosuvastatina. The results were compared with a "control group" without the treatment of the statin Results: The therapy with Rosuvastatina was associated with significant decrease ($P < 0.0001$) of: LDL-cholesterol (43 % versus 5 %), TNF-α (22 % versus 3 %), and the levels of CPR > 3 mg/dL (25% versus 6% of the control group) ($P < 0.0001$). Conclusions: Rosuvastatina diminishes the LDL-cholesterol levels, a fact previously demonstrated. The significant decrease of CPR and TNF-α levels after a month of treatment shows its benefits in patients with Acute Coronary Syndrome.

18

Staphylococcus aureus strains susceptibility against oxacillin - 2´, 4´-dihydroxychalcone combinations

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Previous researches allowed us to establish the flavonoids bacteriostatic action against Gram positive and Gram negative microorganisms and synergism between compounds of this family. The aim of the present work was to determine the antimicrobial effect of a clinical interesting conventional antibiotic, oxacillin (Ox), in combination with 2´,4´-di-hydroxychalcone (Ch) as enhancer against *Staphylococcus aureus* ATCC 29 213 (MSSA) and *Staphylococcus aureus* ATCC 43 300 (MRSA). Using a kinetic turbidimetric original method, growth kinetics in Müller-Hinton broth with Ox variable amounts and Ox variable –Ch constant in different combinations were made. The application of an activity mechanism previously proposed allowed the evaluation of minimal inhibitory concentrations (MICs) of Ox and its combination with the chalcone studied. For *S. aureus* ATCC 29 213 (MSSA) MIC Ox was 25 $\mu g.mL^{-1}$ and the growth of this microorganism for Ox (from 2 to 8 $\mu g.mL^{-1}$) with Ch (10 $\mu g.mL^{-1}$) combinations was totally inhibited, showing synergism. Study of *S. aureus* ATCC 43 300 (MRSA) growth in presence of Ox allowed to verify its resistance. Nevertheless, when Ox - Ch combinations were used, the bacterial growth was 35% lower. These results let us conclude that 2´,4´-dihydroxychalcone synergize the oxacillin action against *S. aureus* ATCC 29 213 (MSSA) and it is a good bacteriostatic agent for *S. aureus* ATCC 43 300 (MRSA)

19

Mutational analysis of the ligand binding site of the α9α10 nicotinic cholinergic receptor.

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A conserved feature of all nicotinic receptors is the presence of a disulfide bond between Cys-192 and Cys-193, adjacent to the acetylcholine (ACh) binding site. In order to characterize the relative importance of these residues in the α9α10 receptor we performed site directed mutagenesis of the Cys to Ser. ACh-gated currents were recorded in two-electrode voltage-clamped *X. laevis* oocytes injected with the receptor subunits. Mutant receptors showed an increase in the EC_{50} (α9α10: 13.8±1.7μM, n=6; α9α10*: 53.52±2.1μM, n=5; α9*α10: 146.7±5.6μM, n=5). A decrease in blocking potency for nicotine, atropine and strychnine was observed. The desensitization rate was similar to wild type receptors for the single mutant α9*α10 (I_{20sec}/I_{peak}=39.7±3.9%, n=2) but was lower in α9α10* (I_{20sec}/I_{peak}=90.6±5.5%, n=3). Treatment of α9*α10 injected oocytes with the Ca^{2+} chelator BAPTA-AM resulted in a 94.2 ±1.1% (n=4) decrease in ACh-evoked currents, showing that mutant receptors retained the high Ca^{2+} permeability previously described for the wild type α9α10 receptor. The macroscopic channel properties of the receptors were not altered by the mutations as shown by the current-voltage relationships. The mutations C192S/C193S in α9α10 appear to alter the affinity of the ligand binding site or, alternatively, the coupling of ligand-binding to channel opening. Our results demonstrate that both α9 as well as α10 can form the principal component of the ligand binding site of the receptor.

20

***In vivo* induction of P-glycoprotein: impact on ivermectin gastrointestinal disposition**

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Ivermectin (IVM), a broad-spectrum antiparasitic drug has been shown to be a substrate of the drug transporter P-glycoprotein (P-gp). The aim of current study was to assess the comparative effect of well-known enzyme inducer agents, rifampicin (RFP) and phenobarbital (FNB), on the intestinal P-gp activity-dependant IVM disposition kinetics in rats. Male Wistar rats were allocated into three groups of 15 rats each. Animals were treated with RFP (Group B) (160 mg/day) or FNB (Group C) (30 mg/day), both orally administered during 8 days. After this period, untreated control (Group A) and pretreated animals received IVM (200µg/kg) subcutaneously. Animals were sacrificed between 6 and 72 h post-treatment. Blood and gastrointestinal tissues were collected and IVM concentrations measured by HPLC. The plasma and gastrointestinal disposition kinetics of IVM was unaffected by the presence of RFP. However, the pretreatment with FNB resulted in significantly lower IVM systemic concentrations compared to the IVM alone treatment. The peak plasma concentration and the systemic availability were between 149 and 164% lower in the FNB pretreated group. IVM intestinal secretion measured as the ratio between drug availability in the luminal content and gut wall tissues was significantly higher (90%) in the FNB pretreated rats. These preliminary results demonstrate that FNB drastically affects the IVM disposition kinetics, which is likely due to a strong induction of intestinal P-gp activity.

21

BACLOFEN DECREASES SOMATIC EXPRESSION OF MECAMYLAMINE-PRECIPITATED NICOTINE WITHDRAWAL IN MICE

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Nicotine (NIC) is one of the active components of tobacco and play a major role in tobacco addiction. The aims of the present study were: a) to evaluate the possible role of $GABA_B$ receptor in NIC physical dependence; b) to analyze the neurochemical variations in various brain regions of mice during NIC withdrawal syndrome and its prevention with baclofen (BAC, $GABA_B$ receptor agonist). Swiss-Webster albino mice received NIC (2.5 mg/kg, sc) four times daily, for 7 days. To precipitate NIC abstinence, dependent mice were injected with the nicotine antagonist mecamylamine (MEC, 2 mg/kg, ip) 1h after the last dose of NIC on the 8th day. A second group of dependent mice received BAC (2 mg/kg, ip) before MEC-precipitated abstinence. Somatic signs were measured for 30 min. The levels of dopamine, serotonin and its metabolites were determined by HPLC in the striatum, cortex and hippocampus. BAC pre-treatment decreased the incidence of some NIC withdrawal signs, such as wet-dog-shakes ($p<0.01$) and paw tremors ($p<0.001$). The global withdrawal score was also attenuated by BAC ($p<0.001$). There were no differences in the monoamines and metabolites levels in the brain areas studied. These results indicate that BAC administration attenuates NIC withdrawal. This would not be related to changes in dopaminergic and serotonergic activity measured 30 min after MEC. *Supported by UBACYT B021*

22

Thyroid hormone regulation of mammary adenocarcinoma cells. Valli E[1], Barreiro Arcos ML[2], Cricco G[1], Cremaschi G[1,2] and Martín G[1]. [1]Lab de Radiosisótopos, FFyB, UBA, [2]CEFYBO–CONICET, Buenos Aires, Argentina. eduardovalli@yahoo.com.ar

Thyroid hormones (TH) exert a broad range of effects on development, growth and metabolism. Their actions on lymphoid cell growth were recently analyzed and we have described that both 3,5,3'-L-triiodothyronine (T3) and thyroxine (T4) are able to increase T lymphoma cell proliferation through the increment of both nitric oxide synthase (NOS) activity as well as of its inducible isoform expression at the protein and mRNA levels. To further characterize TH actions on other tumor cells, the aim of this work was to study their effects on the mammary adenocarcinoma cell line MDA MB231 (MDA). Through cell count by clonogenic assay and by carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling, dose response curves for both T3 and T4 actions upon cell division were performed. We found a dual action of TH on MDA cell growth, with physiologic concentrations of TH leading to inhibition, while lower concentrations stimulating cellular growth. To further analyze NOS participation at any of these doses, NOS activity was evaluated by conversion of [^{14}C]-Arginine to radiolabeled citruline. No modification of NOS activity was observed. TH were also able to regulate metalloproteinase levels in MDA cells. From these results it can be concluded that TH are able to modulate mammary tumor cell growth as occur in lymphoma cells, but with no involment of NOS activity and affecting angiogenic factor production.

23

Altered pharmacokinetic parameters of *p*-aminohippurate (pah) and oat1 renal expression in rats with ischemic acute renal failure (arf).

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Several drugs of pharmacological interest are organic anions. The present study evaluated the elimination of PAH (prototypical organic anion) in response to an early stage of ARF in rats. ARF was induced in adult male Wistar rats by occlusion of both renal pedicles during 60 min, followed by 60 min of reperfusion. Pair-fed sham operated rats served as controls (S group). A pharmacokinetic study of PAH was performed. The expression of the organic anion transporter 1 (Oat1) was assayed in renal cortex homogenates by Western blotting and by immunohistochemistry. ARF rats displayed a significantly lower ($P<0.05$) systemic clearance of PAH (mL/min/100g b.w., S: 3.01 ± 0.15, ARF: 1.06 ± 0.04). Also a decrease ($P<0.05$) in the elimination rate constant from the central compartment was observed in ARF rats ($K_{1\text{-}0}$, min^{-1}, S: 0.47 ± 0.11, ARF: 0.15 ± 0.02). As PAH metabolism and biliary excretion are negligible, the decrease of $K_{1\text{-}0}$ indicates a lower renal elimination of this drug. The total and peripheral volumes of distribution were reduced ($P<0.05$) in ARF rats (mL/100g b.w., VdT= S: 31.25 ± 3.09, ARF= 18.97 ± 3.75; VdP= S: 23.30 ± 1.94, ARF= 11.85 ± 3.18). Oat1 abundance decreased in a 30 % in kidneys from ARF rats. ARF rats presented a diminished capacity to eliminate negatively charge organic compounds, mediated at least in part, by the lower expression of Oat1.

24

CHANGES IN CEREBELLAR AND HIPPOCAMPAL SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN RATS X-IRRADIATED AT BIRTH.

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Developing Central Nervous System (CNS) is highly sensitive to radiation-induced oxidative damage. With the aim to evaluate if this radiosensitivity is related to an alteration in the endogenous antioxidant system, the levels of SOD were determined in cerebellum (CE) and hippocampus (Hip) of 30 and 90-days old Wistar rats X-irradiated at birth with 5 Gy.

Hippocampal SOD levels significatively decreased at 30 days post-irradiation (dpi) (C: 0.37 ± 0.010 U/mg, Rx: 0.33 ± 0.009 U/mg of tissue, $p < 0.05$) and returned to control levels at 90 dpi. In contrast, cerebellar SOD activity remained unchanged at 30 dpi and significantly increased at 90 dpi (C: 0.41 ± 0.015 U/mg, Rx: 0.48 ± 0.009 U/mg, $p < 0.05$). These results suggest that hippocampal SOD seems to be more radiosensitive than cerebellar SOD at short term (30 dpi), probably due to radiation-induced reactive oxygen species (ROS) inactivation. Conversely, the increased levels of cerebellar SOD observed at long term (90 dpi) suggest the existence of a compensatory mechanism capable of counteracting excessive oxidative damage.

These specific changes could reflect a differential regional radiosensitivity through the use of divergent adaptable mechanisms of the endogenous antioxidant system.

25

POTENTIATION OF THE HYPOTENSIVE EFFECT OF THE ENDOCANNABINOID / ENDOVANILLOID ANANDAMIDE BY THE ENTOURAGE COMPOUND PALMITOYLETHANOLAMIDE

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The effects of arachidonoylethanolamide (anandamide; AEA) can be enhanced by structurally related, endogenous fatty acid derivatives termed entourage compounds. We have reported that intrathecal (it) injection of AEA to urethane-anesthetized rats induced a hypotensive effect due to the activation of both cannabinoid CB_1 and vanilloid TRPV1 spinal receptors (García et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 368: 270, 2003). The aim of this study was to examine whether palmitoylethanolamide (PEA) behaves as an entourage compound for the hypotensive effect of AEA. AEA (25, 50 and 100 nmol; it) induced a dose-dependent decrease in the mean blood pressure (Δ MBP: -1.2±2.4, -10.5±1.3 and -17.3±1.3 mmHg, respectively; n=4-6). PEA (100 nmol; it) did not modify *per se* the baseline MBP but increased the hypotensive responses to AEA (25 and 50 nmol; it; $p<0.05$). Moreover, PEA enhanced the hypotensive effects of the endocannabinoid/endovanilloid N-arachidonoyldopamine (25 nmol; it), the metabolically stable analog of AEA methanandamide (25 and 50 nmol) and the TRPV1 receptor agonist capsaicin (0.3 nmol; it). PEA did not modify the hypotensive response to the CB_1/CB_2 receptor agonist WIN 55212-2. It is suggested that PEA positively modulates the hypotensive response to AEA in the spinal cord. This facilitatory effect could involve the enhancement of AEA stimulation of TRPV1 receptors. *Supported by ANPCyT (PICT 5-14107) y CONICET (PIP 5695).*

26

***In vitro* effects of non –steroidal anti-inflammatory drugs on cytokine and matrix metalloproteinase production in osteoartritic human articular chondrocytes**. Demurtas, SL, Ricarte Bratti, JP, Montrull, HL, Demurtas, S, Meirovich, CI and Brizuela NY. Dpto. de Farmacología. FCM. Universidad Nacional de de Córdoba. Santa Rosa 1085. Córdoba, Argentina. nildabrizuela@hotmail.com

The osteoartritis (OA) is a chronic articular disease of the locomotive device that compromises both, the synovial and the subcondral bone. This process happens when the collagen is degraded through an increase of: Interleukina-1 (IL-1), Tumor necrosis factor alpha (TNF-α) and metalloproteases 1 and 3 (MMP-1 and 3). The Nonsteroidal Antiinflammatory drugs are used for the treatment of OA. Aims of the study: To investigate *in vitro* the effect of diclofenac (DICLO) and paracetamol (PARA) on the production of MMP-1, MMP-3 and of TNF-α.

Material and methods: Chondrocytes were obtained by enzymatical digestion of cartilage obtained from 10 women patients with an average age of 70 years old. Patients were diagnosed with severe OA and submitted to surgery for knee implant prosthesis. Cells were treated with and without 10 µg/mL of DICLO and PARA. Enzyme-linked immunosorbent assay (ELISA) was used to quantify MMP-1, MMP-3 and TNF-α.

Results: DICLO was associated with significant decrease of MMP-1, MMP-3 ($p < 0.001$) and TNF- α ($p < 0.01$). PARA significantly decreased the production of TNF-α ($p < (0.01)$.

