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CONFERENCIAS

MECHANISMS OF MEMORY CONSOLIDATION

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Evidence accumulated through the past 15 years by our group has shown that memory consolidation of one-trial inhibitory avoidance learning relies on a sequence of molecular events in the CA1 region of the hippocampus that closely resembles that of long-term potentiation in that area. Indeed, very recently LTP has been recorded in CA1 during and after one-trial avoidance training by Bear and his associates. However, abundant additional evidence indicates that other molecular events partly involving the same steps but with different timing and in different sequence in the basolateral amygdala, entorhinal, parietal and cingulate cortex are as important and necessary as those in the hippocampus for memory consolidation of that task. Thus, overall, the findings indicate that memory consolidation of even a task as deceivingly simple as one-trial avoidance does not rely solely on hippocampal LTP or LTP-like events, and requires the concomitant participation of other brain systems and molecular events orchestrated with the former.

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DISCOVERY OF NOVEL TARGETS FOR THE TREATMENT OF CNS DISORDERS.

Jorge D. Brioni Ph.D.,
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There are significant unmet needs in the clinical area for the treatment of CNS disorders including Alzheimer's disease, attention-deficit hyperactivity disorder (ADHD), schizophrenia, depression and pain. Despite the recent technological innovation in the pharmaceutical area that allowed the incorporation of High Throughput Screening assays, Robotics, Antisense technologies, KO animals, Combinatorial Chemistry as well as Molecular Modeling, the number of New Chemical Entities in the US have not increased, they have barely remained constant during the last decade. The process of Target Validation, Hit-to-Lead and Lead Optimization needs to continue to improve in order to identify novel targets and the best preclinical compounds that can represent a breakthrough for the treatment of these CNS disorders. Our experience in several discovery programs like mGluR1, ASIC, KCO, nAChRs, dopamine D4 receptors and histamine H3 receptors will be discussed.

NOVEL NICOTINIC AGENTS WITH POTENTIAL USE IN PAIN AND ALZHEIMER'S DISEASE.

R. Scott Bitner Ph.D., Senior Group Leader, Neuroscience Research, Abbott Laboratories.

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated cation channels widely distributed throughout the peripheral and central nervous system. In the brain, the two predominant native subtypes are the heteropentameric $\alpha4\beta2$ and homopentameric $\alpha7$ nAChRs. At Abbott Laboratories, considerable effort over the years has been aimed at developing novel agonists targeting both subtypes for clinical indications that have included pain and cognitive disorders, including ABT-089 and ABT-894. Along with medicinal chemistry, biochemistry and pharmacological characterization, studies in my laboratory concerning target validation and mechanistic studies have played a key role in the preclinical development of these agents, the topic of the present lecture. The efficacy of novel nAChRs agonists in animal models of pain and cognition will be described that have employed target validation techniques including antisense knockdown, immunohistochemical assessment of pharmacological signaling, and electrophysiological EEG profiling.

SIMPOSIOS

1-UNIVERSALITY ACROSS EVOLUTION OF SOME PRINCIPLES OF MEMORY ORGANIZATION AND THE UNDERLYING CELLULAR MECHANISMS.

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Research on memory has been carried out using animals from different species, distinguishing three levels of study each with diverse grade of universality, namely, the behavioral, systemic and cellular level. At behavioral or phenomenological level, we find a remarkable persistence in the guidelines of organization in spite of the enormous differences between animal species ("universals"); at system level, we have a great diversity of adaptive solutions through the evolutionary history ("particulars"); and at cellular or molecular level, a remarkable evolutionary persistence ("universals"). In this framework, we discuss the switch hypothesis concerning the relationship between reconsolidation and extinction.

PERSISTENCE OF LONG-TERM MEMORY STORAGE REQUIRES A LATE PROTEIN SYNTHESIS- AND BDNF-DEPENDENT PHASE IN THE HIPPOCAMPUS.

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Persistence is the most characteristic attribute of long-term memory (LTM). To understand LTM, we must understand how memory traces persist over time despite the short-lived nature and rapid turnover of their molecular substrates. It is widely accepted that LTM formation is dependent upon hippocampal *de novo* protein synthesis and Brain-Derived Neurotrophic Factor (BDNF) signaling during or early after acquisition. Here we show that 12 h after acquisition of a one-trial associative learning, a novel protein synthesis and BDNF-dependent phase in the rat hippocampus is critical for the persistence of LTM storage. Our findings indicate that a delayed stabilization phase is specifically required for the maintenance, but not the formation, of the memory trace. We propose that memory formation and memory persistence share some of the same molecular mechanisms and that recurrent rounds of consolidation-like events take place in the hippocampus for maintenance of the memory trace.

ABOUT THE EFFECT OF RETRIEVAL ON SPATIAL MEMORY PERSISTENCE

Martín Cammarota

It is known that non-reinforced retrieval can cause extinction and/or reconsolidation, two processes that affect subsequent retrieval in opposite ways. In the rat repeated non-reinforced expression of spatial memory causes extinction which is unaffected by inhibition of protein synthesis within the CA1 region of the dorsal hippocampus. However, if the number of non-reinforced retrieval trials is insufficient to induce long-lasting extinction, then a hippocampal protein synthesis-dependent reconsolidation process recovers the original memory. Inhibition of hippocampal protein synthesis after reversal learning sessions impairs retention of the reversed preference and blocks persistence of the original one suggesting that reversal learning involves reconsolidation rather than extinction of the original memory. In addition, when given systemically or into the CA1 region after non-reinforced retrieval, the partial NMDAr agonist D-cycloserine improves subsequent memory retention. These results suggest the existence of a hippocampal protein synthesis dependent reconsolidation process that operates to recover or update retrieval-weakened memories from incomplete extinction and suggest that, like consolidation, reconsolidation can be not only blocked but also enhanced by appropriate pharmacological treatments.

CHOLINERGIC MECHANISMS AND MEMORY PROCESSES

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Experimental and clinical evidence has given support to the hypothesis that cerebral acetylcholine plays a role in mnemonic phenomena. Thus, central or systemic administration of anticholinergic drugs or lesions of the cholinergic system cause memory impairment while drugs that enhance cholinergic activity improve memory. Most of these studies have dealt with the acquisition and consolidation of memory and less attention has been paid to the retrieval and extinction processes. Several reports suggest that when a well – consolidated memory is recalled it becomes sensitive to disruption to the same treatments that affect consolidation. This new window of susceptibility is now referred as reconsolidation. In the present work it will be presented experimental evidences that suggest a possible role of central cholinergic mechanisms not only on memory consolidation, but also in memory processes that take place after memory recall.

ADDICTION: FROM MOLECULES TO BEHAVIOR. (ADICCIÓN: DESDE LAS MOLÉCULAS AL COMPORTAMIENTO).

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If neurobiology is ultimately to contribute to the development of successful treatments for drug addiction, researchers must discover the molecular mechanisms by which drug-seeking behaviors are consolidated into compulsive use, the mechanisms that underlie the long persistence of relapse risk, and the mechanisms by which drug-associated cues come to control behavior. Alterations in behavior after exposure to addictive drugs are a striking example of chemical alterations of nervous system function producing long-lasting changes in behavior. Considerable study has been given to behavioral and biochemical correlates of addiction over the past 50 or more years; however, our understanding of the cellular physiological responses of affected CNS neurons is in its infancy. This talk focuses on alterations in cellular and synaptic physiology in the mesocorticolimbic (reward) pathway in response to addictive drugs.

STRESS- AND DRUG-INDUCED SENSITIZATION TO PSYCHOSTIMULANT DRUGS: CON NEUROADAPTATIONS IN NUCLEUS ACCUMBENS SHELL AND CORE.

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A single and chronic restraint stress induce sensitization to the stimulating properties of d-amphetamine (AMPH), morphine (MO cocaine (COC), as measured by locomotor activity and/or place preference conditioning test. The mesolimbic system innervating the accumbens (NAc) is implicated in the development and/or expression of sensitization. Different functions have been attributed to the d (DA) in NAc core and shell activated by drugs and/or stress. In addition, many reports have pointed out a dopaminergic-glutar interaction in the development of behavioral sensitization. Our main goals were to study: 1) the long term influence of restraint s psychostimulant drug-induced DA release by microdialysis from NAc core and shell and caudate putamen (CPu); 2) the involvement of receptors in the restraint stress-induced sensitization; 3) the expression of the Activator of G-protein signaling 3 (AGS-3), GluR1 and NR2A in the NAc and/or dorsal PfC in the model of chronic restraint stress 24h and 21 days after restraint. Wistar male rats (250-320 implanted stereotaxically. After 2 days, MK-801 (0.1 mg/kg i.p.) or vehicle (VEH) were administered 30 min before restraint stre Control animals did not receive the stress session. Following 8 days, we evaluated the effect of AMPH (0.5 mg/kg i.p.), MOR (1 mg/kg COC (10 mg/kg i.p.) on DA release from NAc core and shell, and CPu by microdialysis during 3h. AMPH, MOR and COC in significant higher increase in DA release from NAc core and shell in the restraint group, compared with the no restraint group. MK-801 the restraint stress-induced effects of drug on DA release from NAc. Three weeks after of the last restraint stress we found a significant (43.4 %) of AGS3 in NAcShell compared with the no-stress group, while no difference was observed in NAcCore, vPfC, dPfC ar Twenty-four hours after chronic restraint stress the AGS3 protein levels were not modified. Chronic restraint stress did not have any i on the levels of GluR1 24h or 21 days following the last restraint or on NR2a 24h following the last session. It should be noticed that ei or 21 days after the last restraint stress, a sensitized behavioural response to psychostimulant drug was observed in these chronically animals. These findings showed a long-term restraint-induced sensitization to stimulating effect of psychostimulant drug on behaviour and on DA release from NAc core, and an involvement of NMDA receptors on it. Since the up-regulation in AGS-3 could be alter stimulated Gsα signaling in NAcShell, it is likely that this molecular mechanism may be associated to the restraint stress-induced be sensitization to psychostimulant drug. These results support the hypothesis that common mechanisms between drugs and stress under long term effects at the dopaminergic and glutamatergic transmission as well as in specific proteins (i.e. AGS3) in NAc.

PERINATAL PROTEIN MALNUTRITION INCREASES MOTOR STIMULANT AND REWARDING COCAINE EFFECTS IN ADULT RATS.

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Perinatal protein malnutrition induces long-lasting or permanent alterations in the CNS that may account for changes in the reactivity to diverse pharmacological treatments. The aim of the present study was to investigate whether neuronal alterations induced by perinatal undernutrition alter pharmacological reactivity to cocaine. Thus, motor stimulant and rewarding properties of cocaine were assessed, using an open-field or a Conditioned Place Preference (CPP) paradigm respectively, in adult recovered rats submitted to a protein malnutrition schedule at perinatal age (D-rats), and compared with well-nourished animals (C-rats). Dose-response curves obtained with different doses of cocaine used revealed a shift to the left in the locomotor activity curves of D-rats as compared with controls. Thus, D animals evidenced behavioral sensitization with the lowest dose of cocaine used, whereas this phenomenon was observed in C-rats only with the higher dose. A challenge with cocaine in subjects pre-exposed to cocaine, produced a different increase in dopamine output only in nucleus accumbens "core" of D-rats. Furthermore, in the CPP paradigm dose-response curves to increasing doses of cocaine revealed in D-rats a conditioning effect with lowest doses; intermediate doses did not show any conditioning place preference and higher doses revealed a significant aversive effect. In C-rats, cocaine elicited place preference with intermediate doses, whereas higher doses did not show neither conditioning nor aversive effects. Sensitization to the conditioning effect of cocaine was obtained in D-rats with a low dosage of cocaine, which was ineffective in controls. Related to the higher rewarding effects, sensitized D-rats showed a selective and significant increase in FosB expression in nucleus accumbens (core and shell) and basolateral amygdala, brain areas related to the rewarding neuronal circuits. These results demonstrate that a deficient nutritional status during early life may induce in adult subjects an increased responsiveness to behavioral effects of cocaine and/or enhanced its reinforcement properties.

BEHAVIORAL SENSITIZATION TO AMPHETAMINE IN MICE: INFLUENCES OF A SELECTIVE MONOAMINE OXIDASE TYPE-B INHIBITOR (MAOI-B).

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The behavior sensitization is characterized by a progressive enhancement of a behavioral response after repeated treatments followed by a withdrawal period, when the drug is applied again. This phenomenon is frequently applied in studies using animal models of drug addiction. The majority of these drugs promote plasticity in neurotransmission (ex. dopaminergic system) as is the case for amphetamine, morphine and ethanol. However, studies that evaluate drug effects during abstinence and during the induction of behavioral sensitization are scarce. In the current study we evaluated the influences of a selective monoamine oxidase type-B inhibitor (selegiline, 10mg/Kg) on behavioral sensitization for amphetamine (2mg/Kg) in mice. Results show that a) chronic treatment with amphetamine, followed by a withdrawal period, promote behavioral sensitization to amphetamine, b) selegiline administrated during the withdrawal period potentate amphetamine sensitization, c) selegiline treatment followed by withdrawal sensitizate to amphetamine, d) selegiline treatment followed by withdrawal sensitizate to selegiline, e) corticosterone is increased in rats sensitizated to selegiline but no in those sensitizated to amphetamine, f) corticosterone modification does not depend on behavioral sensitization; they are not correlated effects; g) withdrawal is not necessary for the development of behavioral sensitization.

NICOTINIC RECEPTORS OF COCHLEAR HAIR CELLS: FROM STRUCTURE TO FUNCTION

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Acetylcholine (ACh) is the principal neurotransmitter released by medial olivocochlear efferent axons, and existing data suggest a central role for an atypical nicotinic cholinergic receptor (nAChR) located at the synapse between efferent fibers and vertebrate outer hair cells. Current data support a model in which ACh-gated depolarization is followed by activation of small-conductance, calcium-activated potassium channel (SK2) and subsequent hair cell hyperpolarization. We have been able to define the molecular structure of the mammalian hair cell nAChR, and have demonstrated that it is assembled from α9 and α10 nAChR subunits, in a 2:3 stoichiometry. Although homomeric α9 receptors are functional, the □10 subunit serves as a structural component leading to heteromeric α9α10 nAChRs with particular desensitization kinetics, current-voltage dependency and sensitivity to extracellular Ca^{2+}. Moreover, we have demonstrated that recombinant □9□10 nAChR and native mammalian hair cell receptors share similar pharmacological and biophysical properties. Although □9 and □10 are the latest vertebrate nAChR subunits that have been cloned, their identification has established a distant early divergent branch within the nAChR gene family, most closely related to the ancestor that gave origin to the family.During my talk I will provide the experimental evidence, derived from molecular and cellular biology, physiology and gene targeting approaches, which led to the description of the functional role/s of α9 and α9α10 nAChRs.

NICOTINIC RECEPTOR: TARGET FOR THERAPEUTIC DRUGS

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Nicotinic receptors (AChRs) are members of the Cys-loop receptor superfamily. They mediate rapid synaptic transmission throughou the nervous system in both vertebrates and invertebrates. AChRs are involved in muscle contraction and they contribute to a wide rang of brain activities. Their essential function is to couple the binding of agonist at the extracellular domain to the opening of an intrinsi ion pore. This mechanism, which is known as gating, has not been elucidated. However, it is known that mutations that change gatin kinetics lead to neurological disorders, such as slow-channel myasthenic syndromes. To investigate the structural basis for gating, w generated a chimeric receptor composed of the acetylcholine binding protein (AChBP) and the pore domain from the 5-HT_{3A} receptor The chimeric AChBP-5HT_{3A} receptor shows high surface expression on mammalian cells but it does not function, suggesting tha although binding and pore domains are corrected folded, the interface between them is not compatible, preventing inter-domai coupling. Only when amino-acid sequences of three extracellular loops in AChBP are changed to their 5HT_{3A} counterparts does AC bind with low affinity characteristic of the activatable receptor and trigger opening of the ion pore. Thus functional coupling require structural compatibility at the interface of the binding and pore domains of Cys-loop receptors.