Conclusión: DICLO significantly decreased the levels of metalloproteases and of TNF-α. The inhibitory effect of paracetamol upon TNF-α is still not explained, this observation probably justify the long-term performance of clinical trials.

27

Impact of the experimental periodontitis on unstimulated mucin secretion from the rat submandibular gland.

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We observed that 22 days after inducing the experimental periodontitis in the rat, by placing a sterile silk ligature around the two lower first molars, basal mucin secretion and the EC_{50} of isoproterenol were increased while isoproterenol maximal effect was decreased. Basal values of mucin from rats with ligature were returned to control ones in the presence of atenolol. Desensitization following by a down regulation of β-receptors was observed. These results point to an activation of sympathetic system. In this study we investigated whether the mechanism underlying basal mucin secretion in rats with periodontitis was achieved through a cAMP pathway and the participation of nitric oxide, protaglandins and leukotrienes, in the mechanism by which periodontal disease induce an increase of mucin basal values, by using SQ 22536, L-NMMA, indomethacin (Indo), NDGA and cortisol. Results showed that in the presence of SQ basal values of rats with ligature were similar to controls pointing a β-receptor pathway. Rats with ligature, treated with cortisol, (sc 1mg/ k, 3 days), showed similar mucin values as controls while *in vitro* L-NMMA and Indo had no effect, suggesting that neither NO nor PGs were involved. NDGA, blocked the periodontal impact on basal mucin release. It was concluded that experimental periodontitis increases sympathetic activity through an inflamatory mechanism that involve lipoxigenase products

28

Th1/Th2 immunity and cognitive deficit associated to chronic stress exposure. Involvement of nitric oxide production. **ML Palumbo, MA Zorrilla.Zubilete, MR Wald, AM Genaro. CEFYBO-CONICET-UBA. Paraguay 2155, Bs. As. Argentina molecula_21@yahoo.com.ar**

Stress and imbalance of Th1/Th2 immunity has been implicated in psiquiatric disorders. Nitric oxide (NO) has been involved in many pathophysiological brain processes including hippocampal responses to stress. Here we investigated the effect of mild chronic stress (CMS) on learning and memory and NO participation in Th1-biased C57Bl/6 (C57) mice and Th2-biased Balb/c mice. We observed that CMS exposure induce an increase of Th1- or Th2-type cytokine production in C57 or Balb/c mice respectively. CMS-Balb/c mice showed a poor learning performance in open field test respect to control mice. In contrast, stress did not effect on learning performance in C57 mice. NO production by constitutive isoforms of NO synthase (NOS) was significantly disminished in the hippocampus of CMS-Balb/c mice but not in CMS-C57 mice respect to control. Moreover protein kinase C (PKC) activity was increased in CMS-Balb/c mice and significantly decreased in CMS-C57. However, treatment of normal mice with general NOS inhibitor (L-NAME) induced a memory impairment in both strain of mice. These results suggest an important role for NO and the regulation of its production by PKC, in the cognitive deficit associated to chronic stress exposure. Moreover, Th1 response appears as a protective mechanism preventing NO decrease and memory impairment.

29

Effects of *beta*-cyclodextrin on water flow in urinary bladder isolated from the toad *Bufo arenarum*

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The urinary bladder isolated from toads (TB) exhibits great similarity to mammalian transport epithelia, particularly those of the distal kidney tubule, and are extensively used in studying water transport processes. We measured the effect of *beta*-cyclodextrin, (BCD, a cholesterol scavenger) on water passage across the TB exposed to an osmotic gradient (Jw) by a gravimetric technique, and cholesterol and PO_4^{3-} (from phospholipids) by standard biochemical techniques in the membrane isolated by selective centrifugation. When present in the basolateral bath, although devoid of effect *per se*, BCD inhibited Jw in the TB exposed to oxytocin or norepinephrine (both of which increase the intracellular generation of cyclic AMP [cAMP] by interacting with membrane receptors) but did not alter the response to theophylline (THEO, which increases cAMP by inhibiting cyclic nucleotide phosphodiesterase, an intracellular enzyme that hydrolyzes cAMP). Exposure to BCD also reduced the membrane cholesterol/phospholipid ratio. The present data, together with previous results from our laboratory, confirm that the effect of agents that increase Jw by interacting with membrane receptors (which have been suggested to have a cholesterol-rich lipid environment in the membrane) are selectively altered by BCD-mediated cholesterol extraction from the cell membrane, whereas agents acting intracellularly are not affected by the alteration of the membrane lipid environment.

30

***"In vitro"* ACTIVITY OF A NOVEL ANTIMICROBIAL PEPTIDE CECT7121 AGAINST HUMAN MULTIRESISTANT BACTERIAL STRAINS.**

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The emergence of Gram (+) and Gram (-) multi-resistant bacteria, involves a serious therapeutic concern in clinical practice. ATM peptide CECT7121 is a purified bacteriocin isolated from an environmental strain of *Enterococcus faecalis CECT7121* with 5,000 kDa of MW and high lipofilicity. The goal of this work is to investigate *"in vitro"* the bactericidal action of this compound against ATM multi-resistant bacteria isolated from hospitalized care unit patients who failure to the standarized treatment. The inhibitory action of diverse pathogen multi-resistant bacteria sourced from man was performed as follows: Four strains of resistant vancomycin *E. faecalis (RVE)* (ATCC 51299) and three coming from the Hospital Ramón Santamarina (Tandil, Argentina). *E. faecium (EF)* (Blood culture), multiresistant *Staphylococcus. aureus (MSA)*(Brain Liquid) and oxacilin resistant *Streptococcus pneumoniae (ORSP)* (Pleural liquid). Bactericidal activity of ATM peptide CECT7121 was assessed by killing curves. A rapid killing effect on bacterial population (30 minutes) was observed for *RVE*, *E. F.* and *ORSP*. However the bactericidal effect against *MSA* was slower (after 60 min.). Ninety nine percent of the bacterial population was killed before 2 h of incubation. Developing of ATM peptide CECT7121 may be a potential tool for the treatment of Human multi-resistant bacterial infectious diseases.

31

TRYPANOCIDAL NAPHTHOIMIDAZOLES: STUDIES ON MITOCHONDRIAL RAT LIVER TOXICITY AND MUTAGENICITY

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Naphthoimidazole (NPH) derivatives from β-lapachone: N1, N2 and N3, were shown to have important trypanocidal activity. The aim of this study was the evaluation of the mitochondrial rat liver toxicity and the potential mutagenicity of these compounds. It was found that N1 and N3 (25 and 50μM) produced a significant increase in oxygen uptake with succinate (site II substrate) and mitochondria in metabolic state 4 (resting respiration). On the other hand, state 3 (active respiration) was not modified. Also the respiratory control index (RCI) decreased significantly in the presence of either N1 (25 and 50μM) or N3 (10, 25 and 50μM). Mitochondrial membrane potential (MMP) was measured by rhodamine 123 fluorescence with malate-glutamate as substrate. Both, N1 (50μM) and N3 (50 μM) decreased significantly this parameter after 25 min. incubation 69,9 and 72,8 %, respectively. N2 addition did not significantly modify the respiratory states, RCI or MMP. None of the three NPH was found to be mutagenic when assayed with the Salmonella/microsome test. From these results it can be concluded that of the three NPH studied, N2, because its lack of toxicity, can be considered as the most potentially effective agent for Chagas chemoterapy.

32

Gata-1 and epo-receptor cooperate to promote erythroid expansion by bcl-x_l up-expression in acute anemic stress

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Acute anemic cause stress erythropoiesis for prevent the tisular hypoxia. Relationships between proliferation and differentiation of erythroid bone marrow (BM) and the expression of survival related proteins upon acute anemic stress are less known. To address this aim, CF-1 Swiss mice were injected with a single dose of 5-Fluorouracil (150mg/Kg ip) and apoptosis (TUNEL assay), BM architecture organization (scanning electronic microscopy), proliferation (DNA assay), differentiation (clonogenic cultures), expression of erythroid (EpoR, GATA-1, Bcl-X_L) and apoptotic (Bax, Caspase-3) related proteins by Western blotting were evaluated. Experimental data showed that within the first period of acute stress (1-3 days) apoptosis, arrest of cell proliferation and disruptions of BM architecture were maximal. Bax and caspase-3 overexpressions were also coincident. Interestingly, from day 5 upon drug challenge BM responds to acute stress through Epo-EpoR/GATA-1 system prompting the expression of Bcl-X_L. Erythroid proliferation rates and red cell commited progenitors enhanced in a coordinated way to restore the function of the red cell compartment. The dramatically increment of CFU-E population was concomitant with the cooperation of EpoR and GATA-1 for up-regulation Bcl-X_L in BM erythroid compartment during acute anemic stress. We assumed that these facts are crucial for acquiring proper erythroid cell functionality without delay to respond to tisular hypoxia.

33

CHRONIC TREATMENT WITH THE PHYTOESTROGEN GENISTEIN POTENTIATES THE ANANDAMIDE-INDUCED VASODILATION IN MESENTERIC BEDS OF FEMALE RATS

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Previous evidence showed that the vasodilation caused by the endocannabinoid anandamide is higher in mesenteric arteries isolated from female than from male rats and it is facilitated by estrogens. The aim of the present work was to analyze the *in vitro* and the *in vivo* effects of the phytoestrogen genistein on anandamide-induced vasodilation. The studies were fulfilled in mesenteric beds isolated from Sprague-Dawley rats by measuring the variations in the noradrenaline-induced contractions. The *in vitro* perfusion with 1μM genistein reduced the anandamide-induced relaxation in mesenteries isolated from females but not from males. The blocking effect was also observed when genistein was acutelly administered *in vivo* (10 mg/kg, p.o., 2 h before the assay performance). On the contrary, chronically administered genistein (10 mg/kg, p.o., for 3 days) increased anandamide-induced vasodilation in mesenteries isolated from intact as well as from ovariectomized female rats without modifying anandamide effects in males. Chronic *in vivo* administration of 17β-estradiol (450 μg/kg, i.m., for 3 days) also potentiated anandamide effects in ovariectomized rats. Since the facilitatory genistein effect in the female rats was observed only after its chronic administration, it is proposed that it could depend on adaptive changes in the mesenteric vasculature, that somehow resembled those induced by the long term administration of 17β-estradiol. Supported by grants PICT 5-14107 (ANPCyT) and PIP 5695 (CONICET).

34

Functional characterization of □4□2 recombinant nicotinic receptors with point mutations in the TM2 domain.

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The most abundant heteromeric neuronal nicotinic receptor, is formed by the □4 and □2 subunits. Mutations in these subunits are linked to a monogenic familial epilepsy known as ADNFLE. To reproduce the human phenotype of ADNFLE, Klaasen et al. generated 'knock in' mice that carry one of the two most extensively characterized mutations in the □4 subunit: S248F and 776ins3. Both mutations are located within the second transmembrane domain.

The goal of the present work was to study, using the two electrode voltage clamp technique, the functional properties of the recombinant receptors that result from the expression, in Xenopus oocytes, of mouse clones with the S248F or 776ins3 mutations in the □4 subunit. Both mutations affected the sensitivity of the receptor for acetylcholine (ACh). The pronounced effect caused by the S248F mutation allowed the discrimination in the ACh dose response curve of three different populations of receptors present in the membrane. The S248F mutant receptors desensitized more than wild type and 776ins3 mutant receptors. Responses to ACh were potentiated by the increase in extracellular calcium in both wild type and mutant receptors. The results presented here are in agreement with published data for human wild type and mutant receptors. These results support the use of the knock-in mice as an animal model for the study of ADNFLE.

35

Zinc deficiency affects T lymphocyte activity depending on the PKC isoenzyme pattern displayed in the studied cell type.

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Zinc (Zn) is essential for all highly proliferating cells, especially those of the immune system. We have shown that Zn depletion in mitogen stimulated-T cell cultures lead to the inhibition of proliferation through a decrease in PKC isoenzymes crucial for T cell activation. To understand zinc actions on these signaling proteins, the effect of its deficit was analyzed in the T cell lines LBC and BW 5147 (BW), that mainly expressed conventional and atypical PKC isoforms respectively. These cells were cultured in the absence or presence of specific intra-(TPEN) or extracellular (DTPA) Zn chelators and proliferation, PKC activity and the pattern of PKC isoenzymes were evaluated. Both chelators inhibited LBC cell proliferation, effect that was reverted by Zn addition. BW growth was not affected by Zn chelators. In both cell lines chelators affected PKC activity. DTPA decreased PKC □ and TPEN diminished PKC □in LBC cells, while both chelators decrease □, but exerted no effect on the atypical PKC □ isoform in BW cells. These results show that Zn action on T cell proliferation, depend on the PKC isoenzyme pattern of the cell type. Thus, Zn deficiency affect only T cells (normal and LBC cells) whose proliferation depends on novel or conventional isoforms, with two Zn-finger structures in their regulatory site, with no action on PKÇ□depending-BW cells.

36

TAM receptors are pleiotropic inhibitors of the innate immune response. Carla V. Rothlin[1], Sourav Ghosh[2,4,5], Elina I. Zuniga[3,4,6], Michael B. A Oldstone[3], and Greg Lemke[1] [1]Molecular Neurobiology Laboratory, [2]Molecular and Cell Biology Laboratory, The Salk Institute, La Jolla, CA 92037. [3]Molecular and Integrative Neuroscience Department, Scripps Research Institute, La Jolla, CA 92037. [4]Denotes equal contribution [5]Present address: Dept. of Basic Medical Sciences, Univ. of Arizona, Phoenix, AZ 85004. [6]Present address: Div. of Biological Sciences, Univ. of California San Diego, La Jolla, CA 92093. The activation of Toll-like receptors (TLRs) in dendritic cells (DCs) triggers a rapid inflammatory response to pathogens. However, this response must be tightly regulated, since unrestrained TLR signaling generates a chronic inflammatory milieu that often leads to autoimmunity. We have found that the TAM receptor tyrosine kinases - Tyro3, Axl, and Mer - broadly inhibit both TLR and TLR-induced cytokine receptor cascades. Remarkably, TAM inhibition of inflammation is transduced through an essential stimulator of inflammation - the type I interferon receptor (IFNAR) and its associated transcription factor STAT1. TLR induction of IFNAR-STAT1 signaling upregulates components of the TAM system, which in turn usurp the IFNARSTAT1 cassette to induce the cytokine and TLR suppressors SOCS1 and SOCS3. These results illuminate a self-regulating cycle of inflammation, in which the obligatory, cytokine-dependent activation of TAM signaling hijacks a pro-inflammatory pathway to provide an intrinsic feedback inhibitor of both TLR- and cytokine-driven immune responses.

37

Cephalotin penetration into muscle tissue fluid and muscle tissue.

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Cephalotin is an antibiotic belonging to the cephalosporin group. The aim ot fhe present work was to compare the penetration in muscle tissue fluid (MTF) and muscle tissue (MT) of cephalotin administered in rabbits. New Zealand White male rabbits weighing approximately 2 kg were used. Animals were distributed at random in Trial 1 (n= 15) and Trial 2 (n= 6). In the Trial 1 the animals were implanted in ischiotibial muscles with non reactive material cages. Animals with stabilized cages recibed subcutaneously (sc) a single doses cephalotin (20 mg/kg) and blood, MTF and MT samples were recolected at controloded time. In Trial 2 the animals recibed a single dose of cephalotin (20 mg/kg) intravenously (iv) and blood samples were recolected to time controled. The pharmacokinetics analysis of the data were performed using non-compartmental analysis. Results. Terminal disposition rate constant (λ_z) (serum iv) 1.43 ± 0.54, (serum sc) 1.9, (MTF) 0.57 and (MT) 1.8 h^{-1}; elimination half-life ($t_{½}$) (serum iv) 0.53 ± 0.13, (serum sc) 0.36, (MTF) 1.2 and (MT) 0.38 h; the area under the curve [$AUC_{(0-6)}$] (serum iv) 60.4 ± 38.2, (serum sc) 45.9, (MTF) 21.6 and (MT) 3.0 $\mu g.ml^{-1}.h$. and the bioavailability (F) was 76%. The concentration-times of cephalotin in muscle tissue fluid and muscle tissue were 47.7 and 6.43% of the ones obtained in serum, respectively.

38

COMPARATIVE STUDY OF THE HYPERTENSION TREATMENT IN PRIMARY HEALTH CARE AND SOCIAL SECURITY

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Our objective was to make a comparative study of the distribution of the hypertensive patients by sex and to analyze the prescriptions made in two hospitals and a social security of San Luis. An observational, cross-sectional and retrospective study was carried out in the hospitals Cerro de la Cruz (CC) and del Sur (HS) and in the social security of the provincial state (SS). Age, sex, diagnoses and prescriptions were registered for a month. The medicines and the diagnoses were classified according to ATC and ICD-10. Statistic: difference of proportions. Results (%). Sex: CC, HS, SS respectively: F 77, 76.3, 64.7; M 23, 23.7, 35.3. Specific prescriptions for hypertension (antiHT): CC 85.1, HS 76.3, SS 77; nonspecific CC 14.9, HS 22.3, SS 23. AntiHT: CC: enalapril 66.2, furosemide 8.1, enalapril+antiHT 8.1; enalapril HS 42.1, furosemide 22.4, atenolol 6.6, enalapril+antiHT 5.2; SS: enalapril 17.8; enalapril+hydrochlorotiazide (HTZ) 7.8, amlodipine 6.9, carvedilol 5.8, bisoprolol 4.6, atenolol 3.6, losartán+HTZ 3.4, nifedipine 3.3, losartan 3. Fixed-dose combinations (CDF): CC and HS 0, SS 21.1. The hypertension was prevalent in women. Enalapril and furosemide like monotherapy were the more prescribed in the hospitals; and single and combined enalapril with HTZ in the SS. In the SS many drugs "me too" and CDF were used. The Angiotensin-converting enzyme inhibitors are drugs of high therapeutic value, being the thiazidic diuretics those of first election. A high percentage of no related drugs to the hypertension were prescribed.

39

Milk excretion of eprinomectin in dairy sheep: residues in cheese and suckling lambs

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Eprinomectin (EPM) is a broad-spectrum endectocide antiparasitic drug registered as a topical formulation for use in dairy animals. The pattern of EPM excretion in milk was comparatively characterized following its topical administration to lactating dairy sheep from two different breeds (Pampinta and Istrian Pramenka). A pool of milk collected from the experimental animals group was used for cheese elaboration. EPM concentrations were measured in plasma, milk, cheese (elaborated with milk from treated ewes) and in the plasma of lambs suckling from treated ewes. EPM residual concentrations (measured by HPLC with fluorescence detection) were recovered in milk up to 15 (Pampinta breed) and 32 (Istrian Pramenka breed) days post-treatment. The drug was measured up to 14 days post-treatment in the bloodstream of their suckling lambs. A high concentration of EPM was recovered in cheese elaborated with milk obtained from treated animals, which increased during ripening to reach the highest residual level at 40 days of cheese maturation. The impact of these findings on the health of the consumer is under consideration. The presented work was performed within the scope of Argentina–Slovenia cooperation in science and technology.

40

Transcriptional control of erythroid bone marrow recovery post Paclitaxel: Roles of GATA-1 and EKLF.

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A cooperative cohort of broad-spectrum and specific transcription factors regulates the formation, survival, proliferation and differentiation of erythrocytes. Among them, GATA-1 is critical for erythroblast survival and terminal maturation. Besides, EKLF (erythroid Krüppel like factor) is responsible for ultimate erythroid differentiation. This study was focused to dilucidate the relationships between these factors and Epo-R during erythropoietic recovery after a cytotoxic challenge during a time course study of 10 days. Paclitaxel (Px, 29 mg /kg) was ip injected to normal female CF-1 mice (n=6/lot). Bone marrow (BM) samples were obtained to determine erythroid commited progenitors growth (CFU-GEM, BFU-E and CFU-E) by clonogenic cultures, and the expressions of GATA-1, EKLF and Epo-R by immunoblottings. Epo-R was over expressed from the 3rd day to the 7th days, GATA-1 enhanced from day 5 until the end of the experience and EKLF was over expressed from day 5 reaching maximal values at the end of the schedule. Erythroid commited colonies were depleted between days 1 and 3, showing a noticeable increment by day 10 post Px dosing. These results suggest that Epo-R expression induces the up regulation of GATA-1 and the over expression of its down stream target, EKLF, to trigger the molecular events necessary for erythropoietic recovery post Px insult.

41

Training decreases the contractile response induced by electrical stimulation in mesenteric arteries from obese rats. Del Campo L, Sagredo A, Arroyo-Villa I, Manso R, Ferrer M, and Balfagón G. Departamento de Fisiología, Facultad de Medicina, UAM. C/Arzobispo Morcillo 4, 28029-Madrid. España. E-mail: gloria. balfagon@uam.es

Rat mesenteric artery possesses nitrergic, sensory and adrenergic innervations which participate in the regulation of vascular tone. Since neurovascular control is altered in obese subjects and the exercise improve vascular resistance, our objective was to study the effect of training (three months of moderate exercise during 20 min / three days per week) on vascular response induced by electrical field stimulation (EFS) in mesenteric arteries from the obese Zucker rats, as well as the participation of nitrergic, sensory and adrenergic innervations in that response. For this purpose, endothelium-denuded mesenteric artery was used. Training decreased the EFS-induced contraction. The nitric oxide (NO) synthase inhibitor N^{w}-nitro-arginine-methyl ester (L-NAME) increased EFS-elicited contraction in similar extent in arteries from sedentary and training rats. The calcitonin gene-related peptide (CGRP) receptor antagonist, CGRP(8-37) did not modify the vasomotor response to EFS in both groups of rats. The alpha adrenergic receptor antagonist, fentolamine, decreased the EFS-induced response more in arteries from sedentary than from trained rats. Training increased the vasoconstrictor response elicited by exogenous noradrenaline. Our results indicate that in mesenteric arteries from obese Zucker rats, the training decreased the vasoconstrictor response induced by EFS. This effect seems to be mediated by a decreased noradrenaline release. Supported by DEP2006-56187-C04-04.

42

Anti-inflammatory activity and citotoxicity of pomolic acid isolated from *Cecropia pachystachya* (Ambay).

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In the present work we studied the anti-inflammatory and cytotoxic activities of pomolic acid (PA) isolated from Ambay. PA (125 mg/kg) reduced the carrageenan-induced mouse paw oedema by 37% (1 h), 39% (3 h) and 34% (5 h). Moreover, PA inhibited the *in vivo* production of IL-1β by 39% but did not affect the production of TNF-α. PA (10 μM) displayed no cell toxicity and did not inhibit the PGE_2 production whereas it slightly inhibited the nitrite production (14%) in macrophages RAW 264.7 stimulated with LPS. Mature human neutrophils viability was assessed using the MTT assay. PA (200 μM) produced the death of approximately 60% of the cells after 3 h of incubation. Lytic activity was measured by the exclusion of propidium iodide (IP) assay using flow cytometry. The integrity of cell membrane was altered in 10% IP^+ cells in the presence of PA. These results reveal that the treatment with PA did not produce cellular necrosis. To further evaluation of the nature of the cytotoxic activity of PA, we quantified the development of hypodiploid nuclei. Apoptotic nuclei increased from 4% (0 h) to 43% at 3 h after incubation with PA. The apoptotic cells were quantified by the binding of Annexin V-FITC. At 200 μM PA, the PS exposure was of 71% vs. 17% IP^-/An^+ of the control group after 3 hours of incubation. We concluded that PA has anti-inflammatory activity by the mechanisms probably related to the inhibition of the IL-1β production and/or to the regulation of neutrophil apoptosis.

43

Antioxidants restore endothelium-dependent relaxation in fructose fed rats. Linares LM, Ricci CR, Planells FM, Smart, D, Rosón, M, Reyes Toso CF. Departamento de Fisiología. Facultad de Medicina. UBA. Paraguay 2155 Piso 7. Bs As. Argentina. creyesto@fmed.uba.ar

Rats fed a high fructose diet (Ff) -10 % in water- develop hypertension, hyperglycemia, hypertriglyceridemia and high plasma free fatty acids within 12-15 weeks. In a previous study we have shown that a decreased acetylcholine-induced relaxation (Ach-IR) is observed in intact aortic rings obtained from these animals. This effect was amplified by pre-incubation of rings in a high (44 mmol/l) glucose solution. Vitamin E incubation (VEi) partially restores aortic relaxation. This study was undertaken to evaluate whether the improvement of vascular reactivity seen with VEi was shared by two other antioxidants: Tiron and superoxide dismutase (SOD). Several experiments were performed: In endothelium-intact rings, Ach-IR of aortic rings was studied while in endothelium-denuded rings concentration-response curves to sodium nitroprusside (SNP) (a nitric oxide -NO- donor) were obtained (10^{-10}-10^{-5}M). Ach-IR and SNP relaxation were reduced ($P< 0.01$ factorial ANOVA). Tiron and SOD restored the altered vascular response obtained ($p< 0.01$, factorial ANOVA). Fasting blood glucose and oral glucose tolerance tests (OGTT) were performed. Glucose was measured and lipid peroxides in plasma and heart tissue homogenates were estimated colorimetrically by evaluating thiobarbituric acid reactive substances (TBARS). The heart tissue results were expressed as nmol per g protein. OGTT was altered in Ff rats 60 and 90 min after glucose load ($P<0.05$). TBARS in plasma and in heart were higher in Ff than in control rats ($P< 0.05$ and $P< 0.01$ respectively).

Conclusions: These results support further evidence about the ability of antioxidants to restore altered vascular reactivity, probably through the scavenging property of superoxide anion accumulation.

44

Evaluation of the anti-ulcerogenic effect of *Aristolochia argentina* in rats

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Aristolochia argentina (family Aristolochiaceae) is popular know as "charrúa". The roots of this plant are used in folk medicine. Their infusions and tinctures are reputed to have diuretic, antidiarrheic, astringent and antihemorroidal properties. The aim of this study was to asses the anti-ulcerogenic effect in rats. Infusions of the roots of the plant at 20% were prepared according to Pharmacopea Argentina VI ed. Phytochemical assays were performed. Wistar rats of either sex (200-250g) were employed. We examined the effect of *Aristolochia argentina* on gastric damage induced by oral administration of absolute ethanol (EtOH) and NaCl. The results were expressed in terms of an Ulcer Index (UI) from 0 to 5 (maximal damage). Phytochemical screening indicated the presence of flavonoids, alkaloids, saponins, tannins among others compounds. EtOH and NaCl produced gastric ulcers in all the animals treated. *Aristolochia argentina* prevents the formation of gastric lesions induced by EtOH (UI: 0.57 ± 0.2, $p<0.001$ vs. damage control: 4.9 ± 0.1) and NaCl (UI: 2.6 ± 0.5, $p<0.01$ vs. damage control: 4.78 ± 0.13). Several reports have shown that flavonoids inhibit gastric acid secretion and also protect against experimental ulcer. This anti-ulcerogenic effect of *Aristolochia argentina* could be due, in part, to the presence of flavonoids in this plant.

45

Consumption of benzodiazepines in Pavón Arriba.

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Benzodiazepines (BZDs) family is one of the most used drugs in Argentina. Objectives: to evaluate the consumption of benzodiazepines in Pavón Arriba, a town with about 2000 inhabitants, located in the south of Santa Fe, during the first six months of 2007. Methods: It was studied the consumption of BZDs as monodrugs through the calculation of defined daily doses per 1000 inhabitants per day (DIDs), using the medical prescriptions from all the community pharmacies and the office of dispensation of the only Community Medical Assistance Service (SAMCo). It was evaluated the percentage of patients which shown extended use (EU) of these drugs. Results: Consumption (DIDs): lorazepam (L): 34,9; alprazolam (A): 17,7; midazolam (M): 1,09; clonazepam (C): 4,9; diazepam (D): 4,1 y bromazepam (B): 2,3. Diazepam, the only BZD dispensed in SAMCo, had a significantly lower mean consumption than private dispensation ($p<0.01$). Women are the great majority of users of BZDs: 76% (70-81) %. The percentages of EU found in BZDs without hypnotic activity are (%, 95% CI): L: 28% (18-40)%; A: 31% (22-42)%; C: 21% (14-31)%; D: 10% (3-22)%; B: 17% (5-40)%. There was a significant difference of EU among these BZDs ($p=0,05$). Conclusion: It is necessary to perform research to explain the high consumption of BZDs compared with other towns and the elevated percentage of EU of them, considering the current guidelines. Nowadays, the social overload that women carry would justify, at least partially, their prevalence among the users of BZDs.

46

Loss of protection of diazoxide on the injury by ischemia-reperfusion in adult rat hearts under high K-low Ca-cardioplegia.