AChR function is modified by drugs, which can act through different mechanisms and at different conformational states. Nematod muscle AChRs are targets for anthelmintic drugs, which produce spastic paralysis and ultimately death. We have shown that levamisol and pyrantel are very low-efficacy agonists of mammalian muscle AChRs. By combining site-directed mutagenesis with single- an macroscopic-current recordings we identified residues located at different faces of the binding site that govern the selectivity of thes agents. Mutations at these sites in the parasite subunits may lead to anthelmintic resistance. We also have characterized AChR activity in muscle cells from *C. elegans*, which is a model for the study of parasitic nematodes. We determined that the main channel activity i mediated by levamisole-sensitive AChRs, for which levamisole is a more potent agonist than ACh.

AChRs are also targets for many neuroactive drugs, and consequently, these receptors may be involved in their therapeutic or adverse actions. We have elucidated the action of antidepressants at the molecular level. Our results reveal that these drugs inhibit muscle an neuronal α7 AChRs by different mechanisms. In muscle AChRs, they increase the desensitization rate from the open state, and in α7 AChRs, they interact with the closed state and allosterically inhibit channel opening. For designing new drugs targeted to a specifi AChR subtype, mutated receptor or conformational state, we require an intimate knowledge of the structure-function relationship. New understanding of the molecular pharmacology of AChRs will lead to a new generation of more selective drugs with improved safety profiles. *Supported by grants from CONICET, FONCYT, UNS.*

NICOTINE ADDICTION: THE REINSTATEMENT AND THE MEMORY. (ADICCIÓN A LA NICOTINA: LA REINCIDENCIA Y LA MEMORIA).

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Nicotine induces craving, aversive effects of withdrawal, and aberrant incentive-salience that are associated with addiction and relapse. As anybody who has ever struggled with repeated attempts at smoking cessation can attest to, nicotine is exceptionally intractable to quitting interventions. Only 3 % of smokers that try to quit are successful, indicating a high relapse for nicotine. Molecular and cellular neuroadaptations are often invoked to explain tolerance, sensitization and dependence, but they do not readily explain the long-lasting craving that arises after years of abstinence. A central question of addiction is what mechanisms underlie the long-term changes of the brain that lead to craving and relapse years after abstinence from the drug. It is hypothesized that during the addiction process the drug misdirects the mechanisms that usually subserve learning and memory. Using conditioning place preference, to study addiction at behavioral level, and phosphorylation of CREB (pCREB) and expression of *c-fos* protein, as neuron activity markers, we observed that nicotine induce an increase of pCREB and *c-fos* expression in structures of the mesocorticolimbic pathway and in structures involved in memory consolidation. These changes were evident in addict and relapsing animals, suggesting that during relapse some structures involved in long-term memory are activated.

ABSTRACTS

1

Accumulation of cAMP evoked by pilocarpine stimulation in rat submandibular gland: mechanism involved.

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In previous studies we observed that pilocarpine-induced mucin secretion was decreased when the increase of intracellular calcium concentration was blocked and besides, in the presence of inhibitors of ciclooxygenase (COX), adenylyl cyclase (AC) and nitric oxide synthase (NOS). In this study we explored: 1- the ability of pilocarpine in inducing PGs production, cAMP accumulation and activation of NOS; 2- the mechanism underlying to evoke these responses and 3- the relation between these second messengers among them and in relation to mucin secretion. The methods used were "ELISA" for determination of PGs and cAMP and NOS activity was measured using L-[U-^{14}C] arginine as substrate. Results showed that pilocarpine (10^{-6} M) increased PGs production in 92%, cAMP accumulation in 640% and NOS activity in 50%. Both, PGs and cAMP, induced by pilocarpine, were decreased in the presence of verapamil and indomethacin but not in the presence of L-NMMA. On the contrary, the increase of NOS activity was only inhibited by L-NMMA. A significant correlation was observed between PGs and mucin release (r: 0.9657), between PGs and cAMP (r: 0.9496) and between cAMP and mucin release (r: 0.9667). It is concluded that the increase of calcium concentration induced by pilocarpine results in the activation of COX and PGs production, which in turn, induces cAMP accumulation. This cAMP, together with calcium evokes exocytosis.

2

Toxicity of trifluralin in a mice model for the treatment of chagas disease

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Trifluralin (α,α,α-2,6-dinitro-N-N-dipropyl-p-toluidine, TFL) is effective for the treatment of experimental Chagas disease. Preclinical toxicity studies should be performed. Cell toxicity was studied in HEP G2 hepatoma and Vero C76 cells treated with 50 and 100 μM TFL: cell protein and cell protein/DNA values were normal. Histological, hematological and chemical parameters were measured in CF1 mice treated orally for 30 days: control (n=30), 50 (n=18) and 200 mg/Kg/day (n=20). Renal (urea, creatinine), hepatic (GOT, GPT, ALP, proteins, albumin) and pancreatic (amylase, glycemia) function were normal. MCV, hemoglobin and hematocrit decreased. CK, LDH and GOT increased, suggesting lesion in myocardiac tissue. Histology was normal, excepting the myocardium (mild myocarditis). In view of the published TFL therapeutic effects and these results, TFL would be a moderately toxic drug with potential selective action for the myocardium.

3

Anxyolitic effects of testosterone is not induced by aromatization of estradiol.

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It has been reported that in males testosterone (T) and its metabolites dihydrotestosterone and 3α-androstanediol exhibit all of them anxiolytic properties. However, and since T is also usually aromatized to estradiol (E_2), it has not been ruled out the possibility of E_2 being at least partially responsible for the aforementioned effect. Adult Sprague-Dawley male rats (n = 8 animal/group) were used. Anxiety (total time spent exploring the open arm: TOA) and locomotion activity (number of total arm entries: TLA) were tested on an elevated plus-maze. Our results showed that TOA was significantly shorter in castrated animals, irrespectively of being treated or not with E_2 10 or 25 μg ($p < 0.05$, Student´s *t* test). On the other hand, castrated males treated with T did not show any change in their anxiety levels regarding control animals. Also, it is worth mentioning that by comparing validated measures we can assert that the anxiolytic effect is not a mere –unspecific– increase of motility. Summarizing, in this report and by using intact and castrated male rats, with or without E_2 or T replacement, we could conclude that the anxiolytic effect is not due to E_2 but to T and, eventually its non aromatizable metabolites. Whether or not T action is linked to a direct effect of the steroid on its intracellular receptor and/or via modulation of $GABA_A$ or glutamate receptors remains to be established.

4

Electrocardiographic model to assess drugs in mice with chagas disease

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Aiming at finding new drugs for the treatment of experimental Chagas disease in mice, surface electrocardiography has been used to evaluate the progression of myocardial damage and its response to treatment. We studied electrocardiograms from 178 CF1 mice (25-30 g) under pentobarbital anesthesia (30mg/kg): 19 healthy mice (control), 158 infected with 10^5 *T cruzi* trypomastigotes (clone H510C8C3), 8.3% in acute phase, 51% in early chronic phase and 41% in late chronic phase. Alterations in cardiac frequency (sinus tachycardia or bradichardia) predominated in both the early chronic and acute phases. Alterations on P wave and PR interval (first-degree AV block) were present in the late acute and chronic phases. Prolonged QRS was only observed in the late chronic phase. There were no significant differences in other arrhythmias or alterations of the electric axis. This model proved therefore useful to evaluate new drugs.

5

Hypoxia-stimulated erythropoietin secretion in mice with different types of induced polycythemia.

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Studies performed in our laboratory have shown that sustained exposure to hypobaric air induces an "erythropoietin-hypersecretory state" (EPO-HS) that determines that hypobaria-induced polycythemic mice secrete a large amount of EPO when re-exposed to hypobaric air. The purpose of the present investigation was to compare hypoxia-induced EPO secretion in mice with different types of polycythemia. Adult female CF#1 mice were used throughout. By estimating the total circulating red cell volume (TCRCV) by the dilution of homologous red cells labeled in vivo with ^{59}Fe, it was calculated that experimental mice have to be ip transfused with 1.33 ml of packed red cells, or exposed to a simulated high altitude equivalent to 6,360 m during a 2-wk period, or sc injected with 5.55 IU/d for 10 d of rh-EPO, in order to increase by 80% the TCRCV. When mice were so treated and exposed to air maintained at Torr 337 mmHg for 6 h, data obtained were as follows (expressed as EPO in plasma, pg/ml, as determined by immunoassay, NX = normoxia, HX = hypoxia, N = normocythemic, P = polycythemic): 1) N-NX 124.8 ± 20.73 (ES); 2) N-HX 992.6 ± 79.41; 3) TP-NX 7.98 ± 0.5; TP-HX 49.7 ± 14.42; EPOP-NX 7.9 ± 0.4; EPOP-HX 31.78 ± 13.91; PH-NX 20.02 ± 6.39; and PH-HX 647.1 ± 82.35. These results confirm our previously reported findings and suggest that exposure to hypobariacould be taken as an inducer of EPO-HS. *Supported by UBACYT O-012.*

6

Study of pharmacokinetic interactions between cephalexin and meloxicam

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The purpose of the present study was to investigate if previous administration of meloxicam affects cephalexin pharmacokinetics after its intravenous (iv) administration to healthy dogs. Seven Beagle dogs were included in this study. Each dog received iv 25 mg/kg cephalexin (group 1) or cephalexin following iv 0.1 mg/kg meloxicam (group 2), with a 2 week wash out period. Cephalexin plasma concentrations were determined by microbiological assay. Disposition curves were analyzed by a non compartimental model (PcNonlin software). Pharmacokinetic parameters were compared performing Wilcoxon's paired *t* test ($p \leq 0.05$). Results are reported as mean±standard deviation. Group 1: terminal half-life ($t_{½\lambda}$) = 2.33±0.42 h, apparent terminal rate constant (λ) = 0.3±0.06 h^{-1}, total body clearance (Cl_t) 3.31±0.32 mL/kg.min, volume of distribution (V_d) = 0.66±0.11 L/kg, mean residence time (MRT) = 2.15±0.22 h, area under the curve extrapolated to infinity ($AUC_{0\text{-}inf}$) = 126.9±13.21 µg·h/mL. Group 2: $t_{½\lambda}$ = 1.87±0.3 h, λ = 0.37±0.05 h^{-1}, Cl_t 3.37±0.25 mL/kg.min, V_d = 0.54±0.11 L/kg, MRT = 1.93±0.32 h, $AUC_{0\text{-}inf}$ = 124.2±9.6 µg·h/mL. No statistical differences were found in cephalexin pharmacokinetic parameters between groups. Consequently, no dose adjustment should be required in cephalexin treatments when meloxicam is coadministered.

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Nifurtimox and Benznidazole metabolism in rat heart. Ultrastructural and biochemical observations.

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There are available two drugs for the ethiological treatment of Chagas' disease, Nifurtimox (Nfx) and Benznidazole (Bz). They are being used in the acute phase and recently they were used in the indeterminate phase. Both nitroheterocyclic drugs have serious toxic side effects. The mechanism of toxicity is associated with their nitroreduction and the generation of reactive metabolites. Their potential effects on cardiac function are yet not known, in spite of the well known cardiopathy generally produced by the disease. We describe initial experiments to test the acute effects of both drugs on rat heart. Male Sprague Dawley rats were treated ig with either Nfx or Bz. They reached the tissue at 1, 3 and 6 h after treatment. In vitro studies on Nfx and Bz microsomal and cytosolic nitroreductase activities showed that only the microsomal fraction had the ability to nitroreduce both drugs. CYP and P450 reductase would be involved as suggested by the CO, SKF 525-A and DPI inhibition. Nfx increased the protein carbonyl content at 1 and 3 h and decreased the protein sulfhydryl content at 3 h. In addition, 24 h after treatment ultrastructural alterations such as marked cytoplasm vacuolization, separation and loss of myofibrils and mitochondrial swelling were observed. Results suggest that Nfx administration might aggravate pre-existing adverse cardiac conditions. Bz effects are under study.

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Metabolism of carcinogenic nitrofuranes in rat mammary tissue

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Nitrofurane compounds are widely used as antimicrobial drugs. Also, they were found as food contaminants. It is known that they are carcinogenic in rodents and their mechanism of action is not fully understood. Previous studies had shown that Nifurtimox (Nfx), a 5-nitrofurane used to treat Chagas' disease, is metabolically bioactivated by nitroreduction. Present work intend to study the metabolism of Nitrofurazone, Nitrofurantoine and Furazolidone in cytosolic and microsomal fractions of rat mammary tissue. Comparison against Nfx was made. Female Sprague Dawley rats were used to obtain subcellular fractions of mammary tissue. In vitro studies on nitrofurane nitroreductase activities either with pure xanthine oxidoreductase (XOR) or cytosolic and microsomal fractions were done. Our results indicate that all nitrofuranes were nitroreduced by the pure and cytosolic XOR as evidenced by the requirement of hypoxanthine as co-substrate and its response to the allopurinol inhibition. The intensity of the nitroreductive XOR and cytosolic metabolism for all of them was higher than that for Nfx. Also, drugs were nitroreduced by microsomes (NADPH-dependent), suggesting a CYP and P450 reductase action. These results might suggest that nitrofurane nitroreductive metabolism could be related to the carcinogenic potential of these drugs. Other environmental nitroderivatives are under study.

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Cis-diammine dichloroplatinum nefrotoxicity in rats. Prevention by Diallyldisulfide

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Cis-diammine dichloroplatinum (CisPt) is an effective chemotherapeutic agent against several human cancers but it produces important nephrotoxicity. In this work we analize the possibility that the diallyldisulfide (DADS) could block this toxicity without risk or enhancement of this therapeutic.
Four groups of male Sprague Dawley rats were used (control, DADS control, CisPt and CisPt + DADS). CisPt was administered subcutaneously as a single dose (10.5 mg/kg) in saline. The DADS was administered daily intragastrically in olive oil (292.5 mg/Kg). The animals were sacrificed 5 days after cisPt administration. Seric creatinine and urea determinations were performed and samples of kidneys were taken for histological and chemiluminiscence studies. DADS significantly decreased mortality and blocked CisPt nephrotoxicity. CisPt renal toxicity involved the production of oxygen reactive species (ROS); DADS blocked ROS production without changing Pt levels in kidney. In hands of other laboratories, DADS exhibited an ability to inhibit cellular replication and to promote apoptosis of tumoral cells and thus, we envisage a potential application of DADS as adjuvant of CisPt chemotherapy.

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Pharmacokinetics and penetration into tissue fluid of cefepime administered to hyperthermic rabbits.