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Previous experiments in which it was evaluated the participation of the mitochondrial KATP channels (mKATP) in the protection by a 25 mM K-0.5 mM Ca cardioplegia (CPG) against ischemia-reperfusion (I-R) in rat hearts had demonstrated that 5-hydroxidecanoic (a specific blocker) increased resting heat (Hr) under CPG without changes in the contractility recovery during R Since these results suggested that CPG may open the mKATP it was evaluated a possible synergism between diazoxide (Dzx, a selective opener of mKATP) and CPG. Perfused rat hearts were pretreated with CPG before exposing to 45 min I- 45 min R while measuring intraventricular pressure (P) and total heat release (H_t) in a flow-calorimeter. The addition of 30 µM Dzx to CPG did not modify Hr (+0.064 ± 0.25 $mW.g^{-1}$, n=7). At the start of R, Dzx induced a diastolic contracture (ΔPr: 15.4 ± 3.3* mm Hg) and a reduction in P recovery (to 20.4 ± 6% of the pre-I*) but an increase in H_t (to 18.4 ± 3.1 $mW.g^{-1}$, 177 ± 10% of pre-I*). After 45 min R with Krebs without Dzx, ΔPr fell to 5.0 ± 6.7 mm Hg (NS from 0) and P recovered until 40.3 ± 6% of pre-I* with H_t = 22.5 ± 3.6 $mW.g^{-1}$ (215 % of pre-I*). Results suggest that the pretreatment of hearts with CPG and Dzx is not synergic but avoid the protection of CPG from the injury upon I-R. Dzx would inhibit the mitochondrial re-uptaking of cytosolic Ca^{+2} at the start of R and the contribution of Ca^{+2} from mitochondria to the SR, which are induced by CPG.
*(*p<0.05) X-408 UNLP-2005/07*

47

Cross reaction between bothropic venoms and IgG anti-crotalic phospholipase A_2

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We examined the ability of IgG antibodies obtained in rabbits against PLA_2, from *Crotalus durissus terrificus* venom to cross react with *Bothrops* venoms (*Bothrops alternatus, Bothrops neuwiedii and Bothrops jararacussú*) species that inhabit in the noreastern of Argentina.

ELISA and Immunoblotting were performed between bothropic venoms and a desired amount of IgG anti-crotalic PLA_2. Bothropic venoms preincubated with IgG anti crotalic PLA_2 were i.m. inoculated in mice and then CK level were determinate in sera. Similar mixtures were assayed in red-blood lecithin agar plaque to evaluate the neutralization of indirect hemolytic activity exhibited by these whole venoms.

Immunochemical assays showed that IgG anti-PLA_2 recognized antigens of Bothropic venoms, and demonstrated the cross reactivity among them. However, biological activity neutralization tests reveled that the amount of IgG anti-PLA_2 that efficiently neutralize PLA_2 activity of *C.d.t.* venom was insufficient to neutralize biological activities of Bothropic venoms

These results reveal that cross reaction occurs, but large amounts of proteins are needed to neutralize bothropic PLA_2s. Since that those proteins are responsible for adverse reactions, the use of these antibodies alone as antivenoms or to enrich commercial antivenoms is not recommendable.

48

Synergistic effect of rutin on the nalidixic acid antibac-bacterial activity against *Escherichia coli* ATCC 35218.

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In the last years, due to defensive mechanisms in bacteria, virus, fungi and protozoa, the effect of isolated antimicrobial agents has decreased considerably. The regular natural antibiotics are preferred because these not generate microorganism resistance, they stimulate natural elimination mechanisms, benefit epithelial regeneration process, suit organ functions, inhibiting pathogenic germ growth and increasing organism defenses.

The aim of the present study was to determine synergism between the conventional antibiotic nalidixic acid and rutin, inactive flavonoid, against *Escherichia coli* ATCC 35 218.

Microbial growth curves of *E. coli* in Müller-Hinton broth with isolated nalidixic acid or nalidixic acid-rutin combinations were carried out. Specific growth rate values allowed obtaining the minimal inhibitory concentrations (MIC) of this antibiotic and its combinations with rutin.

These results suggest that rutin synergize the nalidixic acid action against *E. coli*, decreasing its MIC to 45 % of its initial value (CIM $\cong$ 12 $\mu g.mL^{-1}$). The nalidixic acid-rutin combination could be considered as alternative for the infection treatment caused by *Escherichia coli* ATCC 35 218.

49

Transdermal administration of probenecid: concentration importance in the vehicle.

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Gout is a disease caused by uric acid excess in the articulations. In their treatment uricosurics, as probenecid, whose pharmacological effects consist of increasing uric acid elimination by urine, are used. This active principle promote high gastrointestinal irritation unsuited to its administration in patients with digestive disorders. In the present work the design of a formulation, which makes possible its administration by transdermal route was performed.

Probenecid solubility in different solvents and its combinations was evaluated. On the basis of probenecid solubility in an ethanol-water, a carbopol gel with this solvent mixture containing different probenecid concentrations was produced. Permeation studies were carried out by triplicate using Franz type cells and phosphate saline buffer with a 10% (v/v) of ethanol as receiver-medium. Probenecid permeated amount (Q) was determined using UV-Visible spectroscopy at 245 nm. Permeation profiles ((Q/Area) versus time) at different probenecid concentrations allowed to obtain flux (Jm) and permeation (P) and diffusion (D) coefficients values. The results indicate that a 0,66 % (p/p) probenecid concentration assures a higher permeation in a formulation of carbopol gel with a 15% (v/v) of water-ethanol. The permeation parameters are J_m= 3.73 x 10^{-7} g cm^{-2} seg^{-1}, P=1.68 x 10^{-4} cm seg^{-1} and D=1.81 x 10^{-5} cm^2 seg^{-1}.

50

Activity of *Tessaria absinthioides* on inflammatory processes. Effect on spleen and thymus.

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Previous studies allow us to attribute anti-inflammatory activity to *Tessaria absinthioides* (*TA*) (Asteraceae). The aim of the present work was to compare the effect on acute and chronic inflammatory models and also on spleen and thymus. Methods. 20% infusion from aerial part of *TA* was prepared, lyophilized and tested on: a) Paw edema induced by carrageenan: Wistar rats (200-250g), divided into groups, received by ip: saline (control); phenylbutazone (75 mg/kg) or 75 mg/kg, 200 mg/kg and 500 mg/kg of *TA*. One hour later, all animals were injected in left paw with 2% carrageenan suspension. Edema was measured at 1, 3, 5 and 7 h using a plethismometer. b) Granuloma test: a cotton pellet was implanted in Wistar rats (200-250g). The groups received during 6 days by sc: saline (control); dexamethasone (7 mg/kg) or 75 mg/kg, 200 mg/kg and 500 mg/kg of *TA*. On 7th day, all animals were sacrificed and the granuloma, thymus and spleen were weighted. Results. a) *TA* showed anti-inflammatory activity in both evaluated models. b) On acute model, each tested dose showed anti-inflammatory effect. c) On chronic model: the highest dose showed effectiveness but each one provoked an increase on spleen and thymus weights a different effect from dexamethasone. Conclusion. The results demonstrated that *TA* can act like anti-inflammatory agent on acute and chronic processes showing a different effect of dexamethasone on immunologic organs.

51

The *T. cruzi* cyclophilin A enhances the activity of hearth hexokinase activity.

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Cyclophilins (CyPs) are a multigenic family of enzymes that mediate cellular folding events, acting as chaperones by its PPIase activity and are Cyclosporin A binding proteins. We have studied a *T. cruzi* cyclophilin A of 19 kDa, the *Tc*CyP19, that is secreted to the culture medium. We know this protein can bind and inhibit a human calcineurin though the complex *Tc*CyP19-CsA. Its also known that parasite PPIases are involved in infection to host cells. The mitochondrial hexokinase activity has been proposed as a preventive antioxidant defence by an increase of the rate oxygen consumption reducing the rate of H_2O_2 generation. In this work we show that the *T. cruzi TcCyP19* increases the activity of the cardiac mitochondrial hexokinase of Wistar rat aged 3-10 days. Purified mitochondrial membranes by differential centrifugation were used to perform hexokinase activity in buffer 150 mM Tris-HCl pH 8 in the presence of glucose, NADP+, ATP and glucose-6 phosphate-dehydrogenase. Hexokinase activity was recorded at 340 nm during and after 30 min of incubation at 37°C. *TcCyP19* was added at different concentrations to 67µg/ml of protein homogenate. 41.6 pM of *TcCyP19* showed the best effect on the increment of hexokinase activity V_0, 357% ± 20.51 (n=3). The total amount of glucose conversion to glucose 6 phosphate was 13% ± 8,77 (n=7) after 30 min incubation. When *TcCyP19* was incubated with specific antibodies a 50% inhibition of glucose conversion to glucose 6 phosphate was shown. Our perspective is to study if the action of *TcCyP19* on the cardiac hexokinase activity is inhibited by CsA and its analogues which showed experimental anti-*T.cruzi* pharmacological effects.

52

EFFECT OF *Larrea divaricata* **AND** *Acacia visco* **METHANOLIC EXTRACTS, ON ACETIC ACID -INDUCED CHRONIC GASTRIC ULCERS IN RATS**

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In previous studies we showed antioxidant and acute antiulcerous gastric activity of leaves of *Larrea divaricata* Cav. (J. of Ethnopharmacology Vol. 105:415-420, 2006) and antioxidant activity of *Acacia visco* (Biocell Vol 27, 2003) in rat. The aim of this study was to asses the effect of *Larrea divaricata* methanolic extract from leaves (*Ld*MEL) and *Acacia visco* methanolic extracts from leaves (*Av*MEL) and bark (*Av*MEB) on acetic acid-induced chronic gastric ulcers in rats The ulcers were produced according Okabe S. and Pfeiffer C.J. (1972) employing acetic acid as ulcerogenic agent. Wistar rat, both sex, 200-240g, were fasted for 24h, water *ad libitum.* Animals were anesthetized and subserosal injection of 20µl 30% acetic acid was made to the border of the corpus and antrum in the ventral wall. The tested extracts *Ld*MEL, *Av*MEL or *Av*MEB. 200 mg/Kg and reference (omeprazol) 20 mg/Kg were given orally, daily for 8 days. On the 9th day following production of the ulcer, animals were killed and their stomachs removed. The ulceration grade was determined by area (mm^2).The methanolic extracts of *Larrea divaricata* and *Acacia visco* decreased significantly the ulceration compared to the control (ANOVA test).The accelerated healing effect of *Larrea divaricata* y *Acacia visco* extracts on chronic gastric ulcer might be related with their free radical scavenging capacity.

53

Evaluation of cephalexin absorption in cats after administration in two different muscular sites

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Cephalexin (CPX), a first generation cephalosporin, is active against common pathogens isolated from cat´s infections (*Staphylococcus* spp, *Streptococcus* spp, some enterobacteria and anaerobes). It could be administered by oral or parenteral routes. Absorption process depends on drug properties and administration site features (e.g. blood perfusion). Different muscles have different blood supply rate and, this may influence the antibiotic pharmacokinetics profile. The aim of this study was to determine differences in absorption rate of cephalexin in cats after its intramuscular administration within two different muscles.

CPX was administered intramuscularlly (10 mg/kg) to eleven adult cats. Six animals were injected in the *Semitendinosus* muscle (A) and five animals in the *Longissimus dorsi* muscle (B). Blood samples were collected over a 6 hours period. CPX concentrations were determined by microbiological assay. Absorption pharmacokinetic parameters (C_{max}, T_{max} and $AUC_{(0-6)}$) were statistically analyzed by a non-parametric t test (Mann-Whitney test) ($P \leq 0.05$).

Cmax, Tmax and $AUC_{(0-6)}$ for the A and B administrations were 7.72±1.48 µg/mL and 6.54±0.91 µg/mL, 2.2±1.3 h and 2.7±1.2 h and, 33.28±5.68 µg.h/mL and 28.41±3.63 µg.h/mL, respectively. There were not significant differences ($P>0.05$) between A and B for the three pharmacokinetic parameters. Cephalexin absorption rate seems to be equal for the two muscles in cats.

54

Inhibitory effect of fluoxetine on lymphoma growth through the modulation of antitumoral T cell response by serotonin dependent and independent mechanisms.

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Fluoxetine, a selective serotonin reuptake inhibitor, is widely used for the treatment of depressive symptoms of cancer patients, but there are contradictory evidences about its effects on immunity and neoplastic processes. Thus, we studied the effects of fluoxetine on a T-cell lymphoma growing in syngeneic mice and on antitumoral T-cell immunity. Chronic fluoxetine administration improved tumor prognosis, i.e. reduced tumor growth, and increased latency of apparition and survival. In addition, fluoxetine treatment also enhanced T cell proliferation to the selective mitogen Con A, evaluated by [^{3}H]thymidine incorporation. It also increased the expression of the antitumoral cytokines TNF-□ and IFN-□, measured by Real Time RT-PCR, without affecting $CD4^+$ and $CD8^+$ T lymphocyte subsets analyzed by dual fluorescence flow cytometry. In vitro, fluoxetine did not affect tumor cells proliferation, but it exerted a direct effect on T lymphocytes. Both fluoxetine and serotonin stimulated proliferation induced by a suboptimal mitogen concentration but inhibited proliferation at the optimal one. Both effects were directly related to the concentrations used for serotonin but indirectly related for fluoxetine. When both drugs were combined, the results suggested that the effects of fluoxetine are in part independent of its ability to elevate serotonin extracellular levels. These findings indicate that fluoxetine inhibits tumor growth through modulation of T-cell mediated immunity by direct serotonin-dependent and independent mechanisms.

55

TRICLABENDAZOLE RESISTANT *FASCIOLA HEPATICA*: PHARMACOKINETIC AND EFFICACY ASSESSMENTS OF A DRUG COMBINED TREATMENT

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The altered drug influx/efflux and enhanced metabolic capacity identified in triclabendazole (TCBZ)-resistant *Fasciola hepatica* contributes to the development of resistance to TCBZ. The aim of this work was to evaluate the pharmacokinetics (PK) and clinical efficacy (CE) of TCBZ administered alone or co-administered with ivermectin (IVM, drug efflux inhibitor) and methimazole (MTZ, metabolic inhibitor) in TCBZ-resistant *F. hepatica* parasitized sheep. Sheep infected with TCBZ-resistant *F. hepatica* were divided into three groups (n= 4): untreated control, TCBZ-treated and TCBZ+IVM+MTZ treated sheep. Plasma samples were collected (PK study) and analysed by HPLC. In the CE study, the animals were sacrificed at 15 days post-treatment to evaluate the comparative efficacy against TCBZ-resistant *F. hepatica*. The presence of IVM and MTZ did not affect the plasma PK behaviour of TCBZ metabolites. The combined drug treatment was not sufficient to enhance the poor efficacy of TCBZ against resistant *F. hepatica*. Finally, the enhancement of TCBZ concentrations in *F. hepatica* induced by both IVM and MTZ in *ex vivo* assays, did not reach a measurable effect under the current *in vivo* experimental conditions.