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The pharmacokinetic characteristics in serum and tisular fluid of cefepime given intravenously (20 mg/kg body weight) were assessed in healthy rabbits and in rabbits with hyperthermia induced by lipopolysaccharide of *E. Coli* (LPS), with cages implanted subcutaneously. Ten adult rabbits were used in two trials (T1 and T2). The kinetic and statistic analysis were performed by means of a non-compartmental model and multifactorial ANOVA, respectively. Pharmacokinetics results in serum (S) and tisular cage fluid (TCF) (means ± standard error): $t_{1\backslash 2}$ (T1 S)= 1.5 ± 0.2 and (T2 S)= 2.0 ± 0.2 h, AUC (T1 S)= 181.6 ± 17.5 and (T2 S)= 192.3 ± 18.5 µg/ml/h; V_{ss} (T1 S)= 0.31± 0.05 and (T2 S)= 0.69 ± 0.28 l/kg (P< 0.05); CL(T1 S)= 118.3 ± 17.7 and (T2 S)= 93.1± 19.9 (ml/h)kg (P< 0.05); C_{max} (T1 TCF)= 23.5 ± 3.4 and (T2 TCF)= 27.6 ± 3.6 µg/ml; time to reach C_{max} [t_{max} (T1 TCF) = 2.3 ± 0.4 and (T2 TCF)= 1.7 ± 0.4 h]; $t_{1/2}$ (T1 TCF)= 2.4 ± 0.3 and (T2 TCF)= 3.4 ± 0.3 h (P< 0.05); AUC (T1 TCF)= 122.0 ± 12.7 and (T2 TCF)= 156.9 ± 13.6 (µg/ml/h); penetration (T1 TCF)= 67.3 ± 8.7 and (T2 TCF)= 88.5 ± 8.7 %. In conclusion, the pharmacokinetic changes of cefepime observed in rabbits with hyperthermia induced by LPS, could be clinically significant if not taken into account when designing the dosing regimens.

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Concentration of cefepime in inflammatory tissue cage fluid.

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The pharmacokinetics of cefepime in serum and inflammatory tissue cage fluid were determined in rabbits. Ten adult healthy rabbits were used. Concentrations of cefepime were measured in serum and inflammatory tissue cage fluid (ITCF) by biological methods. The kinetic variables were analyzed by means of a non-compartmental model. Pharmacokinetic results in serum (S) and ITCF (means ± standard error) were: half life of elimination [$t_{1\backslash 2}$ (S)]= 1.6 ± 0.2 and (ITCF) 3.7 ± 0.3 h; area under the curve [AUC (S)]= 225.3 ± 21.4 and (ITCF) 208.0 ± 13.6 (µg/ml/h]; maximum concentration [C_{max} (ITCF)]= 37.7 ± 3.6 µg/ml and time to reach C_{max} [t_{max} (ITCF)]= 1.8 ± 0.4 h and the penetration into ITCF (P)= 100.4 ± 8.7 %. In conclusion, cefepime administered to rabbits penetrates rapidly and completely into the inflammatory tissue cage fluid.

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Neural basis of the analgesic action of the striatum.

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In previous papers from this laboratory we demostrated that stimulation of the striatum inhibited the nociceptive response (jaw opening reflex, JOR) to tooth pulp stimulation in rats. This effect is mediated by the activation of dopamine D_2 receptors in the striatum. Our objective was to search the neural structures involved in that action.The experiments were carried out in rats anesthetized with urethane (1,2 g/kg, i.p), implated in the lower incisors with electrodes for stimulation of the dental pulp. The JOR was recorded as the electromiographic response, of the digastric muscles, by means of electrodes positioned into the muscles.The unilateral electrolytic lesion of the globus pallidus (GP) and the substantia nigra reticulata (SNr), suppressed the JOR inhibition induced by a microinjection (7 µg/ 0.5 µl) of apomorphine (an agonist of dopamine receptors) into the striatum. GP: control 100 ± 4.4 %; post-apomorphine 38.9 ± 7.3 %*, post-lesion 71.7 ± 6.2 %; one way ANOVA for repeated samples, Dunnett test *P< 0.05. SNr: control 100 ± 11 %; post-apomorphine 21.3 ± 7.8 %*; 82.2 ± 10.6 %, one way ANOVA, Dunnett test, *P< 0.05. The electrolytic lesion of the rafe magnus nucleus (NRM) also suppressed the inhibition of the JOR; control: 82 ± 6.7 mv.; post-apomorphine: 41 ± 14.2 mv*.; post-lesión: 97.25 ± 44 mv.; one way ANOVA, Dunnett test *P< 0.05. From above results we concluded that GP, SNr and NRM are involved in the analgesic action of the striatum.

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Effects of celecoxib and glucosamine on nitric oxide and prostaglandin e_2 productions in osteoarthritic human cartilage

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The integrity of articular cartilage is determined by a balance between chondrocyte biosynthesis of extracellular matrix and its degradation. Osteoarthritis (OA) is characterized by a degeneration of articular cartilage. Nitric oxide (NO) and prostaglandin PGE_2 are autacoids that contributes to inflammatory and arthritic tissue destruction. In this study, we examined the effects of the glucosamine (GS) and sodium celecoxib (CELE) on the óxid nitric (NO) and prostaglandin E_2 (PGE_2) production in cultured human OA articular chondrocytes. The aim of this study was to investigate the effects in vitro of CELE and GLUCO on levels of NO and production of prostaglandin E_2 (PGE_2), by human articular chondrocytes. Chondrocytes were cultured in the absence or presence of 1-10 µg/ml of CELE and GLUCO. PGE_2 concentrations were determined using HPLC, the Griess assay was used to quantify NO. Enhanced PGE_2 production in degenerative and OA cartilage could be decreased by CELE and GLUCO, whereas no effect on enhanced NO production was found.
Our studies demonstrate :1- differences between CELE and GLUCO with respect to their ability to modulate the PGs. 2- had not significant effect on NO production. These drugs do not slow down the progression of OA.

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The effect of aspartame metabolites on acetylcholine induced-relaxation of aortic rings of rats.

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In a previous study we have shown that an impaired vascular reactivity is obtained in aortic rings of normo-glycemic rats incubated with Aspartame (ASP) L-Aspartyl-L-phenylalanine methyl esther. The aim of the present study was to evaluate acetylcholine (Ach) induced relaxation of aortic rings, after incubation with ASP metabolites which are commonly measured in blood after ASP ingestion: L-phenylalanine (Phe), methanol (Met) and aspartic acid (Aspt). Rings of thoracic aorta were mounted on stainless steel hooks and suspended in tissue baths. Tension development was measured by isometric force transducers connected to an amplifier. At the end of the equilibration period, the maximal force generated by adding a depolarizing solution of KCl was determined. After washing, two rings were used as control and two were incubated in the presence of ASP metabolites -concentration corresponding with 17 mg/kg and 34 mg/Kg of the sweetener ingestion- (Met: 0.07 and 0.14 mM; Aspt: 1.4 and 2.8 mM; Phe; 0.07 and 0.14 mM). Two sets of experiments were performed incubating with: a- the sum of ASP metabolites and b- each one separately; and then cumulative dose-response curves to phenylephrine (PhenE) and (Ach) were performed. When incubations were carried out with the sum of ASP metabolites a reduced Ach induced-relaxation was observed ($P< 0.05$), but no significant differences were obtained with Met, Phe or Aspt separately (Fact. ANOVA). Conclusions: These results support the previous findings that incubation of aortic rings with ASP induces a decreased relaxation to Ach.

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Endothelium-dependent relaxation is decreased in fructose fed rats possibly due to oxidative stress.

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Rats fed a high fructose diet (Ff) -10 % in water- develop hyperglycemia with normal circulating insulin levels, 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, a situation which induces oxidative stress through superoxide anion accumulation. The present work was designed to continue the study of vascular response and evaluate the presence of oxidative stress in plasma and heart tissue of Ff rats. 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). 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 min after glucose load ($P<0.001$). TBARS in plasma and in heart were higher in Ff than in control rats ($P< 0.05$ and $P< 0.01$ respectively). SNP relaxation was decreased in Ff rats $P< 0.01$. Conclusions: The decreased Ach-IR and SNP relaxation obtained in aortic rings incubated in a HG medium could be related to an increased oxidative stress and a decreased bio-ability of NO

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Ischemia-reperfusion performance and ATP and PCr contents in neonatal rat hearts pretreated with cardioplegia-high K: effects of caffeine and KB-R7943.

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Rat neonatal hearts have higher contribution of sarcolemmal (SL) rather than sarcorreticular (SR) Ca than adults. Then, it was studied the consequences of an ischemia-reperfusion (I-R) without and with pretreatment of high-K cardioplegia (CPG). Perfused beating hearts from neonatal rats (10-12 days old) were pretreated with CPG-Ca0.5 mM or Krebs (C) and exposed to 15 min I-45 min R. Contractile force (F) was measured during R and contents of ATP, PCr and their metabolites were analyzed by HPLC in frozen hearts. The ATP energy charge (EC) was calculated as (ATP+ 0.5.ADP)/ (ATP+ADP+AMP)). During R, F recovered until 58.4 ± 10% of pre-I in C and to 69.8 ± 9.6% in CPG-0.5 (NS). EC fell from 0.373 ± 0.039 in pre-I to 0.29 ± 0.04 in I and to 0.219 ± 0.035 in R of C hearts, but increased to 0.389 ± 0.099* in R of CPG0.5 hearts. A pretreatment with CPG-2 mM Ca increased F% in R until 90.7 ± 9.4 %* and PCr from 7.0 ± 1.8 to 14.0 ± 2.7* µmol/g dw, without modifying EC (0.232 ± 0.025). The pretreatment with CPG+ caffeine-10 mM increased F to 97.5 ± 9%*, while CPG+5µM KB-R7943 (a selective inhibitor of the reverse NCX) recovered F until 88.3 ± 11.3%*. Neither caffeine nor KBR changed ATP, PCr nor EC. Neonatal hearts regulate Ca homeostasis during I-R in a way different to adults: CPG0.5 did not increased F recovery but raised the EC of ATP, while caffeine and KBR improved F recovery by increasing Ca store without changing energy resynthesis.
*(*p<0.05 vs.C) X-408 UNLP-2005/07.*

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Spasmolytic effect of cedron *(Alloysia citriodora)* is partially due to vitexin and isovitexin: study on isolated rat duodenum.

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Previously we showed the antispasmodic effect of an acqueous extract (AEC) from cedrón *(Alloysia citriodora* Palau, Verbenaceae) and we identified two components: vitexin and isovitexin. The AEC non-competitively inhibited the dose-response curve (DRC) of Ach and the Ca-DRC in high-K. Now, we studied the mechanism of such effects on rat isolated duodenum: AEC potentiated the non-competitive inhibition of Ca-DRC produced by W-7 (a calmodulin inhibitor) and by papaverine. In high-K media, AEC relaxed muscles (CE50: 2.6 ± 0.2 mg lyophilized/ml) until 81.0 ± 3.2% of papaverine effect and to 78.1 ± 5% (NS) of quercetin maximal relaxation, which is a most selective inhibitor of PDE. The relaxant effect of AEC at 1 mg/ml was inhibited by previous hypoxia, but not that at 2 mg/ml. Vitexin non-competitively inhibited the Ach-DRC with an affinity (pD'_2) of 5.7 ± 0.4 until 48.6 ± 12% of Emax, but significantly increased the pD_2 of Ca-DRC from 2.5 ± 0.1 to 2.8 ± 0.2 ($p<0.05$). Isovitexin slightly inhibited the Ach-DRC with a pD'_2 of 6.8 ± 0.7 until 81.4 ± 9.5% of Emax and did not modify the Ca-DRC (pD_2 3.2 ± 0.1). The present results suggest that: **a)** the spasmolytic effect of AEC could be due in about 80% to PDE inhibition and less to inhibition of the aerobic metabolism, **b)** vitexin is only in part responsible for the AEC effect, since it non-competitively inhibited Ach but not Ca and sensitized muscle to Ca, **c)** isovitexin only slightly inhibited Ach- but not Ca-DRC. *UNLP X-408-2005/07.*

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Melatonin and amphetamine a potential neuro-protector agents against 2,4-dichlorophenoxyacetic acid injured cerebellar granule cells in cultures

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2,4-Dichlorophoxyacetic (2,4-D), a worldwide-used herbicide, has been associated with a range of adverse health effects on human being and different animal species. We laboratory has previously reported apoptosis in rat cerebellar granule cell (CGC) cultures by 2,4-D, a process generally believed to be mediated by oxidative stress. Since melatonin has remarkable antioxidant properties and amphetamine has accelerate recovery of function in animals with brain injury, the objective of this study was to determine if the 2,4-D (1 mM) toxicity mechanism involves an alteration in the oxidative cellular homeostasis and if melatonin or d-amphetamine acts as a neuronal protector. Cellular viability, generation of reactive oxygen (ROS) and nitrogen (RNS) species, reduced glutathione (GSH) levels, and the activities of the antioxidant enzymes were measured. In CGC exposed to 2,4-D, cell viability, GSH levels and catalase activity decreased significantly whereas ROS generation and selenium-glutathione peroxidase activity augmented. Whereas, these effects of were partly reverted in cultures exposed to 2,4-D plus melatonin (0.1 and 0.5 mM) or d-amphetamine (1 and 10 μM).In conclusion, melatonin and amphetamine could act as neuroprotector of the 2,4-D toxic effects because they decrease the oxidative stress and cell death.

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Pharmacokinetic and phototherapeutic studies of zinc phothalocyanine CF_3 in a mouse tumoral model

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Photodynamic therapy (PDT) is emerging as an alternative modality for cancer therapy. It induces neoplasic cell death for photoachievable sensitizers. The aim of this work was evaluate the pharmacokinetic and phototherapeutic effects of Zinc (II) 2, 9, 16, 23-tetrakis (4-trifluoromethylbenzyloxy) phthalocyanine (PT) in a Balb/c mouse tumor model. Biodistribution studies were carried out by intraperitoneal injection of 2,0 mg/kg PT. Histological studies and serum biochemical parameters were used to test hepatic and renal toxicity and functionality. An analysis of tumoral necrosis degree was used to evaluate the phototherapeutic effects. It was achieved 1, 2, 3 y 4 days after PDT. Vital staining was performed by intraperitoneal injection of 0,35 ml 1% Evans Blue solution. Six hours later, tumors were excised and examined macroscopically through a magnifying glass. The unstained area was attributed to tissue necrosis, whereas the stained area showed tissue with preserved blood supply. The dose of 0,2 mg/kg PT resulted in a low acute toxicity with revertible damages. It indicates that this dose can be used to PDT. The photosensitizer is accumulated in spleen, liver and duodenum. It suggests that PT is eliminated from the body, via bile-gut. It do no cross the blood-brain barrier and do no produce toxicity in the skin. The tumoral death was of 89 % four days after PDT. It indicates that PT is effective in PDT.

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Dapsone-induced toxicity in hepatic human cells lines is attenuated by antioxidants: Possible role of CYP450-mediated dapsone metabolism.

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Dapsone (DDS) is the main therapeutic agent for leprosy. Clinical experience has indicated that DDS may cause hepatotoxicity. Activation of the drug to dapsone hydroxylamine (DDS-OH), by CYP450 isoenzymes is cause of hemotoxicity. We examined the role of oxidative stress in DDS- and DDS-OH-induced toxicity in human liver cell lines. SK-Hep1 and HepG2, cultured in micro-plates, were exposed to DDS (0,23, 0,31, 0,47, 0,63, 0,94, 1,25, 1,88, 2,50 mM in DMSO) or DDS-NOH (0,14, 0,19, 0,28, 0,38, 0,56, 0,75, 1,13 1,50 mM in DMSO) over 24, 48 and 72 hrs. DDS and DDS-OH diminished cell viability (MTT assay) in a dose-dependent manner in both cell lines. LDH activity in the incubation medium after a 48-hr exposure to the same DDS or DDS-OH concentrations confirmed MTT results. The anti-oxidants, vitamin C (5 mM), vitamin E (2.5 mM), glutathione (5 mM) and N-acetyl cysteine (4 mM) all attenuated DDS- and DDS-OH-induced cell death in both cell lines. DDS's deleterious effect on cell viability was exacerbated by the CYP450 inducer, rifampicin (0.1 mM), whereas the CYP450 inhibitor, cimetidine (0.15mM), attenuated this effect in both cell lines. We conclude that DDS citotoxicity involves, at least in part, an oxidative-stress mechanism where CYP450 activity may participate through the formation of its pro-oxidant metabolite, DDS-OH.

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Gastroprotection induced by *Baccharis polifolia* Griseb. in rats: role of sulfhydryls groups

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Baccharis polifolia Griseb, known as "quincha mali", is commonly used for its digestive properties. Gastroprotective activity and mechanism of action by *Baccharis polifolia* Griseb. extract (*Bp*E) were investigated. Role of sulfhydryls groups (SH) in the gastroprotection induced by *Bp*E was evaluated.

Methods and Results: Twenty four hours before the experiments, Wistar rats were fasted. Absolute ethanol (EtOH) was employed as ulcerogenic agent (Method of Robert *et al.*, 1979). *Bp*E reduced ethanol-induced gastric mucosal damage ($p<0.001$ vs. control of EtOH). Pretreatment with *N*-ethylmaleimide (NEM), a sulfhydryl-blocker, reduced gastroprotection afforded by *Bp*E ($p<0.001$ vs. *Bp*E + EtOH).

Conclusion: *Baccharis polifolia* Griseb prevents the formation of gastric lesions induced by absolute ethanol at a dose of 250 mg/kg. These facts support the use in traditional medicine of *Baccharis polifolia* to treat digestive disorders. Sulfhydryl compounds may be important in maintaining gastric mucosal integrity, and endogenous sulfhydryl compounds might mediate gastric cytoprotection induced by prostaglandins. We conclude that endogenous sulfhydryls may be involved in the gastroprotection of *Bp*E.

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Effect of early stimulations on some immune parameters in prenatal stress rats

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The application of stressors in the pregnancy produces an alteration of the axis hypothalamic-pituitary-adrenal (HPA), what would induce a long-term alteration of the immune function in the offspring. Early postnatal stimulations produce beneficial effect on long term emotional reactivity and HPA axis activity that could revert the effect of prenatal stress. The objective of this work was to investigate the effect of early postnatal stimulations in prenatal stressed animals on the distribution of the subpopulations of leukocytes in response to acute stress in rats. Males of three months of age were used, offspring of mothers immobilization (IMO) stressed (EP) and non stressed (CP) during pregnancy. Half of the EP animals were manipulated (M) during the first week of life. Previous extraction of blood for basal determinations, the animals of both groups were under acute IMO (20 minutes), after that it was extracted blood at the 0, 40, 70, 100, 130 and 310 minutes post-stress, to count white blood cells, and the subpopulations of leukocyte. The profile of the leukocytes, lymphocytes and neutrophils are similar in EP and C, however the response is depressed in EP animals under postnatal IMO. Also, postnatal stimulation reverts the effects of prenatal stress on the distribution of the subpopulations of leukocytes.

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Increases in TRPV1 protein abundance and CGRP content in endotoxemic rats

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The vasorelaxant effects of the endocannabinoid anandamide (AEA) are potentiated 6 hours after intraperitoneal administration of 5 mg/kg of lipopolysaccharide (LPS) in the rat vascular mesenteric bed (Orliac et al., *J Pharmacol Exp Ther.* 304:179-84, 2003). Since anandamide acts through the activation of TRPV1 vanilloid receptors and the consequent calcitonin gene-related peptide (CGRP) release, the aim of the present work was to study whether modifications in the TRPV1 expression as well as CGRP content and release could be linked to the enhanced anandamide-induced relaxations in endotoxemia. In tongues used as a representative systemic model of TRPV1 receptor expression, a band of 100 kDa equivalent to the estimated molecular weight of TRPV1 was increased 6 h after LPS administration. The CGRP-immunoreactivity in mesenteric beds was also increased 6 h after LPS. The overflow of CGRP increased in LPS-treated rats when mesenteries were perfused during 10 min with 10 µM anandamide. Moreover, relaxations to anandamide were potentiated by the protein kinase C activator 0.1 µM PMA in untreated mesenteries. The effect of PMA was accompanied by an increase in the overflow of CGRP induced by anandamide. It is proposed that the overexpression of the TRPV1 receptors and the increased content of CGRP could contribute to the enhancement of anandamide effects during the endotoxemic shock. An eventual phosphorylation event linked to the overflow of CGRP could also participate in the enhanced relaxation caused by anandamide in endotoxemia.

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Biological behaviourn of 99mTc-ENS in a lung injury (LI) animal model and its comparison with 99mTc-DTPA.

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Objective: To study the biodistribution of 99mTc-ENS in a LI animal model and to compare the results with the ones obtained for 99mTc-DTPA. Materials and Methods: LI was induced in rats according to the model reported by Mortuza et al (2003). Radiopharmaceuticals were administered endotrachealy and the animals were sacrificed after 30 min. Lungs, kidneys, liver, spleen, gastrointestinal tract, heart and blood were removed for its measurements in a gamma counter. The results were expressed as activity concentration percentage (AC%) (activity/organs weight %). Results: AC% values obtained in the lungs were (96,9 ± 4,1)% and (98,5 ± 0,8)% for 99mTc-ENS, and (78,8 ± 18,9)% and (92,9 ± 1,9)% for 99mTc-DTPA in the control and treated groups respectively. Conclusions: The biological behaviour of the 99mTc-ENS is not affected in a pharmacological LI model. In contrast, 99mTc-DTPA is concentrated in the lungs in that condition, with a consequent reduction of the activity in the kidneys.

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99mTc-RBC biodistribution in the iron deficiency anemia

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Objective: Determine if iron deficiency anemia modifies the biodistribution of 99mTc-RBC. Materials and methods: 60 Sprague-Dawley female rats were fed for 3 weeks with different iron content diets to induce different grades anemia: A, severe anemia (6.5 ppm); B, moderate anemia (18 ppm); C, control (100 ppm). Hemoglobin blood content was determined before and after treatment. Labeling of red blood cells (RBC) was performed by the in vivo method. Labeling efficiency was determined by measuring the activity percentage in RBC. Biological distribution was made immediately after RBC labeling and after 24 hr in each group. Blood, liver, spleen, gastrointestinal tract, kidneys, heart and lungs, were extracted and their weight and activity was determined. The results were expressed as activity percentage (A%) and activity concentration percentage (AC%). Results: Labeling efficiency of 99mTc-RBC was higher than 98 % for each studied group. The A% and AC % for group A was higher in spleen at 24 hours compared to the other groups (A%=1.3± 0.6, $p<0.01$, AC%=4.7±2.4, $p<0.01$). In addition, an increase in AC% in kidney was obtained at 24 hours for all the groups.
Conclusion: Biodistribution of 99mTc-RBC is affected by the severe iron deficiency anemia, showing an increase in spleen activity which could be a consequence of splenic uptake. Our findings could be of relevance in clinical diagnosis.

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the construction of a limphocytic PGP activity data base with covariables.

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The aim of the work is to link the PGP activity with the covariables measured in a sample of individuals. The sample size, in this initial stage was of 35 healthy individuals, men and women, between 20 and 60 years. The possible associations between the covariables and the PGP's fluorescence could allow us detecting regulatory factors. The analysis of limphocytic PGP functionality is accomplished by measuring the extrusion of Rodamina 123 followed by flow cytometry. This gives a value to which we call Fluorescente (FR). There were also measured three kind of covariables: biochemistries (hemogram, erythrocyte-sedimentation, hepatic enzymes, glycemia, total cholesterol, creatinine, triglycerides and bilirubin), anthropometric (age, weight and sex) and clinics (diet, smoking habits, mate ingestion, coffee, tea and alcoholic drinks). The information was analyzed by means of stepwise multiple regression. The method express one variable (FR) like linear function of others in order to identify the important explanatory covariables (those with $P< 0.05$). We have found an association between fluorescence and cholesterol ($P=0.008$) and between fluorescence and age ($P=0.04$) No other registered covariable was found related with PGP activity.

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Analysis of limphocytic PGP activity

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The aim of this work was to construct an histogram with a sample of 35 values of PGP and to study the performance of a binormal distribution adjustment. The sample size, in this initial stage was of 35 healthy individuals, men and women, between 20 and 60 years.
PGP activity in lymphocytes (FR) was measured by the extrusion of Rodamina 123 determined by flow cytometry in the presence and absence of verapamil, a PGP inhibitor.
We have obtained a normal distribution N (2,36; 0,52) when fitting a normal to the PGP histogram and we verified that this adjustment was overcome by the mixture of a proportion p=0,55 of a normal distribution with mean m_1 = 1,98 and standard deviation s_1=0,25 and another proportion (1-p)=0,45 of a normal distribution with mean m_2 =2,83 and standard deviation s_2=0,34. Two Goodness of fit tests were calculated, Chi-Square and Kolmogorov-Smirnov. For the first one there was obtained the statistic Ch-S-binormal=0.0007 (P=0,99 N.S); for the second K-S-binormal=0,09 (P=0,95 N.S). Therefore according to these tests, it is not rejected the supposition that the observed values come from the distribution F= 0,55 x N (1,98; 0,25) + 0,45 x N (2,83; 0,34).

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Study of the mechanisms underlying the enhanced cardiovascular responses to anandamide after prolonged nos inhibition

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In Sprague-Dawley rats the vasorelaxant effect of anandamide (AEA: 1; 5 and 10 μM) in isolated mesenteric beds as well as the hypotensive responses to i.v. administered AEA (5 mg/kg) were potentiated by chronic treatment with the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine-methyl ester (L-NAME; 40 mg/100 ml in the drinking water; 4 weeks) (*Mendizabal et al.; Eur. J. Pharmacol. 427, 251-262; 2001*). Since the vascular relaxation caused by AEA is related to the activation of vanilloid receptors (TRPV1) and the release of calcitonin gene-related peptide (CGRP) from perivascular sensory nerve endings, this study examined whether increases in either TRPV1 expression or tissue CGRP content could participate in the enhanced depressor effects of AEA observed after long-term inhibition of NOS. In the tongue, used as a representative model of TRPV1 protein, the receptor expression was 60% lower in rats chronically treated with L-NAME than in untreated rats ($p<0.001$). CGRP-like immunoreactivity in mesenteric arteries did not change after L-NAME treatment. It is concluded that the enhanced depressor responses to AEA in L-NAME treated rats is unrelated to either overexpression of TRPV1 receptors or increased CGRP content in vascular tissue.
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Cardioprotective effect of 17β-estradiol on reperfusion-induced arrhythmias

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Increasing evidence indicates that acute administration of 17β-estradiol has a cardioprotective action. The purpose of this study was to elucidate the effect of this estrogen on the incidence of reperfusion-induced arrhythmias (RA) in isolated rat hearts perfused with the Langendorff technique. We performed 3 series with 10 experiments each: Normal solution as control (C); 17β-estradiol 1 μM (E1) and 5 μM (E5). All the hearts were submitted to 10 min occlusion of the left coronary artery and subsequent 15 min of reperfusion. We recorded and measured epicardial action potentials (AP) from left ventricle, ECG and coronary flow. We only took into account severe RA as ventricular tachycardias and fibrillations. Data were analyzed statistically with ANOVA I and $\chi 2$. The results showed that only 10% of hearts did not develop RA in C, meanwhile in E1 40% and in E5 60%, ($p<0.05$) of cases did not show alterations of the rhythm. At 5 μM concentration a lower frequency and a slight prolongation on AP duration were observed along the entire experimental period. All the other variables did not show differences. We conclude that 17ß-Estradiol significantly reduced reperfusion-induced ventricular arrhythmias and it seems that this effect is associated with the heart rate reduction.

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diet influence on P-Glycoprotein activity in the rat

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P-glycoprotein (P-gp) is an efflux transporter whose activity has been reported to change after different drug treatments. However, little is known about protein intake and P-gp. In order to evaluate a potential variation in P-gp activity, male Wistar rats received either normal diet (control group) or protein free diet (treated group) for two weeks. Both food and water were supplied *ad libitum*. Body weight and diet intake were individually controlled along the treatment. On the 15th day P-gp activity was determined using the everted sac of ileum *in vitro* technique and Rhodamine 123 as P-gp substrate. Ileon exposed surface, weight and total tissue proteins were also assessed to correct any additional variation, and plasma proteins were measured as well. A significant difference was detected for the Pgp activity between control (13.4 ± 5.9 picomol/min) and treated (22.4 ± 2.5 picomol/min) groups. Although there were neither significant differences between groups for ileon exposed surface, weight and total tissue proteins nor in plasma proteins, body weight in control group raised from 208g to 259g while fell in treated group from 206g to 153g. These preliminary results indicate that Pgp activity could be induced after two week protein free diet treatment. Further investigation is required to evaluate other nutritional aspects that could led to this rise in P-gp activity, and to elucidate whether this phenomenon has local or global implications.

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Role of CGRP and prostacyclin in the sex-linked differences of the relaxant effects of anandamide in rat mesenteric arteries

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The aim of this work was to study the mechanisms involved in the greater relaxations caused by anandamide (AEA) in mesenteric beds isolated from female compared to male rats (Peroni *et al.*, *Eur. J. Pharmacol.* 493:151, 2004). The AEA-induced relaxations were abolished by the nitric oxide synthase inhibitor 100 μM L-NAME in intact as well as in de-endothelized male and female mesenteric beds. Sensory *in vivo* denervation also markedly reduced the relaxation caused by AEA in either male or female mesenteries. On the other hand, the remotion of the endothelium enhanced the relaxations caused by AEA in mesenteries isolated from male and ovariectomized female but not from sham-operated female rats. The calcitonin gene-related peptide (CGRP) content in rat mesenteric beds was higher in female than in male rats, faded by ovariectomy and restored to control values by chronic treatment with 17β-estradiol. This latter procedure also increased CGRP content in males up to the same levels observed in females. With regard to prostanoids, the ratio prostacyclin / thromboxane A_2, that did not differ between male and female mesenteries under control conditions, it was reduced in males after exposure to AEA, due to the decrease in the prostacyclin tissue content. Moreover, the cyclooxygenase-2 inhibitor 0.1 μM NS-398 reduced the relaxations caused by AEA solely in female rats. It is proposed that relaxing factors such as CGRP and prostacyclin contribute to the higher relaxations caused by anandamide in the vasculature of female rats.

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Plasma and urine concentrations of cephalothin and cefazolin after intravenous administrations to cats

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Cephalothin (CPL) and cefazolin (CFZ) are first generation cephalosporins active against *Staphylococcus* spp, *Streptococcus* spp and some enterobacteria. Like other beta-lactamic antibiotics, they are largely eliminated by renal mechanisms as active drugs. Their kinetic profiles were extensively studied in human beings and in some domestic animals. However, they have not been characterized in cats. The aim of this study was to analyze the plasma and urine concentrations (cc) profile of CPL and CFZ after their intravenous (IV) administration to domestic cats.

For CPL and CFZ, 9 and 10 adult cats (4.36 ± 0.98 kg and 4.09 ± 0.94 kg, respectively) received 30 mg/kg (CPL) and 20 mg/kg (CFZ) by IV route. Blood samples and total urine elimination were collected over a 6 hours period. CPL and CFZ cc were determined by microbiological assay.

Maximum plasma cc was 353.79±118.92 μg/ml for CPL and 126.84±26.61 μg/ml for CFZ, respectively. At 6 hours post-administration, both drugs were over 1 μg/ml. Plasma elimination half-live were 1.07±0.23 h for CPL and 1.30±0.21 h for CFZ. For both drugs, urine cc exceeded plasma cc more than 10 times in all the measured samples. 66.50% (CPL) and 84.22% (CFZ) of the administered dose were recovered in the urine collected. The high cc found in plasma and urine would be efficacious for infections treatment with CPL or CFZ in domestic cats.