56

EFFECTS OF ALBENDAZOLE AND TRICLABENDAZOLE ON THE TESTICULAR MORPHOLOGY OF RATS WISTAR

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Albendazole (ABZ) and Triclabendazole (TCBZ) are benzimidazole antihelmíntics used in human and veterinary medicine. Its mechanism of action consists in the despolimerization of cytoplasmatic microtubules (MT) of the parasite. The MT in the host are important in the spermatogenesis and is possible its alteration by the action of these drugs. In this work were determined the effects of ABZ or TCBZ in the probable modifications of the rat testis morphology. Three groups of sexually mature Wistar rats were used. ABZ and TCBZ were administered orally in a dose of 2 g/Kg of body weight and to the group control an equivalent amount of sterile physiological solution. The organs were extracted to 48 h. post-treatment. Samples were processed by routine techniques until embedded in Histoplast. For the morphologic analysis of the testicular architecture, 5 µm slices were stained with hematoxilin and eosin.
The average diameter of the sperm ducts was determined and it evaluated the histological appearance and height of his epithelial. ABZ caused a significant diminution of the tubular diameter with a 23.6% of altered tubules, absence of spermatozoa, partial loss of the spermatogenic line and appearance of membranous vacuoles between the cells of Sertoli. For the case of TCBZ histologics alterations were not observed. This work shows that ABZ, at the highest dose rate, affect the spermatogenetic function in mouse..

57

Chemical stability of veterinary drugs residues in feces: adverse environmental impact?

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The macrocyclic lactones (ML) are broad-spectrum antiparasitic drugs excreted in large concentrations as unchanged parent drugs in feces. The current work evaluated the comparative chemical stability of different ML drugs in feces from treated sheep and cattle exposed to environmental field conditions. Fecal depositions from sheep and cattle subcutaneously treated with different ML were kept in the field during different time periods (between 6 and 180 days). Drug fecal concentrations were measured by HPLC. The ML drugs showed a sustained chemical stability. Long persistence of fecal drug residues were observed in the feces exposed to field conditions. Although a rapid decrease in ML fecal concentrations were observed over the first 14 days (cattle) and 32 days (sheep) post-deposition, a slow chemical degradation accounting for the long persistence of the active parent molecules in the environment was registered. Doramectin was the ML compound recovered at the highest residual concentration in feces from both animal species. Even after 100 days of field exposure, doramectin concentrations were 308 (sheep) and 425 (cattle) ng/g dry faeces. The potential adverse effects of the fecal residual concentrations against non-target organisms are under evaluation in an Argentina-Slovenia bi-national collaborative project.

58

Evaluation of psychopharmacological effects of *Artemisia copa* aqueous extract in mice.

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Artemisia copa Phil. (Asteraceae) is a small shrub native of the northwestern of our country, commonly known as "copa-copa", and the infusion of the aerial parts are used in popular medicine as analgesic and anti-inflammatory, between others. In the present study the aqueous extract from aerial parts of *A.copa* (AC) administered p.o., was evaluated for its psychopharmacological activities in several experimental models using female Swiss albino mice. AC at doses up to 1.5 g/kg produced a dose dependent sleep induction and potentiation of sub-hypnotic and hypnotic doses of pentobarbital (PB30: 0.5 ± 0.35 min, AC 1.5 g/kg + PB30: 18.24 ± 3.88 min, $P<0.01$. PB40: 34.83 ± 5.90 min, AC 1.5 g/kg + PB40: 51.33 ± 3.22 min, $P<0.03$, respectively). AC produced a dose dependent increase and decrease in the spontaneous motor activity (0.5-1.5 g/kg) and a decrease on exploratory (holeboard) behavioral profiles (1.5 g/kg). AC displayed dose-related anxiolitic-like activity as indicated by increases in the percent of marbles they left uncovered in the marble-burying test. In addition the extract (1.5 g/kg) produced a significative decrease in the duration of seizures and mortality induced by PTZ 75 mg/kg, without effects in the latency time. These results suggest that the extract may contain sedative principles with potential anxiolytic and anticonvulsant activities.

59

THROMBOLYTIC AND ANTICOMPLEMENTARY PROPERTIES OF LOW MOLECULAR MASS DERMATAN SULFATE.

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Low molecular mass dermatan sulfate (LMMDS), obtained by dermatan sulfate (DS) peroxy-radical depolymerization, acts as anticoagulant by accelerating the inhibition of thrombin heparin cofactor II. The objective of the present study was to evaluate its activities over anticoagulant system and over the classical activation pathway. Anticoagulant activities were studied using a rat experimental venous thrombosis, induced on the vena cava by stasis. DS (0.3 mg kg^{-1}) or LMMDS (0.2 mg kg^{-1}) was intravenous (IV) administered 2 hours later (protocol 1) or simultaneously (protocol 2) of venous occlusion in order to evaluate thrombolytic or antithrombinic activities, respectively. *In vivo* and *in vitro* inhibition of the classical complement activation pathway by LMMDS was assessed using a hemolytic complement assay. DS and LMMDS significantly reduced thrombus weight, protocol 1, 2.4±0.48 mg and 1.38±0.52 mg, respectively vs the vehicle (6.23±1.19 mg). In protocol 2, thrombus weight after DS, LMMDS and vehicle was 3.00±0.06 mg, 3.23±0.22 mg and 4.75±0.05 mg, respectively. After LMMDS IV administration, the lowest rat serum dilution (corresponding to the maximum complement system inhibition) was achieved at 60 min ($P<0.001$, basal vs 60 min). LMMDS was less effective than heparin in *in vitro* complement inhibition (1250 µg/ml vs 25 µg/ml). LMMDS seem to be more effective in stabilized thrombus reduction than in prevention of thrombus formation. Furthermore, it is an effective *in vivo* inhibitor of the complement classical pathway activation.

60

In vivo 5-Fluorouracil induced apoptosis on murine thymocytes: involvement of CD95, Bax and Caspase-3

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Apoptosis has shown to mediate chemotherapeutic drug-induced cell death in vitro and in vivo. Unfortunately many of these drugs are non-specific and cause severe side effects. To study the effects of the chemotherapeutic drug 5-Fluorouracil (5-FU) over normal thymus, and the contribution of the pro-apoptotic proteins CD95, Bax and Caspase-3 to 5-FU induced apoptosis in vivo, a single dose of 5-FU (150mg/kg ip) was injected to Swiss CF-1 mice in a ten days time course study. Total organ weight and cell number were reduced. The thymic architecture assessed with electron scanning microscopy, was altered leading to the lost of cell-cell contact disposition. Apoptosis was measured with different methods, including optical and fluorescence microscopy and TUNEL assay. Cell death in thymocytes enhanced among the first six hours and the fifth day, reaching values 3 times over control at the second day. Furthermore, in the 5-FU treated thymus, the pro-apoptotic proteins CD95 and Bax were strongly up-regulated during the first two days, showing a timely coincident pattern of expression with the maximal apoptotic values. Moreover, Caspase-3 activity was also greater at the same period. Thus, a significant portion of in vivo thymocytic apoptosis after 5-FU treatment is mediated, at least in part, by CD95, Bax and Caspase-3. Our findings therefore contribute to the understanding of how 5-FU exerts its function in vivo upon normal thymic tissue.

61

THE EFFECT "IN VIVO" OF THE ANTHELMINTIC TRICLABENDAZOLE ON FASCIOLA HEPATICA. ITS IMPORTANCE IN THE EFFICACY TEST

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The efficacy test are the main tools for the characterization of drug and the test recommended for the characterization of anthelmintics. The parasite, *Fasciola hepatica*, has a worldwide distribution and infects a wide variety of mammalian hosts, including the human. Triclabendazole (TCBZ) is an antihelmintic benzimidazole widely used to control the fluke *F. hepatica*. In this work were evaluated through histopatological and immuno histochemical techniques the alterations generated in *F. hepatica* exposed "in vivo" at TCBZ. Four healthy bovines without antecedents of fasciolasis was inoculated with 400 metacercarias each one. Two of the inoculated bovines (experimental) were treated to the 90 days post infection with 8 mg/Kg. of TCBZ and sacrificed jointly with the controls to day 105. The flukes of the bovine controls showed testicular tubes full of cells in different states from the espermatogenesis with abundant viteline cells and uterus with mature eggs. The fluke exposed to TCBZ displayed their testes with abundant peripheral vacuolización, significant diminution of the number of viteline cells and uterus without detection of egg. These findings support the fact that despite the survival of the trematode to the drug exposure, they showed a marked infertility, which would impede them to continue their biological cycle. These findings induced us to review the validity of the controlled efficacy test, which is based only on the counting of live trematodes at the moment of host sacrifice.

62

Involvement of the opioid receptors in the sedative, antinociceptive and potentiating effects with benzodiazepines of the glycosilated flavonoid hesperidin.

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Previous studies performed in our laboratories reported the sedative and the sleep enhancing activities of hesperidin (hesperetin-7-rhamnoglucoside). These properties are greatly increased when the glycoside is injected together with diazepam and this interaction has been shown to be synergistic. In the present work we demonstrate that this effect is shared with various benzodiazepines (like alprazolam, bromazepam, midazolam and flunitrazepam), which were used to study the potentiation with hesperidin as measured in the hole board and the locomotor activity tests. We have also proved that the behavioral and synergistic effects induced by hesperidin do not involve classical $GABA_A$ receptors. In addition, it has been demonstrated that hesperidin possesses anti-inflammatory and analgesic activities. In order to get going in the study of the mechanism of action of hesperidin, we explore the possible participation of other brain receptors, namely opioid, serotonin and alpha 1-adrenergic. The use of opioid antagonists in the current work provided the first experimental evidence of the participation of those receptors in the sedative, antinociceptive and potentiating effects of hesperidin with benzodiazepines in mice.

63

EVOLUTION OF ORGANIC ANIONS RENAL EXCRETION IN RATS WITH BILATERAL URETERAL OBSTRUCTION (B).

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The organic anion transporters play an important role in renal elimination of anion drugs, including β-lactam antibiotics, cytostatic agents, diuretics and antiviral drugs. The aim of the present study was to evaluate the evolution of *p*-aminohippurate (PAH) renal excretion and cortical renal expression of the organic anion transporters (OAT1 and OAT3) in rats with B. Both ureters were ligated during 24 h, then they were released and the studies were done after 1 (B1,n=5), 2 (B2,n=6) and 7 days (B7,n=5). A parallel group of sham rats (S,n=16) was employed. PAH renal clearance (Cl, mL/min/100g b.w.) was determined employing conventional techniques, and OAT1 and OAT3 expression using immunoblotting and immunohistochemical techniques. Data were analysed with ANOVA and Newman-Keuls $P<0.05$: [a]vsS, [b]vsB1, [c]vsB2, [d]vsB7. B1 and B2 rats showed a clearance of PAH lower than S (S: 3.77±0.41; B1: 0.49±0.18 a,d; B2: 0.83± 0.18 a,d; B7: 2.79±0.23 b,c). Immunoblotting revealed significant decreases in both OAT1 and OAT3 expression in basolateral plasma membranes in B1 and B2. The expression of OAT1 and OAT3 recovered to control level in B7. Immunohistochemical assays confirmed these results. These data demonstrate that modifications in OAT1 and OAT3 expression could explain the evolution of organic anions renal excretion in rats with B.

64

INTERACTION BETWEEN NEURONAL Na^+, K^+-ATPase AND NMDA RECEPTOR

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Ouabain increases the activated population of NMDA receptors. The participation of NMDA receptor in the ouabain neurotoxic effect has been suggested. Herein the effect of ouabain on the expression of NMDA subunits and Na^+, K^+-ATPase α3 subunit, as well as on the activity of neuronal Na^+, K^+-ATPase was evaluated. Wistar rats of 250 g were administered via *i.c.v.* with 1 µl 10 mM ouabain or saline solution (control). Two days later, membranes of cerebral cortex and hippocampus were isolated. Western blots with antibodies for the NMDA receptor subunits: NR1; NR2A; NR2B; NR2C and NR2D and with the specific antibody for Na^+, K^+-ATPase subunit α3 were carried out. Na^+, K^+-ATPase activity was measured in synaptosomal membranes. The treatment produced the following changes: subunits NR2B and NR2C diminished in cerebral cortex and hippocampus, NR2A decreased only in hippocampus, whereas subunit NR1 did not changed in the studied areas. NR2D increased in cerebral cortex, but remained unaltered in hippocampus. Na^+, K^+-ATPase α3 subunit increased in cerebral cortex whereas Na^+, K^+-ATPase activity diminished 50%. It is concluded that ouabain administration led to a differential decrease in the expression of NMDA subunits. These results may be correlated with the modulatory action of ouabain on NMDA receptor. Enhancement of Na^+, K^+-ATPase α3 subunit may be a compensatory response to the inhibition of the Na^+, K^+-ATPase activity induced by ouabain.

65

Assessment of pharmacological response of diclofenac and gemfibrozil in an atherogenic experimental model by hyperfibrinogenemia induction.

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Pharmacological response to diclofenac and gemfibrozil was evaluated by hyperfibrinogenemia(PF), nitric oxide(NO) and superoxide and the effect on mitochondrial damage in smooth muscle cells from thoracic aorta with atherogénesis. Wistar male rats were used: control(I), multiple injury(MI)x60days(II), MIx60 days+diclofenac(III) and MIx60 days+gemfibrozil(IV). MI group were induced by injection of adrenaline(0,1mg/rat/day). Diclofenac (0,2mg/rat/day) and gemfibrozil (3mg/rat/day) were administered for 45 days. We measured PF(mg/dl), NO(uM) and SOD activity by spectrophotometry. Slices of thoracic aorta were studied by electronic microscopy (EM). Results were analysed by ANOVA and Chi Square and $p<0.05$ level of significance was established in all cases. PF and SOD activity significantly increased in II (400±17 and 217±14) compared to I (209±4 and 141±4)($p<0.001$). NO significantly decreased in II(16.73±2.55) respect to I(24.05±2.42)($p<0.001$). PF from III(253±13) and IV(251±21), NO from III(19.11±1.1) and IV(22.53±0.7), and SOD from III(227±22) and IV(218±24) presented similar values at the control after administrating the drugs. EM showed involution of mitochondrial lesions in (III) and (IV) respect to (II). Probably PF would generate changes in oxidative stress with low availability of NO an increased SOD. Diclofenac (by inhibition of ciclooxigenase) and gemfibrozil (by reduction of expression of proinflammatory citocins and PF in vascular cells) would modify the inflammatory process present in atherogénesis.

66

Secondary renal hypoxia induced by adriamycin: Expression of HIF-1α and tisular changes.