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Ciprofibrate effects upon oxidative stress in an atherogenic experimental model. FloresL, RitzerA, BaézM, BalcedaA, CampanaV, MoyaM. Cát Física BiomédicaFacCs.Ms.UNC.SantaRosa1085.Córdoba monicamoya@hotmail.com.

Hyperfibrinogenemia could induce atherogenesis modifying nitric oxide(NO)pathway and generated mitochondrial changes.
We studied the ciprofibrate response upon the diminished value of NO and the probable regression of mitochondrial damage in the smooth muscle of thoracic aorta. Hyperfibrinogenemia was induced by parenteral injection of adrenaline (0,1 mg/rat/day) during 30 days in the untreated (B) and treated with ciprofibrate (0,05 mg/rat/day) in the last fifty days after posinduction (group C). Both groups were compared with control (A). Plasma fibrinogen level (mg/dL) was determined by spectrophotometry and NO (μM) by Griess Reaction. Slices "in toto" of thoracic aorta of all mentioned groups were studied by electronic microscopic(MET). Plasmatic results were analized by ANOVA and the Axiovision 3.0 program was employed for MET. In group B fibrinogen increased significantly compared with the control (A) and the treated group (C) ($p<0.001$). NO diminished significantly in (B) in respect to control (A) and treated group (C) ($p<0,001$). MET showed mitochondrias in (B) with increased size,intermembranose space dilatation and crest desorganization compared with (A) and (C). Maybe, ciprofibrate modified the genetic code transcription of hepatic proteins, activating transcription factor of peroxime proliferator activated receptors. As fibrinogen is an hepatic protein its synthesis might be decreased by a similar mechanism; and consecuently normalize the NO synthesis and allow the partial regression of the lesions.

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2,4-dichlorophenoxyacetic acid (2,4-d) effect on hypothalamic dopaminergic neurons of adult rats subchronical or chronically exposed

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Dopaminergic system alteration in rats exposed to 2,4-D through lactancy has been reported in previous histological and biochemical studies from our laboratory. The aim of this work was to perform an immuno-histochemical quantitative study of the hypothalamic dopaminergic neurons 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 16th 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}. Serial coronal sections -from plates 18 to 35 of the Paxinos and Watson atlas- were immunostained according to Sternberger's PAP technique using a monoclonal anti-TH primary antibody. Data showed a decrease in the number of dopaminergic neurons from the arcuate hypothalamic nucleus, with both doses, in 2,4-D chronically exposed rats.

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"analysis of in vivo/ in vitro correlation in two acetaminophen`s formulations "

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The aim of this work was to analyze the existence of a correlation *in vivo* o *in vitro* in two different Paracetamol's formulations.
The test was carried out using as active Paracetamol principle in microparticles of alginato in capsules of gelatine, in one case without cover, and in other one, covered with a sheet of alginato.
The experimental determination was made in vitro, taking for each formulation three batches of 900 ml containing a similar gastric intestinal medium without enzymes, to which the pH was changed from 1.5 up to 7.2 during the trial, producing the dissolution (%Diss.) curve.
The formulation without cover was administrated in twelve healthy volunteers and the other, in six healthy volunteers .
The *in vivo* absorption curve (%Abs) was calculated by Wagner Nelson's Method, based in the salivary Paracetamol concentration.
The regression equation (%Abs) = $\alpha + \beta$ x (%Diss) gave as a result, $\beta = 1.21$ and a 95% confidence interval for β (0.017; 2.417) (without cover) and $\beta = 2.53$, (0.628; 4.432) (with cover).
Conclusion: As the confidence interval for β, contains in both cases the value 1, we conclude that exists correlation level A (relationship between in vitro dissolution and in vivo absorption) for both

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Inhibition of prenatal angiotensin-converting enzyme interferes alveolarization in the rat lung development

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The renin angiotensin system (RAS) may reside within several individual organs or tissues, such as lung, kidney, heart, and vascular smooth muscle cells, where it is believed to act in a functionally independent paracrine/autocrine fashion. Angiotensin II can act as a modulator of growth in a variety of cells and tissues. Different components of the RAS, including angiotensin conver-ting enzyme (ACE) and both angiotensin type 1 and type 2 receptors, are expressed in lungs endothelial and epithelial cells. The aim of the present work was to investigate if prenatal ACE inhibition influences morphological changes in postnatal lung tissue development. Pregnant Wistar rats were treated with ACE inhibitors. Mini-osmotic pumps with Captopril, Enalapril and saline solution were implanted subcutaneously. Structural changes in lung pups were evaluated at light microscopical level at different postnatal stages: PND1, PND8, PND15, PND30 and a semiquanti-tative respiratory airspace was measured. Lung histology revealed striking differences in lung structure between the treated groups and the control group. Enalapril treatment caused widening of respiratory airspaces and thinner alveolar septa whereas captopril produced thinning of it and increase connective tissue in alveolar septa. Sections per each lung were evaluated for semiquantitative assessment and the statistical analysis indicated significant differences (ANOVA, $P< 0.0001$). The results suggest that prenatal ACE inhibition in rats interferes with lung development.

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The superior ovarian nerve (SON) modifies the steroidogenic ability of splenocytes on the polycystic ovary in rats

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Polycystic ovary (PCO) is a complex endocrine disorder associated with ovarian hyperinnervation. We have previously shown that the ovarian steroidogenic response in rat is differentially regulated by the splenocyte secretions (Ss) through the neural connection involving ovary-SON-coeliac ganglion-spleen. Here, we study if the SON transection affects the steroidogenic ability of splenocytes (S) on the PCO. Two groups of rats of 60 days age were used: **PCO**, induced by estradiol valerate (2mg/rat) and killed 2 months latter and, **PCO+SONt**, PCO rats subjected to SON transection seven days before sacrifice. $1x10^6$ S from PCO and PCO+SONt rats were isolated and cultured in RPMI medium. Culture medium were used to stimulate PCO and PCO+SONt ovaries in incubation (3h, 37°C, 95%O2-5%CO2). The released androstenedione (A) and progesterone (P) were determined by RIA. The mRNA nerve growth factor (NGF, neurotrophin) expression level from ovaries PCO and PCO+SONt were determined by RT-PCR. Ss from PCO+SONt rats on PCO+SONt ovaries decreased A ($p<0.001$) and P ($p<0.01$) release in relation to PCO. The PCO+SONt ovaries NFG expression was minor than that of PCO and the ovarian stimulation with Ss from PCO+SONt did not altered the NGF expression. The SON section modifies the ovarian steroidogenic response by changes in the Ss and also in the ovary, contributing to decrease the high androgen levels which are characteristic of the PCO condition.

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Modulation of the ovarian progesterone response after stimulation of celiac ganglion with VIP in rat

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We know that celiac ganglion (CG) in the ex vivo integrated system CG-superior ovarian nerve(SON)-ovary (O) previously described in our laboratory, regulates the ovarian steroidogenesis. In this work we study the effect of CG stimulation with 10^{-7} M Vasoactive Intestinal Peptide (VIP) on the ovarian release of Progesterone (P) and Androstenedione (A), and its relation with nitric oxide (NO) releasing and ovarian nerve growth factor (NGF) expression. Holtzman cyclic rats on diestrus 2 (D2) were used. CG and O were placed in different cuvettes containing Krebs-Ringer buffer, and incubated in a metabolic bath. The SON connection was always preserved. After VIP addition to CG, samples from the ovarian cuvette were taken at 30, 60, 120 and 180 min, to measure the P, A and NO release. Basal values were obtained without VIP addition. Hormones were measured by RIA and NO by Griess reaction. The expression of NGF mRNA the ovary was determined by RT-PCR. VIP induced a significant increase of P ($p< 0.001$) at 60 min which was mantained until 180 min of incubation. This was associated to a significant decrease in the ON release, at all studied times. VIP did not modified the A release, compared with basal values. The ovarian NGF mRNA levels decreased with the VIP addition to CG. VIP on CG has a differential effect on the hormone release from the ovary. The effect on ovarian P response could be mediated by NO and NGF.

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ILEX PARAGUARIENSIS effect on intestinal PGP activity

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The intestinal P-glycoprotein (Pgp), an ATP-dependent multidrug efflux pump, can be an active secretion system or an absorption barrier by transporting some drugs from intestinal cells into the lumen. Some components were reported to modulate P-glycoprotein activity. Many of herbal constituents, in particular flavonoids, have been were reported to modulate P-glycoprotein (Pgp). Pgp interacts with a broad range of substances, and limits oral drug absorption. We have analyzed the influence of mate decoctions and mate infusion on intestinal Pgp activity, considering the wide use in our society of the same one, and possible importance that it would have in the bioavailability variability that it is observed in different drugs that are substrates of this transporter. We have taken as a model to begin this study, the isolated and everted rat intestine sac. It was validated with one of its recognized substrates (^{3}H-digoxin, 0.2µCi- 50uM) and one inhibitors (verapamil 100µM). To this end, the isolated tissues were incubated with the substrate and the efflux kinetics analyzed during 1 hour. The lineal transport was verified by liquid scintillation counter. Verapamil (100µM) inhibited the ^{3}H-digoxin efflux by 44.3 % ($p<0.001$). When evaluating the effect of the decoctions of mate (2 % P/V), we observed a 44 % inhibition of Pgp activity ($p<0.001$). In relation with mate infusion experiments, we also observed an inhibitory effect of 35.2 % ($p<0.01$)

Ours results suggest that decoctions of mate and mate infusion could contribute to the variability of bioavailability of drugs that are substrates of this efflux pump.

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Cytoprotective effects of flavonoids on the gastrointestinal tract: Structure-activity relationships

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Flavonoids comprise a class of natural products which are found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers, tea and red wine, and are consumed regularly as part of human diet. In the present study we have explored the effect of flavonoids on gastric ulcer (Method of Robert *et al.*, 1979) and ulcerative colitis (Method of Le Duc *et al.*, 1993) in rats. The protective effect of flavones (nepetin, nevadensin, 7-O-methylsudachitin) and flavanol (quercetin) was similar in both models ($p<0.05$ vs. respectives controls). These compounds showed good antiulcerogenic and gastroprotective effects and ameliorated the severity of the inflammatory lesions and reduced the damage area in colitis. Several structural requeriments for cytoprotective effect include: 3',4'-hydroxyl groups (B ring), 3'-hydroxyl and 4'-methoxyl groups (B ring) or 7-hydroxyl and 8-methoxyl groups (A ring) with a 4'-oxo function. The presence of a double bond between C_2-C_3 is crucial for cytoprotective effect since 7-O-methyleriodictyol was without effect. Morin, santin and genkwanin were not active in these assays. The evaluation of compounds with antioxidant properties, such as the flavonoids, may be interesting in the development of new strategies for the treatment of these gastrointestinal disorders.

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Study of acute toxicity of *Acacia visco* methanolic extract in mice.

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Acacia visco Lor. Ap. Griseb (Fabaceae), commonly known as "aromo", "viscote" or "arca", grows in central region and northwest of Argentina, is used how ornamental plant. In previous studies we showed anti-inflammatory (acute and chronic) and antiulcerous gastric activity of *Acacia visco* methanolic extract in rat (Biocell Vol 28 (3), 2004) . The aim of this study was to asses the acute toxicity in mice of methanolic extract from leaves (*Av*MEL) and bark (*Av*MEB) *of Acacia visco*. Mice were fasted for 4 h, water *ad libitum* and given oral increasing doses (100 at 2000 mg/Kg) of *Av*MEL or *Av*MEB. Animals were assigned to nine groups of 6 mice each, both sexes**,** eight which were treated with the extracts and one group control with vehicule (SF). Animals were observed for 14 consecutive days to record body weight (1st, 7th and 14th day), mortality or other toxic symptoms. After 14 days mice were sacrificed. Kidney, spleen and liver were observed macroscopically and the relative weights (organ/body) were determined. No toxic symptoms (restlessness, respiratory distress, diarrhea or convulsions) or death ocurred. There were no significantly differences in body weigth during experiment. Relative wet weigth of organs were not statiscally different from group control. In conclusion, under the present experimental conditions both, bark and leaves methanolic extract of *Acacia visco* showed no toxicity.

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Pharmacokinetics and bioavailability of ceftazidime in dogs

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The purpose of this study was to investigate the pharmacokinetics and bioavailability of ceftazidime after single intravenous (i.v.), and subcutaneous (s.c.) doses in healthy dogs. Six mixed-breed dogs were used in this experience. All animals received i.v. (20 mg/kg) and s.c. (25 mg/kg) ceftazidime in a 2-way crossover design. Blood samples were collected in predetermined times after drug administration. Concentrations of ceftazidime were determined by microbiological assay. Data were analysed by noncompartmental techniques using PCNONLIN software. Results are reported as mean ± standard deviation. For the i.v. route, total body clearance (Cl_t), volume of distribution (V_d), V_d at the steady state (V_{dss}) and terminal half-life ($t_{½\lambda}$) were 2.66±0.1 ml/kg·min, 0.240±0.04 l/kg, 0.208±0.03 l/kg, and 1.06±0.25 h, respectively. For the s.c. route, peak concentration, time to peak concentration and $t_{½\lambda}$ were 55.12±15.28 µg/ml, 0.96±0.33 h and 1.78 ± 0.67 h, respectively. Mean residence time was 1.32±0.29 and 3.13±1.03 h for the i.v. and s.c. routes, respectively. Bioavailability (corrected by $t_{½\lambda}$) following s.c. administration was 74.28±9.9%. No adverse effects related to treatment were observed. Our results suggest that ceftazidime could be used for treating susceptible microorganisms infections in dogs.

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Enrofloxacin pharmacokinetics in pregnant goats and placental transfer.

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The objectives of this study were to study the pharmacokinetics of enrofloxacin and its metabolite, ciprofloxacin, administered by the intravenous (i.v.) route to pregnant goats, and to determine the placental transfer of these drugs. Six female pregnant goats with 106-125 days of pregnancy received an i.v. dose of 7.5mg/kg of enrofloxacin. Blood samples were withdrawn at pre-determined times. At 144-146 days of gestation, 7.5mg/kg of i.v. enrofloxacin was administered previous a cesarean surgery and blood samples from the mother and from the umbilical vein, were taken. Enrofloxacin and ciprofloxacin concentrations were determined by HPLC. Plasma disposition curves of both drugs were analyzed by a non linear methods applying PcNonlin software. Similar to other species, enrofloxacin was partially metabolized to ciprofloxacin. The elimination half life were 1.72 ± 0.26 and 5.19 ± 2.22 h while the AUC were 21.41 ± 7.46 and 4.39 ± 1.17 µg·h/mL for enrofloxacin and ciprofloxacin, respectively. Low ciprofloxacin concentrations (0.08 ± 0.003 µg/ml) were detected in the fetuses, however high levels of enrofloxacin (5.93 ± 1.38 µg/ml) were found in the umbilical blood. These results show an acceptable pharmacokinetic profile, however precautions have to be considered when a treatment with this drug has to be used in pregnant goats.