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Cellular hypoxia has been shown to have relevant effects in the pathogenesis of renal injury. Hypoxia Inducible Factor 1 (HIF-1α) is the prototypical factor involved in transcriptional responses to oxygen tension. The aim of this work was to evaluate HIF-1α expression and its correlation with the secondary hypoxia induced by Adriamycin in murine kidneys along 28 days. CF-1 mice were injected with a single dose of Adriamycin (11μg/g, ip). Samples of serum and urine were collected. Kidney slides were stained with H/E and PAS for histopathological evaluations. Scanning electronic microscopy was used for morphological descriptions in renal outer cortex and medulla. Apoptosis was evaluated with fluorescence microscopy. HIF-1α was determined by Western blotting in nuclear and cytoplasmic kidney extracts. Experimental data showed progressive glomerular, tubular and interstitial injury with mononuclear cells infiltrates. Moreover, creatinine and urea levels were statiscally greater than control at 28 days ($p<0.01$). By the 7^{th} day hematocrits increased and a significant proteinuria was noticed. Apoptotic cells were maximal at 7 days. We noticed HIF-1α translocation from cytosol to nucleus concomitant with the acute hypoxic response (7 days). These results show that structural changes precede the functional renal alterations. Moreover, adriamycin induces the progressive translocation of HIF-1α to nucleus. This fact was coincident with morphological and functional disturbances in renal tissue.
This study opens new insights for the understanding of the renal injury caused by Adriamycin.

67

Signal transduction through Na^+, K^+-ATPase impairement involves high affinity neurotensin receptor

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Phosphoinositide (PI) metabolism is enhanced in neonatal brain by activation of neurotransmitter receptors and by inhibition of the sodium pump with ouabain (Oua) or endogenous inhibitor, endobain E (End E). Neurotensin inhibits synaptosomal membrane Na^+, K^+-ATPase activity, an effect blocked by SR 48692 (SR), a selective antagonist for high-affinity neurotensin receptor (NTS1). To evaluate potential participation of NTS1 on PI hydrolysis enhancement by sodium pump inhibition, cerebral cortex miniprisms from neonatal Wistar rats were preincubated for 0 or 30 min in the absence or presence of SR; then, Oua or End E were added and incubation proceeded for 20 or 60 min. After 60-min incubation with Oua, IPs accumulation vs basal was 500% or 860% if preincubation was omitted or lasted 30 min, respectively; values were reduced 50% in the presence of SR. With 20 min incubation, IPs accumulation by Oua vs basal was 300% or 410% if preincubation was 0 or 30 min, respectively, an effect blocked 23% or 32% with SR. PI hydrolysis enhancement by End E was similarly blocked by SR, being higher when incubation with End E lasted 60 vs 20 min. PI turnover increase by sodium pump inhibition with Oua or End E is diminished by SR suggesting that, at least partially, NTS1 is involved in this cell signaling system.

68

EFFECT OF A PRENYLATED FLAVONOID ON THE ACTIVITIES OF MEMBRANE-BOUND ENZYMES AND ON MITOCHONDRIAL MEMBRANE POTENTIAL. Elingold I[1], Casanova MB[1], Celentano AM[2], Taboas M[1] ,Pérez C[3], Cabrera JL[4], Diez RA[5], Dubin M[1].

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The prenylated flavonoid 2´-4´-dihidroxy-5´-(1´´´-dimethylallyl)-6-prenylpinocembrin (6PP), obtained from Dalea elegans, shows antimicrobial activity. Previous results of 6PP showed dose-dependent anti-radical, and antioxidant activities. 6PP, also inhibited and/or uncoupled mitochondrial respiration and inhibited F_0F_1 ATPase activity (Elingold et al, SAIC 2006). We study the effect of 6PP on succinate deshidrogenase (SDH) and NADH-oxidase activities and mitochondrial membrane potential (MMP). 6PP promoted significantly inhibition of SDH and NADH oxidase activity in a concentration-dependent manner (IC_{50} of 25 μM and 19 μM respectively). The effect of 6PP on MMP was investigated in coupled mitochondria and was measured by rhodamine 123 fluorescence. Using malate-glutamate as substrate, 6PP (50 μM) significantly decreased 32% MMP after 25 min incubation, while 6PP (100 μM) significantly decreased by 47.5 and 56% MMP for 10 or 25 min incubation, respectively With succinate as substrate, 6PP (50 and 100 μM) decreased MMP by 33 and 48.4% respectively after 10 min incubation. On the other hand, 6PP (25, 50 and 100 μM) decreased MMP for 25 min incubation (34.4, 55 and 56%, respectively). In summary, we have partially characterized the activity of the prenylated flavonoid 6PP, demonstrating its toxic effects on isolated rat liver mitochondria.

69

Effect of exercise on the vasodilator and vasoconstrictor response in aortas from obese rats.

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Vascular tone is determined by endothelial vasodilator vasoconstrictor factors, such us nitric oxide (NO) and cyclooxygenase-2 (COX-2) and cytochrome P450 derivates. Since obesity alters endothelial function and the exercise improves it, the aim of this study was to analyze the effect of training on vasodilator and vasoconstrictor responses, and the involvement of NO, COX-2 and cytochrome P450 derivates in those responses. Aortic segments from sedentary and trained (three months of moderate exercise during 20 min/three days per week) obese Zucker rats were used to analyze: (i) the acetylcholine (Ach)-induced NO release; (ii) the effect of NO synthase inhibitor, L-NAME, or COX-2 inhibitor, NS-398 on the Ach-induced response, (iii) the effect of L-NAME, NS-398 or ABZ, a cytochrome P450 inhibitor, on the NA-induced response. The Ach-induced NO release and vasodilator response were similar in aortas from sedentary and trained rats, and L-NAME abolished the Ach-induced response while NS-398 did not modify it. The NA-induced contraction was similar in arteries from both groups; L-NAME or NS-398 increased or decreased, respectively, the NA response in similar extent, while ABZ decreased that response only in aortas from sedentary rats. These results indicate that, in aortas from obese Zucker rats, training eliminates the participation of vasoconstrictor derivated from cytochrome P450 on NA-induced response. Despite this alteration the net vasomotor responses to Ach and NA are maintained.

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Hypoglycemic drugs use in the
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Drug utilization research is essential to promote the rational use of them. Objectives: to evaluate the consumption of hypoglycemic drugs in outpatients of Hospital Provincial del Centenario during the first four months of the current year. Methods: It was studied the consumption through the calculation of defined daily doses (DDDs), of metformin (M) and glybenclamide (G), using the medical prescriptions from hospital's pharmacy. People who use these drugs were defined as diabetics II (DII). On the other hand, it was collected the consumption of NPH insulin (NPHI) and current insulin (CI) from prescriptions which did not include oral hypoglycemic drugs. Such users were defined as exclusive insulin-dependent diabetics (EIDD). Results: M and G consumption (DDDs means ± DS) to each month of study, n= nº of dispensations. First month (nM= 167; nG=157): M: 16±7, G: 26±11*; second month (nM= 123; nG=108): M: 15±6, G: 24±9*; third month (nM= 114; nG=124): M: 15±9, G: 26±16*; fourth month (nM= 131; nG=119): M: 15±8, G: 26±13*; *p<0.00001. NPHI and CI consumption means of 4 months of study: (nNPHI=207; nCI=38): NPHI: 50±16, CI: 28±8*; *p<=0.00001. Conclusion: The current tendencies advise M as the first choice drug in DII, then it must be noticeable the significantly higher consumption of G rather than M each month studied. Furthermore, it should be investigated the excessively low consumption of CI compared to NPHI in EIDD.

71

Antidepressant-like effects of omega-3 fatty acids and fluoxetine are potentiated by combined treatment in rats

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Depression is a psychiatric disorder that affects millions of people worldwide. Current pharmacological treatment has numerous potential side effects, is not effective for all individuals and possesses at least a two-week delay period for its onset of action. In coincidence with the high concentration of polyunsaturated fatty acids including the essential fatty acids (EFAs) in the brain, recent research underscores the important role of omega-3 fatty acids (ω-3) in the central nervous system function and the EFAs potential in the treatment of various neuropsychiatric disorders. The purpose of the present study was to determine whether omega-3 fatty acids, give alone or in combination with the antidepressant fluoxetine (FLX), would produce antidepressant-like actions in rats. We found that compared to control diet, ω-3 diet significantly decreased the immobility time and increased behaviors of swimming during the forced swimming test (FST), indicating that ω-3 has antidepressant-like effects in rats. Moreover, FLX effects were potentiated by dietary supplementation with ω-3 given daily for 16 days which rendered decreased immobility and increased swimming when measured 24 h after the last injection. In any case, ω-3 diet supplementation modified neither rat body weight nor locomotor activity. Our findings suggest that potentiation of FLX with ω-3 fatty acids diet deserves further study as a possible strategy for patients with depression.

72

Vitamin E administration on fructose fed rats partially restores endothelium-induced relaxation. Wallinger ML, Linares LM, Obaya-Naredo D, Saredo S, Reyes Toso, ML, Vázquez MB, Reyes Toso CF. Departamento de Fisiología. Facultad de Medicina. UBA. Paraguay 2155 Piso 7. Bs As. Argentina. creyesto@fmed.uba.ar

Rats fed a high fructose diet (Ff) -60% fructose- develop an experimental Metabolic Syndrome within 12-15 weeks. In a previous study we have shown that a decreased acetylcholine-induced relaxation (Ach-IR) is observed in intact aortic rings obtained from these animals. This effect was amplified by pre-incubation of rings in a high (44 mmol/l) glucose (HG) solution, a situation which induces oxidative stress. The present work was designed to evaluate the effect of vitamin E supplementation (VEs) (50 mg/day) on acetylcholine-induced relaxation (Ach-IR) in Ff rats. Several experiments were performed. In endothelium-intact rings, Ach-IR was studied while in endothelium-denuded rings concentration-response curves to sodium nitroprusside (SNP) were obtained (10^{-10}-10^{-5}M). Fasting blood glucose and oral glucose tolerance tests (OGTT) were performed. OGTT was altered in Ff rats 60 and 90 min after glucose load ($P<0.05$ and $P<0.01$ respectively). Rings obtained from Ff rats exhibited a decreased Ach-IR, while those from rats with VEs showed an improved Ach-IR ($P< 0.05$ two way ANOVA). In endothelium-denuded rings an impaired relaxation to SNP was detected and this effect was partially counteracted by VEs ($P< 0.05$ two way ANOVA). Conclusions: The decreased Ach-IR and SNP relaxation obtained in aortic rings from Ff rats incubated in a HG medium were partially restored with VEs. This effect could be related to its antioxidant activity.

73

CANCER IMMUNOTHERAPY BASED ON THE REGIONAL LYMPH NODES STIMULATION WITH AN ATTENUATED *SALMONELLA*.

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When tumors spread beyond the primary site, they often come to rest in the regional lymph nodes. The host´s immune response, both to cancer and to infectious diseases is also initiated in these nodes.
The aim of this study is to develop an immunotherapy protocol to promote the regression of a subcutaneous (s.c) tumor using a recombinant bacteria attenuated *salmonella* as an anticancer drug. Balb/c mice were s.c inoculated with LM3 tumor cells (mammary adenocarcinoma murine). When tumors were palpable, animals were immunized with bacteria by s.c route, for three times, in either the peritumoral or intratumoral and the periganglionar areas. Mice treated with bacteria display a significant reduction in the tumor size and a significant increase in the medium survival time ($p < 0.05$, Logrank test). In addition, immunization induced draining lymph node cells to produce significant levels of IFN γ ($p < 0.05$), indicating the presence of a Th1-type cytokine response. Our results demonstrated an efficient antitumor therapeutic response elicited by this novel therapy.

74

HYPOXIC HYPERSECRETION OF ERYTHROPOIETIN INDUCED BY FLUTAMIDE IN THE MALE MOUSE. Barceló AC, Martínez MP, Conti MI, Bozzini CE. Department of Physiology, Faculty of Odontology, University of Buenos Aires; and Bio Sidus SA

Hypoxia-stimulated secretion of erythropoietin (EPO) in polycythaemic mice is markedly enhanced by previous treatment with testosterone (T) (androgen induced EPO-hypersecretory state, EPO-HS). Flutamide (FLU) is a pure, non-steroid antiandrogen that also blocks brain androgenic receptors, thus preventing the feedback inhibition of LH by T. FLU thus causes significant increments in serum T and LH. This investigation was designed to test the hypothesis that FLU induces an EPO-HS in the male mouse. FLU (Sigma F9397), 1.5 mg/200µl, was daily injected sc during 4 wks to CF#1 adult male mice. Treatment induced a highly significant ($p<0.001$) reduction in seminal vesicle weight and no change in the hematocrit value. FLU caused a significant 6.7 times increase in serum T. Plasma EPO (pEPO) was determined by immunoassay (Quantikine Elisa Kit, code MEP00 R&D Systems) in both normoxic and hypoxic mice. The latter were exposed to air maintained at Torr 304 mmHg for 6h in a simulated high altitude chamber 24h after transfusion of heterologous (rat) red cells (1.2 ml ip). pEPO were 1054.2 ±185.4 (SD) and 2174.5 ± 191.7 pg/ml ($p<0.001$) in normocythemic control and FLU-treated mice, respectively, after exposure to hypoxia. In polycythaemic mice similarly treated, values were 58.6 ± 12.7 and 508.4 ± 61.2 pg/ml ($p<0.001$). Analysis of data suggests that flutamide induces an EPO-HS in the male mice.

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75

Comparison of L-menthol and D-limonene as quercetin permeation enhancers

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Quercetin is a flavonoid that present interesting properties: reduces arterial pressure and endotelial disfunction and shows anti-inflammatory and antioxidant activity. However, quercetin (Q) is slightly soluble in water, which may be responsible for its limited absorption upon oral administration. Considering efficiency and toxicity balance, terpenes have been extensively studied for their clinical use as penetration enhancers. They cause an increasing drug diffusivity through skin by disruption of the intercellular lipid packing in the stratum corneum. The aim of the present study is to optimize quercetin transdermal permeation in carbopol gel (CG) through ear pig skin. Terpenes such as L-menthol and D-limonene as chemical enhancers were employed. Permeation experiences were carried out using an automatic sampler with Franz-type diffusion cells. The pig skin was mounted between the donor compartment (containing quercetin - carbopol gel - enhancer) and (PBS, pH=7.4). Quercetin cumulative amounts in the receptor medium were determined spectrophotometrically at 255 nm. Diffusion coefficients for the systems Q-GC ($D = 2.083x10^{-7}$ cm^2 s^{-1}); Q-GC-L-menthol ($D = 13.72x10^{-7}$ cm^2 s^{-1}) and Q-GC-D-limonene ($D = 5.458x10^{-7} cm^2$ s^{-1}) demonstrated that for quercetin transdermal release: 1- the L-menthol effect is approximately 2.5 times higher that D-limonene; 2- terpenes such us L-menthol and D-limonene could be promising chemical enhancers.

76

Effect of acetaminophen intoxication on intestinal P-glycoprotein expression and activity in rats.