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Pharmacokinetic interactions between meloxicam and cephalexin in dogs

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The pharmacokinetic interactions between meloxicam (MEL) and cephalexin (CEX) after intravenous (iv) administration to healthy dogs were investigated in this study. Six beagle adult dogs received 0.1 mg/kg MEL (group 1) or 25 mg/kg CEX 10 min after MEL administration (group 2) in a 2-way crossover design. Blood samples were collected in predetermined times after drug administration. Concentrations of MEL were determined by HPLC. Data were analysed by noncompartmental techniques using the PCNONLIN software. Results are reported as mean ± standard deviation. Statistical differences ($p \leq 0.05$) between group 1 and group 2 pharmacokinetic parameters were found for elimination rate constant (0.029±0.009 vs 0.0134±0.004 h^{-1}), terminal half life (26.3±9.5 vs 56.8±19.6 h), volume of distribution (292.3±92.6 vs 717.6±329.2 ml/kg) and volume of distribution at the steady state (197.2±54.0 vs 237.7±55.6 ml/kg). No differences were found for total clearance (7.99±2.4 vs 8.75±2.2 l/kg/h) and area under the last concentration curve (13.45±3.7 vs 12.09±3.2 µg·h/ml). These results show pharmacokinetic interactions in MEL distribution and elimination when administered with CEX.

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Acute Thymic Response and CD95 (APO-1/Fas) Expression at Different doses of 5-Fluorouracil

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5-Fluorouracil (5-FU) is a wide-spread used drug against solid tumors, showing a well documented toxicity during chemotherapy regimens. We used an *in vivo* murine model to investigate the effects of different doses of 5-FU (150mg mg/kg and 200mg/kg, i.p.) in a short time course study (0 - 48hs) to assess the thymic acute response. Data of the thymic weight, cellularity, viability, cell lineages as well as apoptosis were obtained. Immunoblottings studies were made to evaluate the expression of CD95 (APO-1/Fas). Data show that 5-FU caused a deeply reduction in weight and thymic cellularity ($p<0.01$). The correlation between them was direct and significant in both groups ($r = 0.8545$, P value = 0.0029). Viability did not show differences between groups, eventhought both of them diminished from 24hs ($p<0.01$) onwards. Mature lymphocytes and lymphoblasts were affected in both groups ($p<0.01$), showing no differences between them. The analysis of the apoptotic patterns reveals that 5-FU (150mg/kg) caused the maximum apoptotic indexes at 48hs (1.13± 0.09%, $p<0.01$), while the higher doses induced maximal injury at 24hs (5.84± 0.19, $p<0.01$). The expression of CD95 (APO-1/Fas) was strongly up-regulated from the 6th hour of the study ($p<0.01$). Correlating apoptosis with the CD95 expression showed a direct and significant correlation ($r= 0.8475$, $p=0.0079$).

These data suggest that 5-FU produces an acute reduction in the organ weight, cellularity, and viability and cell lineages within the first 48hs. Experimental data also suggest that 5-FU induces cell death through CD95 expression.

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Phytochemical and pharmacological studies of flowers decoction from *Chiliotrichium diffusum* (Asteraceae)

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In this work we studied phytochemistry and the cardiovascular effects of flowers from *Chiliotrichium diffusum*. The flowers were air-dried after its collection and powdered flowers were extracted by decoction. These extract was characterized by chemical reactions and by planar chromatography profiles of phenolic compounds. Antibacterial activity was analyzed by agar diffusion assays. For cardiovascular studies, urethane-chloralose anesthetized male Wistar rats were used. Aqueous extract was dissolved in saline solution for iv administration. The femoral artery was cannulated to register arterial pressure. Mean arterial pressure (MAP) and heart rate were calculated from the registers.

Anthocyanin pigments and condensed tannins were identified. 3,7,4'-trihydroxy-flavylium and apigeninidin were the principal compounds detected. In vitro screening no demonstrated antimicrobial activity. The decoction had a dose-dependent depressor effect (ΔMAP: 3 $mg.kg^{-1}$ -18±3 mmHg; 10 $mg.kg^{-1}$ -26±3 mmHg; 30 $mg.kg^{-1}$ -41±4 mmHg; $n = 5$) without cardiac effects. The depressor effect of 10 $mm.kg^{-1}$ was blocked by the β-adrenergic antagonist propranolol (ΔMAP: 1±3 mmHg, $n= 5$, $p<0,05$) and by atropine muscarinic blockade (ΔMAP: -10±3 mmHg, $n=5$, $p<0,05$). In conclusion, the flowers decoction shows a dual muscarnic and β-adrenergic depressor effect.

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CD 95 and Bax expressions are related to thymic apoptosis triggered by 5-Fluorouracil treatment

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5-Fluorouracil (5-FU) has shown to induce apoptosis mediated by CD95 (APO-1/Fas) in immune cells *in vitro,* but much less is known about these effects *in vivo*. The aim of this work was to evaluate CD95 and Bax expressions (immunoblotting), and their correlations with apoptosis in a time-course study on thymus recovery (0–10 days) using an *in vivo* murine model following a single dose of 5-FU (150 mg/kg i.p).

Data obtained show that 5-FU caused a reduction in the cellularities from the first day onwards ($p<0.01$) showing a direct and significant correlation with the thymic weight ($r=0.8545$, $p=0.0029$). Moreover, this hipocellularity correlates directly with the lymphocytic population ($r=0.6970$, $p=0.0306$). The highest apoptotic indexes were obtained between the first and the fifth day ($p<0.01$) returning to normal values on day 7 post 5-FU. Up regulation of the cell death receptor expression CD95, was noticed within the first five days ($p<0.01$). In addition, CD95 showed a direct and significant correlation with the apoptotic indexes ($r=0.8475$, $p=0.0079$). Analyses of Bax expression, revealed up-regulation of this pro-apoptotic preotein within the first five days. Furthermore, Bax and CD95 exhibit a direct and significant correlation in the acute period of 5-FU injury ($r=0.083$, $p=0.0154$). These results suggest that 5-FU, primarily induces apoptosis during the first five days through CD95 pathway, which is linked to Bax up-regulation.

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Skin-phototoxicity assay *in vivo* of anthraquinone-fraction from *Heterophyllaea pustulata*.

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Heterophyllaea pustulata, popularly known as "cegadera", is a phototoxicity specie which causes dermatitis and blindness (kerato-conjunctivitis) on the cattle. The animals suffer from the toxic symptoms when they consume different parts of this plant and they are exposed to sunlight. Therefore, this toxicity has been defined as a typical photosensitization by Hansen and Martiarena. We have demonstrated that the majority of the metabolites were anthraquinones (AQs) with photosensitizer activity.

The present study were undertaken to evaluate the effects of an anthraquinone-fraction (AQs-frac) on the skin of male mice.

The quali-quantitative composition of AQs-frac was determinated by HPLC. The AQs-frac administration in male Balb/c mice (20 g) was directly on the skin by subcutaneous via. The assay was achieved in the dark and under solar radiation in triplicate.

The AQs-frac produced photodermatitis and the AQs present in the original fraction were detected into the serum mice by HPLC.

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Influence of *boswellia carterii* extract on human neutrophil apoptosis and activation *in vitro*

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The gum resin of Boswellia species have been used as traditional medicine in the east countries and their main constituents has shown anti-inflammatory and apoptotic properties in different cellular lines. The aim of this study was to undertake a detailed characterization of the effects of *Boswellia carterii* extract (BC) on human neutrophils activity in vitro. Cytotoxic activity of BC (10-100µg/ml) was time and dose-dependent. Viability of PMNs was determinate monitoring the uptake of the vital mitochondrial dye MTT. The oxidative burst of PMNs induced by BC (10-200µg/ml.) was evaluated by reduction of NBT and inhibited by Diltiazem (76%), NEM (88%), chelerythrine (48%) and wortmannin (66%). These results show that the activation is calcium-dependant, the superoxide anion is generated for NADPHoxidase and PKC and PI3K are partiality involved in the PMNs activation.

The quantification of apoptotic cells was determined by flow cytometry using the technique of Nicoletti. We demonstrate that BC (100 µg/ml) induced dose-dependent apoptosis (69% vs. 20% of the control). DNA fragmentation was also evaluated by two different assays. (1) the diphenylamine method (53% ± 8.95% incubated 3hs with BC 100µg/ml) and (2) agarose gel electrophoresis showing the characteristic pattern for apoptosis. Our results suggest that the cytotoxic effect of *Boswellia carterii* extract is due to an apoptotic process.

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Dual function of Caspase-3 expression in hematopoietic cells post-5-fluorouracil treatment.

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Caspases, a cysteine proteases family, play a critical role during apoptosis. Moreover, it has been communicated it is crucial for hematopoietic development. However, is not fully understood the timing of caspases activation and its role in the bone marrow (BM) recovery post-chemotherapy. The aim of study was to elucidate caspasa-3 expression in BM cells and to correlate with BM cellularities, apoptotic indexes and proliferation, in a time-course study on murine haematopoietic recovery (0-20 days) following a single dose of 5-Fluorouracil (5-FU, 150 mg/kg). We performed haematological determinations in BM, Western blotting and DNA assays. We observed, on the 3th day post-5- FU, the maximal apoptotic index (6.13±0.73%p<0.01) and the minimal BM cellularity (27.60±0.25% under control p<0.01). Apoptosis returned to control values (1.5±0.47%) by the 7th day, while BM cellularities started to recovery from 10th day post-5FU onwards. Procaspasa-3 (32KDa) expression and its active form (caspasa-3 [17KDa]) showed a significant up regulation at 2nd days post 5-FU (p<0.01 over control). The proliferative indexes (^{3}[H]Thymidine assay) were significantly enhanced between 6th and 10th days concomitant with a new up expression of caspase-3. These results suggest that 5-FU induces a biphasic pattern of caspase-3 activation in BM cells, linked in the first days to apoptosis and it seems to be related to vital processes of proliferation and differentiation towards recovery.

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Heat Shock Effects in Murine Macrophages:Relationship between Fas and Hsp 60 expressions.

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Apoptosis is a major mode of cell death in response to injury. Expression of heat shock proteins (Hsp60) can cause death if the cellular defense mechanisms are insufficient. CD95 (Fas/Apo-1), pathways regulates several physiological and pathological processes. It was communicated that the death cell receptor shows up- regulation with temperature increment.

The aim of this work was to evaluate CD95 and Hsp60 expressions (immunoblotting), in a time- course study at different temperatures (37º– 40ºC). Peritoneal murine macrophages (PMM) suspensions were obtained 3 days post- thioglicolate injection by peritoneal washings with ClNa. These samples were incubated at 2, 4 and 6 hours at 37ºC (control group) and 40ºC (thermical shocked group). Results are expressed as the mean of four single assays from each mice lot. Data suggest that heat stress, rapidly (2 hs) sensitized PMM to induce Fas (p< 0.05) and Hsp60 (p<0.01) expressions. Up- regulations of CD95 and Hsp60, at 40ºC, were correlated with viability, showing a significant (p= 0.017/ (r) = -0.9821, p= 0.0233/(r) = -0.9767 respectively) and inverse correlation. These findings might help to the understanding of death receptor regulation upon stress, fever or inflammation.

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Erythropoietin Induced Delayed Apotosis by BCL-X_L and EPO-Receptor Expressions on Erythroid Bone Marrow Cells after Taxol Treatment

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Bcl-x_L, and Erythropoietin-Receptor (EPO-R) has been proposed to mediate the antiapoptotic action of erythropoietin (EPO) on erythroid progenitor cells.

We investigate in a time course study (1-10 days) Bcl-x_L and EPO-R induction on murine bone marrow (BM) cells after a single dose of Taxol (Tx) treatment (29 mg/Kg i.p). These expressions were compared to those evaluated after *"ex vivo"* EPO stimulation (2 UI/ml EPO for 2 h, 37ºC, 5%CO_2). Bcl-x_L and EPO-R expressions (westernblotting) were correlated with total erythroid cells (x10^{6}/ femur) and hemoglobin-synthesizing erythroblasts (%Fe^{59} uptake).On the 1st day post-Tx, BM erythroid cells fell 4 times compared to control (p<0.001), remained decreased until the 7th day (p<0.05) and returned almost to normality by day 10 post-Tx.

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

These results suggest that Bcl-x_L and EPO-R mediate the antiapoptotic effect of EPO following Tx-induced injury, and are involved in governing in vivo the erythroid cell fate.

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Participation of P-glycoprotein in the central pharmacokinetics of phenytoin in an experimental model of epilepsy.

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The present work examines the participation of the P-glycoprotein (P-gp) in the central pharmacokinetics of phenytoin (PHT) in an experimental model of epilepsy induced by 3-mercaptopropionic acid (MP). Seizures were induced in Wistar rats by injection of MP during 10 days. Control rats (C) were injected with saline solution (SS). A shunt or concentric microdialysis probe was inserted into the carotid artery or the hippocampus, respectively, in order to monitor PHT levels. PHT (30 $mg.kg^{-1}$, i.v.) was injected 30 min after administration of vehicle (V) or nimodipine (NIMO, 2 $mg.kg^{-1}$, ip). No differences were found in PHT plasma levels comparing all groups. In rats pretreated with V, hippocampal PHT levels were lower in MP rats (maximal concentration (Cmax): 2.7±0.3 µg/ml, $p<0.05$ vs C) compared to C animals (Cmax: 5.3±0.9 µg/ml). Whilst pretreatment with NIMO did not modify central kinetics of PHT in C (Cmax: 4.5±0.8 µg/ml), PHT levels were significantly higher in MP rats pretreated with NIMO (Cmax: 6.8±1.0 µg/ml, $p<0.05$ vs MP rats pretreated with SS). Our results indicate that centralkinetics of PHT is altered in MP induced epileptic refractory rats. The effect of NIMO on hippocampal levels of PHT suggest that P-gp is implicated in the reduced central bioavailability of PHT in this experimental model of epilepsy.

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***In vivo* hematoxicity profile induced by 5-fluorouracilo.**

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5-fuorouracilo (5-FU), an antimetabolite drug widely used, is dose-related hematotoxic in antineoplastic treatment and it has been associated to hematopoietic progenitors and blood cells reduction. We wanted to characterize in detail the *in vivo* hematotoxicity of two single doses of 5-FU (150/200mg/Kg ip) in a time-course study (0-20days) following the recovery of the hematological parameters using CF1 mice. We determined the peripheral values of blood cells, Hb, Hct, hematimetric indexs, and bone marrow (BM) viability, apoptosis, mitosis and differential cellularities. Data show that 5-FU caused maximal reduction in BM viability at day 1, cellularity and mitosis at day 2, while apoptosis increased from days 1 to 7 ($p<0.01$). In the firsts 5 days, a significant decreases ($p<0.01$) were seen in erythrocytes, reticulocytes, hematocrit, haemoglobinic indexes and marrow erythroid cells at both 5-FU doses. A maximal depression for myeloid-leucocyte parameters and megakaryocites-platelets levels, were observed from days 2 to 4 ($p<0.01$). The recovery was noticed at day 7 in erythroid index, while at 10^{th} day for the others blood lines. All hematological parameters were restored to control values between 15^{th} and 20^{th} days (150 or 200mg/Kg respectively). It is conclude that the administration of 5-FU induces BM doses-related pancytopenia and anemia with reticulocytopenia, leucopenia and thrombocytopenia in the immediate post-dosing period.

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Cytotoxic activity *in vitro* of natural anthraquinones

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From *Heterophyllaea pustulata* (Rubiaceae) nine anthraquinones (AQ) with photosensitizing properties were isolated. Since several natural photosensitizers have demonstrated potential application in therapeutic, we have initiated the biological effects study of these natural AQs. In a preliminary study, we have demonstrated the antibacterial and antifungal activity "*in vitro*" of different AQ-extracts of *H. pustulata* as well as their low acute toxicity "*in vivo*".

The objective of this work was to examine the cytotoxicity of these AQs with the aim to determine the non cytotoxic concentrations interval, because their future utilization in experimental assays as potential antibacterial, antiviral and antiparasiticals agents.