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Acetaminophen (AP) is the most used analgesic and antipyretic drug. Whether AP affects intestinal barrier, as represented by P-glycoprotein (Pgp), is unknown. The **aim** of this study was to evaluate the effect of AP intoxication on intestinal Pgp expression and activity in rats. **Material and Methods:** Male Wistar rats were injected i.p. with 1 g/kg bw of AP (AP Group) or vehicle (C group). Experiments were performed 24 hr after the injection. Pgp expression was evaluated by western blot and its activity using the everted intestinal sac model. The last 10 cm of intestine (next to the ileocecal valve) were filled with rhodamine 123 (15 uM, serosal side) and incubated in Krebs-Henseleit buffer with or without verapamil (100 uM, a Pgp inhibitor, mucosal side). Mucosal samples were taken for 45 min in 5 min-periods. Data are expressed as means ± SD. **Results:** Intestinal Pgp expression was higher in AP (1244±286) vs C (661±226) (Optical density units, n=3, $p<0.05$). Pgp activity was increased in AP vs C, the slope (nmol rho/g/min, n=4) of the secretion kinetic curve being 67% higher in AP (0.082±0.016) than in C (0.049±0.003) ($p<0.05$). The presence of Verapamil inhibited rho transport in AP and C (slope AP+V: 0.006±0.002 and C+V: 0.007±0.004). **Conclusion:** induction of P-gp by AP leads to increased membrane barrier activity at the intestine.

77

Participation of genetic control of the Th1 –Th2 balance in diabetes-induced immune alteration. R Rubinstein, M Wald y AMGenaro CEFYBO- UBA-CONICET. Paraguay 2155. Bs As. Argentina. roxirubin@yahoo.com.ar

Diabetes predispose to infections supporting an association with immunosuppression, being the hyperglycemia the main factor involved. However, some individuals with diabetes have poorer outcomes after infection and increase incidence of hospital pathogen invasion but others have a normal evolution. Genetic control of the Th1 –Th2 balance has been associated to different resistance to infection. Here we studied the effect of diabetes state in the immune response in Th1-biased C57Bl/6 (C57) mice and Th2-biased BALB/c mice. We observed that diabetes result in a time- dependent decrease of Con A T-cell and LPS B-cell stimulated proliferation in BALB/c mice. In contrast, in C57 mice diabetes did not induce inhibition of lymphocyte reactivity. However, glucose levels in diabetic animals were significantly higher in C57 than BALB/c mice. Therefore, the direct effect of elevated extracellular glucose on lymphoid cells by culturing BALB/c and C57 mice T and B lymphocytes was investigated. Results indicate that T and B-cell proliferation in BALB/c was decrease by high concentration of glucose but not the C57 one. Oxidative stress production, measureared as lipid peroxidation levels increased when T and B cell proliferation was diminished, suggesting its participation in the observed effects. These results indicate that genetic control of Th1-Th2 balance could affect the evolution of infection in subject with diabetes.

78

FLUBENDAZOLE KINETIC BEHAVIOUR: ABILITY TO DIFUSSE INTO HYDATID CYSTS IN MICE

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Flubendazole (FLBZ) has shown efficacy against protoscoleces of *Echinococcus granulosus in vivo* and *in vitro*. The aim of this work was to evaluate the plasma pharmacokinetic (PK) behaviour of FLBZ and its ability to access the hydatid cyst in infected mice. BalbC mice (n= 44) infected with *E. granulosus* (eight months of infection) were orally treated with a FLBZ-cyclodextrin solution (5 mg/kg). Blood and cysts samples were collected between 0 and 12 h post-treatment and analysed for FLBZ/metabolites by HPLC. FLBZ, the main analyte detected in plasma, reached a Cmax value of 1.55 µg/mL and was fast depleted from the bloodstream. A reduced-FLBZ metabolite was the main molecule recovered in plasma. While FLBZ peaked at 15 min post-administration in plasma, its higher cyst concentration (0.1 µg/g) was achieved at 52 min post-treatment. FLBZ concentrations within the cysts were significantly higher than those measured for its reduced and hydrolyzed metabolites. The higher lipophilicity of parent FLBZ may account for a greater diffusion rate through the cyst wall compared to that observed for its more polar metabolites.

79

Postnatal stress: effect of staurosporine in GABA and L-serine uptake. Scolari MJ, Rodríguez CB, Salatino AE, Acosta GB. Instituto de Investigaciones Farmacológicas. Junín 956. 5° piso. C1113AAD. Buenos Aires. Argentina. E-mail: gacosta@ffyb.uba.ar

It is well documented that early stress in life has marked consequences in the adult age. In previous works we demonstrated that postnatal acute stress alters the uptake rate of GABA and L-serine and induces an increase in the activity of protein kinase C (PKC) at postnatal days (PD) 5, 7 and 13. In the present study, we want to determinate if acute postnatal stress induced changes in amino acids uptake and PKC activation are correlated. For this aim, the effects of protein kinase C (PKC) inhibitor (staurosporine) on GABA and L-serine transporters activity were examined in cerebral cortex (CC). Experiments were performed with CC of Wistar male rats in PD mentioned above. Neonates were stressed by maternal separation plus cold exposure during 1 hour at 4ºC. Unhandled neonates, left undisturbed in their home cages, served as control. Upon termination of stress session, neonates were killed by decapitation. The brain was removed and CC was dissected. Examining the time-dependence of this inhibitor, we found no significant changes in the stressed animals at PD13. However, a return to control values was observed at PD5 and PD7, suggesting that in this developmental stages postnatal stress induced changes in GABA and L-serine uptake, could be associated with PKC activation.

80

Enzymatic pathways involved in flubendazole liver biotransformation

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Flubendazole (FLBZ) is a benzimidazole anthelmintic widely used in poultry and swine, which may be an alternative drug for parasite control in ruminants. The objective of this work was to characterise the main enzymatic pathways involved in the hepatic biotransformation of FLBZ. Liver cytosols and microsomes were obtained from control and phenobarbital (PB)-induced female Wistar rats, and from untreated male Corriedale x Merino cross breed sheep. Subcellular fractions were incubated with 40 μM of either FLBZ or its reduced chiral metabolite (red-FLBZ) in presence of NADPH. Incubation mixtures were analysed by HPLC. Liver microsomes from control rat reduced FLBZ to red-FLBZ and oxidised the later back to the parent molecule. Microsomes obtained from PB-induced rats displayed higher cytochrome (CYP) 3A and 2C-mediated N-demethylase activities, which correlated with an enhanced ability to convert red-FLBZ into FLBZ. CYP-mediated oxidative metabolism of red-FLBZ to FLBZ was absent in sheep liver. Both cytosolic and microsomal fractions obtained from sheep liver were able to reduce FLBZ into red-FLBZ at the same rate; the reduction of FLBZ led to the prevalent (~98%) stereospecific formation of one of the enantiomeric forms of red-FLBZ. A NADPH-dependent ketone-reductase may be involved on FLBZ reduction in sheep liver. The study of drug metabolising enzyme activities may help to predict drug-drug metabolic interactions in Veterinary Therapeutics.

81

EVALUATION OF FORMOCRESOL EFFECTS ON APOPTOSIS AND NECROSIS IN MURINE MACROPHAGES (MM).

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Formocresol (Fc) is a widely drug used in pulp therapy in primary teeth, but there are certain concerns about its toxicity and potential carcinogenicity. The aims of this study were: to evaluate apoptosis and necrosis through May-Grunwald Giemsa (MGG) and double fluorescent stainings and to describe the morphological changes of MM incubated with Fc by scanning electronic microscopy (SEM). Briefly, MM were obtained 3 days post-thioglicolate injection by peritoneal washings with saline solution. Cells were incubated in cover slides with Fc 1:100 (Buckley´s formulation) at 2, 4, 6, 12 and 24 hs (37ºC 5% CO_2) against control cultures. Cell viabilities were determined by Trypan Blue exclusion assay at each time of the schedule. Cell viabilities declined between 4 and 6 hs of incubation with Fc. Necrotic MM were noticed with MGG at 4, 6 and 12 hours. Fluorescence microscopy showed significant indexes of necrosis at 4, 6, 12 and 24 hours upon Fc effect. Furthermore, the values of apoptosis indexes were significant at 2 hours of incubation. This work suggests that both methods used for the analysis of cell death (apoptosis and necrosis) were coincident with viability experimental data. However, the fluorescence microscopy technique was more sensitive than MGG staining for the evaluation of necrosis. Moreover, the most acute necrotic effect caused by Fc on MM was noticed between 4 and 6 hours of incubation.

82

Effects of P-glycoprotein inhibition in the central bioavailability of carbamazepine and phenobarbital in an experimental model of epilepsy.

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The present work examines the participation of the P-glycoprotein (P-gp) in central carbamazepine (CB) and phenobarbital (PB) pharmacokinetics in an experimental model of epilepsy induced by 3-mercaptopropionic acid (MP). Seizures were induced in Wistar rats by injection of MP (45 $mg.kg^{-1}$) during 10 days. Control rats (C) were injected with saline solution (SS). A concentric microdialysis probe was inserted into hippocampus in anaesthetised rats, in order to monitor CB or PB levels. CB (10 $mg.kg^{-1}$, i.v.) or PB (20 $mg.kg^{-1}$, i.v.) was injected 30 min after administration of vehicle (V) or nimodipine (NIMO, 2 $mg.kg^{-1}$, ip). No differences were found in CB hippocampal levels comparing all groups. Conversely, in rats pretreated with V, hippocampal PB levels were lower in MP rats (maximal concentration (Cmax): 6.2±0.5 µg/ml, $p<0.05$ vs C) compared to C animals (Cmax: 10.4±1.1 µg/ml). Whilst pretreatment with NIMO did not modify central kinetics of PB in C (Cmax: 13.5±1.1 µg/ml), PB levels were significantly higher in MP rats pretreated with NIMO (Cmax: 10.9±1.2 µg/ml, $p<0.05$ vs MP rats pretreated with V). Our results indicate that P-gp is involved in the reduction of central bioavailability of PB in this experimental model of epilepsy, withouth affecting central pharmacokinetics of CB in MP animals.

83

ASTROGLIOSIS IN THE HYPOTHALAMUS OF ADULT RATS SUBACUTE OR CHRONICALLY EXPOSED TO 2,4-DICHLOROPHENOXY-ACETIC ACID (2,4-D)

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Serotoninergic system alteration and midbrain and hippocampus astrogliosis in rats exposed to 2,4-D have been reported in previous histological and biochemical studies from our laboratory. The aim of this work was to perform an immunohistochemical quantitative study of the hypothalamic astrocytes on adult rats exposed to 2,4-D. Wistar rats were made pregnant and exposed to 2,4-D (50 or 70 mg/kg/day, through diet) from day 16^{th} of gestation to weaning. After weaning, pups were divided in two experimental subgroups: **T_1:** fed with untreated diet until sacrifice at the postnatal day 90 (PND_{90}). **T_2:** treated until PND_{90}. Hypothalamic coronal sections were immunostained with two astrocyte markers: a-GFAP and a-S100 and results were quantified by image analysis. In different ways according to the exposition period, astrogliosis in arcuate and periventricular hypothalamic dopaminergic nuclei in 2,4-D-exposed rats, was detected. Since astrogliosis can be related to neural injury, this work supports previous reports about 2,4-D neurotoxicity.

84

The cytotoxic effects of mifepristone on tumor cell lines MDA-MB-231, MCF7 and T47D involve both extrinsic and intrinsic apoptotic pathways.

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In addition to its use as a contraceptive agent, mifepristone (MF) has been used to inhibit the growth of cancer cells. The antiproliferative effect of MF is observed on MCF7 cells overexpressing estrogen (ER) and progesterone receptors (PR), on MDA-MB-231 cells lacking both steroid receptors and on T47D cells expressing receptor levels similar to those of normal cells. Thus, MF seems to produce an antitumor action independently of the binding to nuclear PR. Our main objective was to investigate the mechanisms involved in the cytotoxicity of MF on cell lines of human breast cancer. Thus, we compared the cytotoxic potency of MF on three tumor cell lines and on the non-tumor cell line MCF10A. We studied by western blot the involvement of the intrinsic or extrinsic cascades of apoptosis. Our results show that cells MDA-MB-231 are more resistant and T47D cells are more sensitive to MF, and that MCF7 cells has a middle sensitivity. Moreover, MCF10A cells showed a high resistance to MF, comparable to that of MDA-MB-231 cells. Thus, the absence of PR is related to a minimal effect of MF, while their presence is associated to a higher cytotoxic response, although without linearity. The cytotoxic mechanism of MF involved both the extrinsic and the intrinsic pathways, since it produced cleavage and activation of procaspase 8, the proapoptotic protein Bid and decreased the expression of the antiapoptotic protein Bcl_{XL}, in a dose-dependent fashion. The later result may indicate that both pathways seem to be activated by MF.

85

Expression and functional evidence of the prostaglandin $F_{2\alpha}$ (FP) receptor mediating contraction in human umbilical vein (HUV). Errasti A, Cesio C, Souza G, Del Rey G, Pelorosso F, Rothlin R. III Cátedra de Farmacología. Facultad de Medicina. UBA. Paraguay 2155, piso 9, 1121. farmaco3@fmed.uba.ar.

Introduction: the aim was to determine the pharmacologically properties of functionally coupled FP receptors in HUV, by using selective agonists ($PGF_{2\alpha}$, latanoprost free acid and bimatoprost free acid) and to determine the antagonist effects of a novel FP-receptor antagonist, AL-8810, to complete the characterization of this receptor. Furthermore, the existence of the FP receptor in this tissue was investigated at mRNA and protein level. ***Methods:*** umbilical cords (n=72) from healthy patients after full-term deliveries were employed. The vein was dissected out of cords and rings used for isolated organ bath experiments or RT-PCR and Western blot. ***Results:*** the pEC_{50} of $PGF_{2\alpha}$ (6.00 ± 0.06, n=11) was significantly ($P<0.05$) greater than that of latanoprost free acid (5.66 ± 0.06, n=13), and bimatoprost free acid (5.56 ± 0.07, n=11). The agonist's maximum responses were not different. AL-8810 antagonized $PGF_{2\alpha}$ (pK_B=5.93 ± 0.05; n=7) and latanoprost free acid (pK_B=6.25 ± 0.03; n=6) accordingly to affinity values reported at the cloned human FP receptor. HUV rings express the FP receptor mRNA and a protein with an electrophoretic mobility (64-kDa) indistinguishable from human placenta FP receptor, a rich source of FP receptor. ***Conclusions:*** collectively, present results indicate that functional FP receptors are expressed in this tissue promoting vasoconstriction in HUV.