The cytotoxicity was measured in Vero cell line using the neutral red uptake assay (NR) for survival cell. AQs dissolved in MEM were tested from 1 µg/ml to 50 µg/ml. From the plot survival cell percentage *vs.* AQ concentrations, we determinate the CC_{50} and CC_{80} (50% and 80% cytotoxic concentration) for each AQ. The CC_{80} values of AQs were among 7 – 25 µg/ml.

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Determination of hypothalamic levels of monoamines in male and female rats exposed pre and postnatally to 2,4-dichlorophenoxyacetic acid (2,4-d)

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Continuing our previous studies on herbicide 2,4-D reproductive and developmental toxicity studies, hypothalamic levels of monoamines and their metabolites were measured. Virgin female 90 day-old Wistar rats were made pregnant, and the treated group was exposed to 2,4-D (70 mg/kg/day, sprayed on food) from gestation day 16 onward. On postnatal day 23, pups were weaned and the treated group continued to be fed with 2,4-D until sacrifice by decapitation at 45 or 90 days of age.

Brains were dissected and the hypothalami separated and weighed. They were homogenized by sonication in 0,1M perchloric acid and centrifuged. Noreprinephrine (NE), dopamine (DA), 3,4-dihydroxyphenyl-acetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were measured in the supernatants by HPLC.

At 45 days of age, we observed significant decreases in DA, DOPAC, NE and HVA levels in the treated females. At 90 days of age, only NE level showed a significant decrease in the treated males.Results show that 2,4-D alters the hypothalamic levels of monoamine in different manners at different ages. These alterations could modify the development of hormonal systems associated with sexual behavior in adult animals.

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Ethanol induced neurodegeneration in bed nucleus of stria terminalis in early postnatal period.

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Studies on the effects of ethanol in embrionic and early postnatal periods, described disfunctions in brain development as in proliferation, migration, diferentiation, synaptogenesis, and alteration in the developmental apoptosis. Our interest is study the ontogeny of neurons of the Extended Amygdala of Temporal Lobe, nerve growth factors and pro/antiapoptotic mediators expression, in normal rats and in models of neurodegeneration induced by ethanol, to analize the neurobiological bases of alterations induced by the drug. Methods: Male rats 7 and 15 postnatal (pn) days were used. Control Group: ClNa 0.9% sc. Experimental groups: Ethanol 20% in saline, sc. 2.5 g/kg at 0 hour, and 2.5 g/kg two hours after. The Amino-Cupro-Argentic technique (De Olmos et al 1994) was used to reveal neurodegeneration. Cells were counted through a microscope with a LEICA DC 200 camera and KS Lite v2.00 program for statistic analysis. Results: neural death was detected 2 hours after ethanol, increasing at 8 and 24 hours in the Lateral Nucleus of Stria Terminalis of Central Extended Amygdala, showing its vulnerability to ethanol in this model to study neuroanatomical and behavioral alcoholic neuropatology in early postnatal periods.

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Increased PKC activity in frontal cerebral cortex of stressed neonatal rats. Scolari MJ & Acosta GB. Instituto de Investigaciones Farmacológicas. Junín 956. 5° piso. C1113AAD. Buenos Aires. Argentina. E-mail: scolarimariano@yahoo.com.ar

In the last years, we demonstrated that the postnatal acute stress modified the neuronal responses through changes in aminoacidergic transporters activity, this fact, appears to be associated with neurobiological alterations. The aim of this study was to investigate if the alterations induced by postnatal stress in rat developing brain is correlated with changes in PKC activity which is proposed as a molecular transporter regulator. Experiments were performed with cerebral frontal cortex (CF) of rats in different postnatal days up to adulthood. Neonates were stressed by cold exposure during 1 hour at 4ºC. Unhandled neonates, left undisturbed in their home cages, served as control. Upon termination of cold exposure, neonates were killed by decapitation. The brain was removed and CF was dissected. PKC activity was assayed on both cytosolic and membrane preparations by measuring the incorporation of ^{32}P to a peptidic substrate (histone). We found significant increase in the stressed animals compared with control groups at 5 ($p< 0.01$), 7 ($p< 0.05$) and 13 ($p< 0.01$) postnatal days (PD). No difference in subcellular distribution of PKC was observed at 21PD and adult stage. These results suggest that PKC activation is related to single acute cold exposure during postnatal development.

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Endothelial dysfunction in hypercholesterolemia induces release of vasoconstrictor arachidonic acid metabolites.

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The aim of this work was to study the influence of arachidonic acid metabolites on the endothelial dysfunction observed in aorta of hypercholesterolemic rabbits. Rabbits were feed with either normal rabbit chow (CD) or a diet cointaining 1% cholesterol for 6 weeks (HD). Thoracic aorta was excised. Rings were cut and mounted in a organ bath to measure NO basal production with Griess reagent and to register isometric contractions. Aortic rings were contracted with noradrenaline and then exposed to increasing doses of acetylcholine (Ach) or sodium nitroprusside (NP) to construct one cumulative dose-response curve (CDRC) in absence or presence of indomethacin or tempol. After washing, indomethacin or 17-ODYA was added 30 min before one CDRC to angiotensin II (Ang II). Transmembrane potential (Pm) was recorded before and after 10 min of Ang II stimulation in absence or presence of indomethacin or 17-ODYA. Results: relaxations induced by Ach were lower in HC rabbits. This effect was blocked by indomethacin but not tempol. NP-relaxations were the same in all cases. NO-release was lower in HD rabbits. The Ang II reactivity-improvement was abolished by indomethacin and 17-ODYA. Ang II stimulation induced depolarization in HD rabbit aorta. This effect was reversed by 17-ODYA but not indomethacin. These results demonstrate that high cholesterol diet would diminish NO production and would increase the release of vasoconstrictor arachidonic acid metabolites that sensitize smooth muscle to Ang II through increasing 20-HETE synthesis.

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Chiral inversion of r-(-) fenoprofen in cats with toxic hepatic disease induced by carbon tetrachloride and acetaminophen.

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Non-steroideal-antiinflammatory-drugs (NSAIDs) are compounds widely used in humans being and animals to treat inflammation. A subgroup of NSAIDs, the 2-aryl-propionic-acids (2-APA) or Profens undergo a chiral inversion (CH.I.) process which enzimatically converts the inactive R-(-) enantiomer into its therapeutically active form, the S-(+) enantiomer.The liver is the principal site for CH.I. A severe hepatic disease should alter the percentage of CH.I. obtained for R-(-) FPF. To test this hypothesis we study the CH.I. of R-(-) FPF in cats with toxic hepatic disease induced either carbon tethrachloride (CCL_4) or acetaminophen (AMF). In the case of CCL_4 the percentage of CH.I. was 90.5 ± 21.1 and the difference with healthy animals was not statistically significant. It was not possible to successfully induce an hepatic toxic disease with AMF. This results suggests an important extrahepatic contribution to the CH.I. process.

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Dissociation of the core and shell sub-regions of the nucleus accumbens in the control of the fixed interval schedule

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In this study we sought to investigate the importance of the nucleus accumbens core (NACc) and NAC shell (NACs), in the regulation of the fixed interval (FI) schedule of reinforcement by microinfusing DA, MK-801, and AMPA in both regions. We also examined the role of the projections from dorsal subiculum (dSub) to NACc, and ventral Sub (vSub) to NACs. Lidocaine (Lido) administered into Sub and the contralateral projection region was used to inactivate these circuits. Intra-core DA administration increased and intra-shell decreased overall rates. On the other hand, the effects of the injected glutamatergic drugs were undistinguished in core versus shell with MK-801 increasing and AMPA decreasing rates, suggesting that both NMDA and AMPA receptor stimulation blunted rates in both areas by different mechanisms. Furthermore, Lido inactivation of dSub/NACc, but not vSub/NACs, reduced FI rates by decreasing run rates. Lido inactivation coupled with injections of MK-801 or AMPA suggests that MK-801, but not AMPA administration into the contralateral subiculum restored and even increased FI response rates by modifying post-reinforcement pause time. These findings reveal the differential involvement of the NAC subregions in DA-mediated maintenance of FI performance and confirm critical roles for NMDA-mediated subicular projections to NACc.

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Anti-inflammatory activity of berenjenol, new 24 methyl cycloartane isolated from *oxandra cf xylopioides* (annonaceae).

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A new triterpenoid derived from 24-methyllanostane was identified in the leaves of *Oxandra cf. xylopioides*. The unusual structure of the new compound was assigned as **1**, for which the name berenjenol is proposed. The leaves also afforded the known monoterpene isoespintanol (**2**). The anti-inflammatory activity of these compounds has been studied both in vitro and in vivo. For the in vivo model, we chose the model of acute inflammation induced by injection of carrageenan in the paws of mice. For the in vitro studies, we monitored the release of nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) in macrophages RAW 264.7 stimulated with LPS.

Compounds **1** and **2** significantly reduced the paw edema induced by carrageenan at 125 mg/kg, but whereas **1** has clear effect at 1 h, 3 h and 5 h, reducing the paw edema by 44%, 64% and 51%, respectively, **2** only had significant effect at 3 h, reducing the edema by 43%. These compounds did not show any toxicity against RAW 264.7 macrophages at 100 μM. Only **2** was active in the in vitro assays and decreased the IL-1β production by 72% at 100 μM. Moreover, it reduced the IL-1β mRNA synthesis.

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Timed changes of Synaptic Zinc in the Bed Nucleus of Medial Extended Amygdala in the Kainic acid model of Epilepsy are suggestive of reactive neuroplasticity.

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Repeated seizures induce permanent alterations of the brain in experimental models and patients with intractable temporal lobe epilepsy (TLE). 15% of brain zinc is in presynaptic vesicles containing glutamate, and is co-released with it, increasing its effects on postsynaptic excitatory neuroreceptors. The changes in Zinc density in the Bed Nucleus of Medial Extended Amygdala (BSTM), induced by Kainic Acid as a model of TLE was studied. Adult male rats (n=4 per group) 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 Zinc, and analysed by densitometry. Images were captured with a Zeiss microscope and a Leica videocamera, using the KS Lite v2.00 program to determine the grey value difference between control and experimental animals. Student t test was used for statistics, with a $p < 0.05$ as a significance limit.
Results: normal dark staining was seen in BSTM sections of control animals. At 10 days post KA inj. a dramatic loss of staining was observed. A slow but steady recovery of Zinc density can be followed in the 4 months period studied. We found significative loss of synaptic Zinc in from 10 days to 1 month exp animals, not observed in the 2 to 4 months animals. This indicates an acute loss of synaptic Zinc in status epilepticus and a chronic neuroplasticity process of recovery through sprouting in a 4 month period post KA induced TLE.

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Environmental factors appear to be non-genetic risks in the progression of type I diabetes. In particular, stressful life events have been seen linked to the onset of diabetes in humans. However the mechanisms involved are not yet well understood. Here, we studied the effect of chronic mild stress exposition (CMS) on the development and evolution of STZ type I induced diabetes in Balb/c mice. This experimental model of diabetes arrives to mild hyperglycemia allowing a good survival. The results indicate that CMS exposition by six weeks previous STZ administration result in a significant delay in the onset of hyperglycemia compared to matched diabetic controls. On contrary, CMS exposition after diabetic induction conduce to a late significantly higher levels of glucose than those observed in non exposed animals. Glucose levels were correlated with glycosilated hemoglobin. Hyperactivation of hypothalamo- pituitary-adrenal (HPA) axis estimated by determination of serum level of corticosterone, a hyperglycemic hormone, was observed in diabetic animals. Corticosterone levels showed an early significant variation according the time when chronic stress was applied. This levels were lower when animals were exposed previously and higher when animals were exposed after diabetes induction. This findings indicate that stress influence the onset and evolution of diabetic disease having an important participation the HPA axis.

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Role of the cannabinoid system in the effects induced by nicotine on anxiety-like behaviour in mice

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The present study was designed to examine the possible involvement of the cannabinoid system in the anxiolytic- and anxiogenic-like responses induced by NIC in mice. Animals were only exposed once to nicotine. The acute administration of low (0.05, sc) or high (0.8 mg/kg, sc) doses of NIC produced opposite effects in the elevated plus-maze, i.e., anxiolytic- and anxiogenic-like responses, respectively. The effects of the pretreatment with the CB1 cannabinoid receptor antagonist, rimonabant (0.25, 0.5 and 1 mg/kg, ip), and the cannabinoid agonist, Δ9-tetrahydrocannabinol (Δ9-THC) (0.1 mg/kg, ip), were evaluated on the anxiolytic- and anxiogenic-like responses induced by NIC. Rimonabant completely abolished NIC-induced anxiolytic-like effects and increased the anxiogenic-like responses of NIC, suggesting an involvement of CB1 receptors in these behavioural responses. On the other hand, Δ9-THC failed to modify NIC anxiolytic-like responses, but attenuated its anxiogenic-like effects. In addition the association of non-effective doses of Δ9-THC and NIC produced clear anxiolytic-like responses. These results demonstrate that the endogenous cannabinoid system is involved in the regulation of NIC anxiety-like behaviour in mice, and provide new findings to support the use of cannabinoid antagonists in the treatment of tobacco addiction.

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Responses, but not motivational manifestations of 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. Previous studies in rodents have shown that NIC modifies locomotion, anxiety, nociception learning and memory. Repeated nicotine administration produces several behavioural responses in animals closely related to its addictive properties, such as reinforcing effects and physical dependence. The aim of the present study was to evaluate the possible role of $GABA_B$ receptor in responses induced by acute and repeated NIC administration by using CD1 mice. Acute NIC (3 mg/kg, sc) administration decreased locomotor activity and induced antinociceptive responses in the tail-immersion and the hot-plate test. Baclofen (BAC, $GABA_B$ receptor agonist) (2 mg/kg, i.p.) increased ($p<0.05$) the hypolocomotion induced by NIC and decreased ($p<0.05$) the antinociceptive effects in the hot-plate test. Finally, BAC administration did not modify the aversive motivational state associated to naloxone precipitated NIC withdrawal evaluated by the place aversion conditioning paradigm. These results demonstrated that some acute effects elicited by NIC, but not the aversive motivational consequences of NIC withdrawal can be modulated by the endogenous GABAergic system.

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AT_1 receptors blockade attenuate the behavioral and neurochemical sensitization to amphetamine.

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It is known that psychostimulants induce behavioral sensitization, a neuroadaptation that implicates cellular and molecular changes in the dopaminergic and glutamatergic systems. The evidences support that brain Angiotensin II interacts with dopaminergic neurotransmission through AT_1 receptors in striatum and substantia nigra. Male rats (250-300 g) were treated during 5 days with candesartan cilexetil (CV, 3mg/kg v.o.), an AT_1 antagonist, and 24h later received one injection of 5 mg/kg amphetamine (Amph). The animals were challenged one or three weeks later with 0.5 mg/kg of Amph and the locomotor activity registered during 2 hours. Other group of animals without the Amph challenge were sacrificed and the striatum was dissected for 3H-DA release induced by K^+(28mM) and amphetamine (10^{-5}M) by *in vitro* superfussion. An additional group treated with CV were implanted with microdialysis probe in striatum and DA release induced by Amph was quantified by HPLC. We confirmed that locomotor sensitization induced by Amph was higher after 3 weeks of 5 mg/kg Amph injection, and found that this effect was attenuated by previous blockade of the AT_1 receptor. The antagonist treatment induced a slightly increase in the locomotor activity when tested 3 weeks later. CV attenuated the increase in 3H-DA release induced by K^+ in Amph treated group although CV by itself increased it. The data are discussed according to the long-term effects induced by the psychostimulants and AT_1 blockers. Additional experiments are needed to clarify the molecular mechanism involved in the brain DA-angiotensin II interaction.