86

Evaluation of the antispasmodic effect of *Eupatorium arnottianum*

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Eupatorium arnottianum Griseb. ("clavel", "tuji") is an herb that grows in the NE and Centre of Argentina and S of Bolivia. It is used by rural populations for gastrointestinal pain and by "kallawaya" healers from the bolivian altiplano against asthm, bronchitis, colds and topically in plasters for bone fractures. The aim of this study was to examine the effect of ethanol extracts from aerial parts of *E. arnottianum* (Ea) and two of its isolated compounds on rat jejunnum. Cumulative concentration-response curves (CRC) for acetylcholine (Ach) and $CaCl_2$ were obtained for the tissues in absence and presence of different concentrations (0.3, 0.5, 1 and 2 mg/ml) of Ea. The extract exhibited an inhibitory effect on the CRC induced by Ach and $CaCl_2$ and significantly reduced the maximal response in a concentration–dependent manner.
Two compounds isolated from the active extract inhibited non – competitively the Ach CRC (0.03 mg/ml) with 70.5±8.2 and 60.3±7.6 of E máx. Quercetin, used as positive control, was capable of completely blocking the contractile response to Ach at the same concentration. The present results demonstrate that ethanol extract of EA exerts antispasmodic activity on rat jejunnum. The isolated compounds, the structure of which is under elucidation, could be responsible of the antispasmodic effect produced by the extract.

87

ANTIOXIDANT, ANALGESIC AND ANTIINFLAMMATORY ACTIVITIES OF *CHILIOTRICHIUM DIFFUSUM* (G.F.) K. (ASTERACEAE).

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In this work we studied pharmacological effects and the antioxidant activity of *Chiliotrichium diffusum* (Asteraceae). The flowers and leaves were collected in Santa Cruz, Argentina and air-dried after its collection. Powdered flowers and leaves were extracted by decoction or by ethyl acetate after hydroalcoholic drawing. Antioxidant activity was analyzed by DPPH method. Carragenin antiinflammatory test was carried out in rats and antinociceptive tests (hot plate and writhing by acetic acid) were carried out in mice.

The antioxidant activity of leaves and flowers fracctions by ethyl acetate after hydroalcoholic drawing showed 94% (SC_{50}= 3.5 µg/ml) and 87% (SC_{50}= 9.5 µg/ml) of inhibition respectively.

Flowers decoction showed antinociceptive activity as it was seen in the hot plate test (30% MIP) and the writhing by acetic acid at the dose of 500 mg/kg ip. This decoction (100 mg/kg ip) also showed anti-inflammatory activity when it was studied by the carragenin test in rats (inhibition 67%).

In conclusion, *Chiliotrichum diffusum* has antioxidant, antinociceptive and anti-inflammatory activities.

88

Comparison of neurotensin effect on Na^+, K^+-ATPase activity in several experimental conditions

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In previous work, we showed that peptide neurotensin inhibits neuronal Na^+, K^+-ATPase activity. This effect involves high affinity neurotensin receptor and is modified by administration of antipsychotic drugs. Herein we evaluated neurotensin effect on synaptosomal membranes isolated from rat cerebral cortex in two pathological conditions: hyperglycaemia by streptozotocin administration and in spontaneous hypertensive rats (SHR). In membranes isolated after hyperglycaemia induction, 3.5×10^{-8}- 3.5×10^{-6} M neurotensin failed to modify Na^+, K^+-ATPase activity, whereas decreases 8%-36% in enzyme activity were recorded in the control. In membranes isolated from SHR, 3.5×10^{-6} M neurotensin, similar to the control, decreased 40% enzyme activity. Previous administration of clozapine (17.8 mg / kg, 30 min) prevented neurotensin inhibitory effect. Findings indicate that alteration of neurotensin effect on Na^+, K^+-ATPase activity may be an adaptive phenomenon which occurs concomitant with the increase in glucose blood level and / or blood pressure.

89

Anandamide (AEA) inhibits *in vitro* kinin B_1 receptor up-regulation in isolated human umbilical vein (HUV) rings through CB1 receptor activation. Pelorosso F, Gago J, Diana Menéndez S, Gagliardo M, del Rey G, Errasti A, Ireizo J, Mendez Garrido F, Brichetti V, Rothlin R. Tercera Cátedra de Farmacología. Facultad de Medicina. UBA. Paraguay 2155, piso 9, CP 1121. farmaco3@fmed.uba.ar.

Introduction: Contractile responses induced by kinin B1 receptor agonists are sensitized over *in vitro* incubation time in isolated HUV rings. This sensitization process is dependent on NF-□B activation and *de novo* receptor synthesis. Taking into account that the endogenous cannabinoid AEA has been shown to inhibit NF-□B in several models, we decided to evaluate its possible effects on B_1 up-regulation in HUV. **Methods and results:** Continuous exposure to 10 microM AEA during a 2.5 h incubation period inhibited concentration-response curves (CRC) to Sar-desArg-D-Phe-bradikinin (SdADPBK), a metabolically stable B_1 agonist, in HUV rings. On the other hand, when applied only 15 min before CRC, 10 μM AEA failed to modify responses to SdADPBK. Moreover, continuous exposure to 10 μM AEA failed to modify responses induced by bradykinin, a kinin B_2 receptor agonist. In addition, 1 and 5 μM methanandamide, a metabolically stable cannabinoid receptor agonist, reproduced the inhibitory effect observed with AEA, thereby ruling out a major role for AEA metabolites (i.e. arachidonic acid and ethanolamine) on its effects on B_1 responses in HUV. Finally, treatment of rings with 1 μM AM251, a CB_1 receptor antagonist, abolished AEA inhibitory effects on SdADPBK-induced contractile responses in HUV. **Conclusion:** These results suggest that AEA is able to inhibit B_1 receptor up-regulation in isolated HUV through CB_1 receptor activation.

90

Changes in Timm's Staining in the Posteromedial Cortical Amygdala in the Kainic acid model of Epilepsy are indicative of synaptic reorganization.

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Temporal Lobe Epilepsy (TLE) is a common form of epilepsy in humans. TLE promotes cell loss and gliosis and synaptic reorganization was observed mainly in the hippocampus. However, in the Amygdala this effect have not been communicated. Changes in Zn density in the Posteromedial Cortical Amygdala (PMCo), induced by Kainic Acid (AK) as a model of TLE were studied and presented here.

Methods: Adult male rats (n=6) were perfused every 10 days after KA ip injection up to 4 months. Controls were injected with saline. The brains were processed by the Timm's method to reveal synaptic Zn, and analysed by densitometry. Images were captured with a Leica videocamera, using the KS Lite v2.00 program to determine differences between controls and experimental animals. ANOVA was used for statistics, with a $p < 0.05$ as a significance limit.

Results: Normal staining was seen in PMCo sections of control animals. At 10 days post KA a dramatic loss of staining was observed. A slow but steady recovery of Zn density could be followed in the 2-4 months period studied. We found significative loss of synaptic Zn from 10 days to 1-month exp animals, not observed in the 2 to 4 months animals. This indicates an acute loss of synaptic Zn in status epilepticus and a chronic neuroplasticity process of recovery through sprouting in a 4-month period post KA induced TLE.

56

Autores

38	Calderón C.
07	Carballo M.
81	Cardoso ML. .
84	Carón R
08-31	Casanova M.
29	Castillo G.
06	Castillo. C
16	Catania VA.
55-68-78	Ceballos. L.
11-30	Ceci. M.
08-31	Celentano AM.
25	Celuch SM..
M01	Ceresetoo M
39-57	Cerkvenik- Flajs V.
85	Cesio C.
31	Chan A
29	Chanampa Y
27	Chiapeta.D.
83	Cholich V.
27	Chuluyan. E
01	Cipolone S.
86	Clavin M.
04	Collia N.
11-30	Confalonieri. A.
13-46	Consolini. A.E.
74	Conti MI.
87	Córdoba. O.L.
07	Cortada C.M.
05-22-35-91	Cremaschi G
22	Cricco G.
84	Cuello D
70	Cuis N.
07	Curras V.
D	
42	Dade M...
31	de Castro SL.
47	De Marzi M.
54	de Olmos J.
54	de Olmos S
38	De Pauw M. .
18-48-49-75	Debattista NB.
01	Decono Ambesi M
41-69	Del Campo L
85	Del Rey G.
76	Dellicarpini G.
17-26	Demurtas. S.
26	Demurtas. SL.
09-68-78	Denegri M. G.
02	Denzoin. L.
50	Derra MR.
23	Di Giusto G.
89	Diana Menéndez S.
08	Diez Ra.
08-31	Dubin M.
03-83	Duffard.R.
E	
19-34	Elgoyhen AB
15-45-70	Elías MM
08-31	Elingold I.
09-68-78	Elissondo M. C.
85-89	Errasti A.
07	Escobar A.
003-83	Evangelista de Duffard A.M.
F	
39	Farias. C.
37	Farina O. H..

51	Fatala Chaben. INP
14	Favier LS.
56-61	Felipe. A.
05-58	Ferraro G;
12-41-69	Ferrer M.
03	Ferri.A.
61	Fiel C.
01	Fillipini B.
65	Flores L.
87	Flores. M.L.
02	Fogel. F
02	Franci. R.
91	Frick, L
51	Fuchs A.
44	Fusco M.
G	
89	Gagliardo M. del
89	Gago J.
11-30	García Allende. N.
14	García Aseff S.
21	Garcia Bonelli C
83	García G.
25	García MC.
45	Gattino S.
28-77-91	Genaro. AM
61	Gentile Ma L.
76	Ghanem CI.
C2-36	Ghosh Sourav
82	Girardi E.
88	Gironacci. M.
04	Goldman C.
82	González N.
86-87	Gorzalczany S.
39	Grabnar. I.
73	Gravisaco M. J.
52	Guardia Calderón CE.
52	Guardia T.
24	Guelman. L.R.
27	Guerrieri. D.
H	
17	Heredia. D .
58	Hnatyszyn O.
82-87	Höcht C.
39	Hodoscek. L
I	
39	Imperiale. F.
89	Ireizo J.
M04	Isoardi N
J	
84	Jahn G
40-60	Juaristi JA.
K	
04	Kaliski M.
19-34	Katz E
35	Klecha A.
57	Kolar L.
39-57	Kozuh Erzen. N.
53	Kreil. V.
L	
37	Lacunza J.
71	Laino. C.H.
53	Landoni. M.
20-39-55-57-68-78-80	Lanusse C.
82	Lazarowski A.

47	Leiva L.
36	Lemke Greg.
04	Leonardi N.
32-0-60-66	Lettieri CN.
49	Lhez L.
20-57-80	Lifschitz A.
43-72	Linares LM.
34	Lipovsek. M. M
01	Lomastro J.
88	López Ordieres. G.
62	Loscalzo LM. .
16	Luquita MG.
M	
92	Miozza V,
27	Mafia. P
47	Malchiodi E.
06	Mancilla. C..
41-69	Manso R.
05	Manuele. M G.
62	Marder M
14	María A.
10	Marra C.
22	Martín G.
83	Martínez A
74	Martínez MP
86	Martino V
12	Martorell A.
47	Maruñak S.
20-80	Maté L
82	Mayer M.
26	Meirovich. CI
89	Mendez Garrido F.
31	Menna-Barreto R.
36	Michael B.
58-88	Miño J.
73	Mongini C.
53	Montoya. L.
26	Montrull. HL
84	Montt Guevara M.
06	Moreira. M.
55	Moreno. L.
58	Moscatelli V.
16-76	Mottino AD
65	Moya M.
06	Muñoz. O.
N	
31	Nagel R.
07	Niselman A.V.
O	
72	Obaya-Naredo D.
59	Oberkersch R.
16	Ochoa JE.
36	Oldstone. A
75	Olivella MS.
82	Opezzo J.
29	Orce G..
35	Orqueda A.
02	Ortega. I.
P	
15	Paciaroni J.
45	Paciaroni J.
62	Paladini AC.
28	Palumbo. ML
18	Pappano NB
48-49-75	Pappano NB
35	Paulazo M.A.

01	Pazo. JH
52	Pedernera AM.
85-89	Pelorosso F.
14-38-44-50-52	Pelzer L.
67	Peña C.
44	Pereira S.
90	Pereno. G.
67	Pereyra-Alfonso S.
08	Pérez C.
33	Peroni RN.
43	Planells FM.
19	Plazas PV.
03	Pochettino.A.
39-57	Pogacnik M.
06	Ponce. J.
65	Pons P
Q	
15-45-70	Quaglia N.
R	
46	Ragone. M.I.
11-30	Ranno. G..
38	Rapisarda M.
71	Reinés. A.G.
89	Rey G.
43-72	Reyes Toso CF.
72	Reyes Toso. ML.
26	Ricarte Bratti. JP.
43	Ricci CR.
42	Ríos J.L
65	Ritzer Lacroix A.
79	Rodríguez CB.
64-67-88	Rodríguez de Lores Arnaiz G.
47	Rodríguez J.
88	Rosin C.
43	Rosón. M.
50	Rotelli AE.
36	Rothlin. C
85-89	Rothlin R.
77	Rubinstein. R
07-76	Rubio M. C.
16	Ruiz ML.
10-37	Rule R.
S	
12-41-69	Sagredo A.
79	Salatino AE.
04	Salgueiro J.
20-57-80	Sallovitz J.
11-30-68-78	Sánchez Bruni. S.
72	Saredo S.
C3	Sardi SP
61	Saumell C..
19	Savino J. .
56-61	Scarcella S.
42	Schinella G.
79	Scolari MJ..
M02	Sifonios L
71	Slobodianik. N..
43	Smart. D.
56-61	Solana H.
02	Soraci. A.
44-50	Sosa A.
85	Souza G.
11-30	Sparo. M.
42	Spegazzini E.
81	Stemberg ER.

92	Sterin-Borda L,
66	Stoyanoff. T
57	Suarez V.
T	
08	Taboas M .
82-87	Taira C.
18-48	Talia JM.
02	Tapia. O.
65	Táran M.
47	Teibler P.
81	Todaro JS
14	Tonn C.
23-63	Torres A. M.
42	Tournier H.
17	Trassovare. Y.
17	Trejo. S
U	
11-30	Urbizu. L..
V	
22	Valli E.
38	Valsecia M.
21	Varani A
84	Vargas Roig L
67	Vázquez C..
72	Vázquez MB
53	Velo. M.
73	Vendrell A.
31	Ventura-Pinto A.
10	Villagra S.
16-76	Villanueva SM.
63	Villar S.
20-80	Virkel G.
37	Vita M.
W	
28-77	Wald M
73	Waldner C.
72	Wallinger ML.
62	Wasowski C
14-44	Wendel G.
Z	
10	Zaidenberg A.
91	Zappia, P
28	Zorrilla.Zubilete MA.
C4 -04	Zubillaga M
36	Zuniga Elina I

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