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SSelective cytotoxicity of Dehydroleucodine on human MDA-MB231 tumor cells and normal lymphocytes

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The sesquiterpene lactone Dehydroleucodine (DhL) is the active principle of *Artemisia douglasiana* Besser, popularly known as "matico". This drug has cytoprotective action in gastrointestinal normal cells but its effect on tumor cells is yet unknown. The aim of this study was investigate whether DhL affects the viability of human mammary tumor cells (MDA-MB-231) and normal lymphocytes. MDA-MB-231 cells were cultured in DMEM (3 independent assays) and Lymphocytes (obtained from 4 donors by venous puncture) were resuspended in RPMI 1640. Cells were exposed to DhL (0, 5, 25, 50 and 100µg/ml) for 15 or 60 minutes. Cell viability was evaluated by trypan blue exclusion test at 0, 4, 8 and 24 hours after DhL addition. Cell viability of MDA-MB-231 cells and lymphocytes was not affected with 15 min of DhL exposure. Viability of tumor cells was significantly decreased with 60 min of DhL exposure; 25µg/ml DhL affected the viability 24h after drug administration *($p< 0.05$)* and 50µg/ml DhL affected the viability 8h and 24h after treatment *($p< 0.05$)*. Surprisingly, no tumor cells were observed in the plates treated with 100µg/ml DhL since the 4h after treatment. Drug administration during 60 min did not affect lymphocytes cell viability. These results suggest that DhL has a strong cytotoxic effect on tumor cells but not on normal lymphocytes at the concentration assayed. *This work was supported by CONICET (PIP 5952)*

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Cardiac basal metabolism: efects of clonazepam and nifedipine on the energetic response to calcium removal.

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In arterially perfused adult rat heart and in the presence of the cardioplegic agent 2,3 butanodione monooxime (BDM), we found that calcium removal (0Ca) from the perfusion media induced an steady increase in basal metabolism (evaluated as resting heat production (Hr)) that was associated to a sodium (Na) influx from extracellular space (SAB, 2005). The increased intracellular Na concentration (Na_i) would activate Na_i-dependent processes such as the NaxK pump and Na_i dependent mitochondrial Ca cycling that would account for the observed change in resting energy cost. Therefore, we studied whether mitochondrial Ca cycling participates in the energetic response to Ca withdrawal. Futhermore, we also tested whether sarcolemmal Ca channels are involved in the Na influx to the cell. Ca removal incresed Hr ($+4.4 \pm 0.4$ mW/gdt, $p<0.05$). Once the steady resting heat rate was achieved in 0Ca media, muscles were perfused with either the mitochondrial NaxCa exchange inhibitor clonazepam (CLO) 10 μM or nifedipine (NIF) 3 μM. Both, CLO and NIF decreased Hr (-1.3 ± 0.2 and $-1,4 \pm 0.4$ mW/gdt, for CLO and NIF respectively, $p<0.05$). The results suggest: a) a contribution of the Na_i-dependent mitochondrial Ca cycling to the energy expenditure in 0Ca, and b) Na entry through L type Ca channels would be at least in part responsible for the increase in Na_i and hence the energy cost of cardiac resting state when Ca is removed. CONICET-PIP02544 and UBACYT O023.

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Thyroid status influence lymphocyte activity via protein kinase C (PKC) isoenzyme modulation.

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Evidences pointing to bidirectional regulation between thyroid axis and the immune system were described. Previously we showed that T and B cells from hyperthyroid (HT) mice upon stimulation with mitogens showed higher stimulation indexes than control cells, while the contrary occurred with hypothyroid (ht) lymphocytes. To characterize the biochemical mechanisms involved in these effects, PKC activity upon mitogen stimulation and the expression of crucial PKC isoenzyme that participate in T (α, β and θ) and B (α, β, δ and ζ) lymphocyte activation were evaluated. PKC total activity and mitogen-induced PKC traslocation was increased in HT, but decreased in ht lymphocytes. This was accompanied by an increment of α, β and θ isoforms in T cells, but only β in B cells from HT mice. In ht T lymphocytes a decrease in β isoenzyme was found, while in B cells diminished expression of β and ζ isoforms were observed. Moreover, spleen cells from HT and ht mice stimulated in vitro with a T selective mitogen displayed a higher or lower IL-2 and IFN-γ secretion respectively, when compared to control euthyroid cells. No differences were found for IL-6 levels. Results indicate that thyroid status modify lymphocyte activity through PKC isoenzyme regulation, thus leading to the modulation of lymphoid cell proliferation and of important cytokines related to cellular immunity

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Qercetin inhibitory effect on metalloproteinases activity and synthesis

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Flavonoids are phenolic plant constituents with a broad range of biological activities. Quercetin is the major dietary flavonoid found in fruits and vegetables. It has anti-inflammatory, anti-oxidant and anti-tumoral properties but the mechanisms through which quercetin exerts their biological actions are not fully understood. Metalloproteinases (MMPs) are endopeptidases involved in tissue remodeling by degradation of the extracellular matrix (ECM). MMP-9 plays an important role in cell migration and extravasation. The activity and synthesis of MMP-9 are regulated at several levels and its production can be induced by growth factors. The exacerbation of MMP-9 activity and synthesis leads to an untimely and accelerated turnover of ECM, which is observed in pathological conditions such as acute and chronic inflammation, vascular diseases and cancer. Thus, inhibition of MMP-9 activity and synthesis may conduce to an alternative therapy in these pathologies. In the present study we investigate the inhibitory effect of quercetin on MMP-9 proteolytic activity using zymographic assays. Quercetin inhibits pro-MMP-9 and active MMP-9 activity in a dose and time dependent manner. We also evaluate the effect of quercetin in the induction of MMP-9 production showing that quercetin blocks MMP-9 synthesis. Our results demonstrate that quercetin inhibits MMP-9 activity and synthesis. Thus, quercetin constitutes a potential therapeutic agent with anti-inflammatory and anti-tumoral properties.

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Mechanisms that mediate the antidepressant effect of enriched environment in rats exposed to inescapable stress

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The objectives of this work were to investigate the effect of an enriched environment (EE) on the helplessness behavior (LH) and on a parameter that we have found altered in this model of depression: the decrease in the immunostaining of the light subunit of the intermediate neurofilament (NFL) in the hippocampus. We also tested if EE induces neurogenesis in animals exposed to stressful conditions.

Male rats in which LH behavior was induced, were kept for 21 days in a special cage with different devices: tunnels, ropes, a running wheel, etc (LH-EE rats). LH animals not kept in the EE (LH) were the control group. Depressive-like behavior (escape latency) was measured in an avoidance task, NFL was evaluated by immunohystochemistry, and the number of neurons that co-expressed BrdU (injected the first three days, 50mg/kg)/ Tuj-1 (an immature and mature neuronal marker) were quantified by double-immunofluorescence and confocal microscopy.

LH-EE rats showed a reversion of the depressive-like behavior and an increment in NFL immunostaining. In addition, LH animals showed higher BrdU+ cells than LH-EE but no differences in the percentage of BrdU/Tuj-1+ cells were observed. Therefore, the EE reverses the depressive-like behavior as well as the NFL reduction but neurogenesis seems not to be implicated in these phenomena. *This work was supported by* ANPCyT (PICT-05-11102),UBA (M013) and CONICET (PIP *5870).*

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Anti-inflammatory activity of *Acmella decumbens*

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Acmella decumbens (Sm) R.K.Jansen (ex *Spilanthes decumbens* (Smith) A.H. Moore) (Asteraceae), is a species which roots have been used in ethnopharmacology as an odontalgic agent. In previous studies we demonstrated the analgesic properties of the hexane extract of *A. decumbens* roots in rats.

In the present work the evaluation *in vitro* of anti-inflamatory activity of the hexane extract of *A. decumbens* is reported, evaluated by using two different methods:
i) inhibitory action of metaloproteinases (MMPs)
ii) inhibitory action of nitric oxide (NO) production.

The hexane extract showed inhibition of MMPs and NO production at 40 µg/ml.

The obtained results lead us to conclude that this plant extract exerts anti-inflammatory properties. In subsequent studies the anti-inflammatory activity of each compound isolated from the hexane extract will be evaluated.

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Alterations in behavioral learning due to prenatal stress in rats.

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The effect of stress during early development stages induces both immediate and latter alterations that are reflected through changes in the neurochemical, cognitive and behavioral systems. Experiments performed in animals have shown that maternal prenatal stress affects their offspring. However, the intracellular mechanisms involved in these alterations have not been fully elucidated.

The aim of the present work was to evaluate behavioural and neurochemical changes induced by prenatal stress (PS) in different regions of the offspring´s brain, such as cerebellum and hippocampus. The PS procedure was performed every day from day 11 of pregnancy until delivery.

Our results show behavioural alterations in PS animals respect to control animals, evaluated by radial-maze test [reduced locomotion and an increase number of errors in PS rats at 30, 60 and 90 days of age] by passive avoidance conditioning task PS rats showed lower latency. The activity of constitutive nitric oxide synthase (NOS) was increased in the cerebellum but not in hippocampus of PS animals at 15 and 30 days of age. These results demonstrate that early changes in NOS activity induced by prenatal stress might be involved in long term learning deficits, evidenced as a pourer performance in behavioral tasks

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Improvement anti-inflammatory activity

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Flavonoids are polyphenolic compounds found in the plant kingdom with a wide range of biological activities. Our investigation is focalized in its potentiality as anti-inflammatory agent. The biological activity was evaluated by inhibition of nitric oxide (NO), a citotoxic mediator in inflammatory processes. In previous works we reported the results concerning of NO inhibition by quercetin; in the present study we investigate a quercetin tetraacethyl derivate (TAQt) in order to analyzed the structural variation as an activity improvement.
Macrophages cellular lines were used and the LPS-induced NO production was measured *in vitro* by a colorimetric method described by Griess, cultived in presence of different concentration of TAQt.
The results demonstrate that structural variation improves the biological activity showing a IC_{50} = 15.2 µM for TAQt while Qt showed a IC_{50} = 62.8 µM.
This effect leads us to confirm that structural variation improves the anti-inflammatory activity in this kind of compounds.

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Therapeutic drug monitoring of lopinavir/ritonavir in pediatric patients

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The aim of this work is to describe the results of therapeutic drug monitoring (TDM) in pediatric HIV – infected patients who are being treated with lopinavir/ ritonavir (LPV/r) containing regimens. Twenty five patients were included in this work. Fifty three lopinavir plasma levels were determined by HPLC. The average LPV dosage used was 19 mg/kg/day. Age range: 8 months to 19 years old.
Only 3 of the patients yielded subtherapeutic levels (through < 1 µg/mL), although these reference levels were determined for adult patients. In two of these patients, dosage was increased by 50%, and a new TDM was performed, leading to levels inside the therapeutic range. Mean concentration between 1.5-2.0 h after drug administration was 5.82 ug/ml (SD: 3.42 ug/ml). The mean concentration of a second peak level obtained between 2.5-3.0 hs was 8.7 ug/ml (SD: 2.94 ug/ml).
Although in previous works with other protease inhibitors we showed that a high percentage of trough levels were below the therapeutic range (75% for nelfinavir, 55% for indinavir/r). the less variability observed with lopinavir/r could be atributted, in part, to the presence of ritonavir in the same formulation, since it could assure the “booster” effect of ritonavir that is absorbed simultaneously with lopinavir.

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Evaluation of a ^{32}P patch designed for the treatment of skin diseases.

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Objective: To design and evaluate a 32P patch for the treatment of skin diseases. Materials and methods: the patch was prepared from [32P]-chromic phosphate and silicone. The (a) activity concentration, (b) homogeneity (c) integrity, (d) therapeutic efficacy for two treatment schemes in an animal model of skin cancer, (e) bioelimination and biodistribution in healthy and treated animals and (f) dosimetry to plan the treatment schemes were determined. Results: The 32P patch demonstrated homogeneity of activity and dose. On the other hand, it showed integrity under degradation conditions like the ones in a treatment. According to the bioelimination and biodistribution studies, no leakage of 32P from the patch was observed. The treated tumors reduced their mean diameter compared to controls. The single dose therapeutic scheme showed higher percentage of complete and partial remissions compared to the fractionated scheme. These results were confirmed by the histopathological analysis of the samples. Conclusion: The 32P patch was designed and produced according to specifications for the treatment of superficial lesions of the skin. Although the 32P patch is an open source, it behaves like a sealed one for its use in brachytherapy treatments.

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Evaluation of a ^{32}P-patch (Silibraq®) in the management of keloids which have recurred after several previous treatments: a case report.

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Keloids are the result of excessive fibroblast proliferation and then over-abundant collagen deposition. There is no method able to guarantee absolute success in the therapeutic approach to keloids. Our case report involves a female patient with six lesions treated with Silibraq®. Pre-treatment and adjuvant treatment of the lesions were performed with Thiomucase, 5-fluoruracile, procaine and triamcinolone. Taking into account the activity contained in each of the patches and the total radiation dose to administer according to clinical practice, dosimetric calculations were done for each lesion. Total remission was achieved in three treated lesions. The other lesions did not achieved total remission yet, but their sizes are diminishing. The differences observed in treatment outcome may be related with lesion features (extensive lesions, resistant, relapsing, etc), adjuvant treatments and/or treatment schedule

Modalidad presentación: POSTER
Trabajo presentado para optar por el premio SAFE.

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Sauroxine and sauroine effect on the acetylcholinesterase activity

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Previous investigation on *Huperzia saururus* plant species showed that its main constituents are alkaloids and the quali-quantitative composition was determined. Besides, the purified alkaloid extract (AE) was assayed and it showed a marked acetilcholinesterase (AchE) inhibitory effect. In relation whit this activity we also tested AE activity on the hippocampal long term potentiation (LTP) generation, and we have obtained positive results.

The present study had the aim to identify which of the compounds is/are responsible for the AE inhibitory action on AchE. Thus, we tested sauroxine and sauroine, two of the majority compounds of the AE by using an *in vitro* colorimetric method described by Ellman

Results show that sauroxine develops an inhibitory effect on AchE. It was evaluated in seven different concentrations showing a IC_{50}= 12 µg/mL On the other hand, sauroine did not produce inhibition on the enzyme at any concentration, not even at 500 µg/mL.

Further studies will make possible the identification of other responsible for the inhibitory activity of AE.

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Sauroxine inhibits the hippocampal long term potentiation (LTP)

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The alkaloid extract (AE) of *Huperzia saururus* is constituted by a mixture of alkaloids, three of which occur as a majority: sauroine, sauroxine and 6-OH-lycopodine. In previous investigations we demonstrated that AE shows a facilitating effect on the LTP generation, as well as it was proved for sauroine, so this alkaloids it is one of the responsible for the AE activity. On the contrary, we reported that sauroxine inhibits the LTP phenomena at a concentration of 1 µg/mL. Literature references indicate that there are compounds that inhibit the LTP generation at certain concentrations whereas at lesser ones they produce facilitation.

In view of these antecedents we evaluate sauroxine at a lesser concentration (0,5 µg/mL). Results showed that at this dose the alkaloid is still inhibiting the LTP. Controls showed an average threshold of 75±11.0 Hz, whereas when it was perfused with sauroxine not LTP generation was achieved, even stimulating until 200 Hz.

Further experiments at other doses will be assayed in order to determine whether sauroxine has a dual effect or definitely produce an inhibition on the LTP generation.

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Effect of sauroine on memory retention

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Our previous *in vivo* experiments imdicated that the alkaloid extract of *Huperzia saururus* facilitates long term memory retention. The main alkaloid present in this extract is sauroine, for which we have also demonstrated that reduces the threshold of the hippocampal long term potentiation generation. In further experiments *in vivo,* this alkaloid showed to modify memory retention at a concentration of 5 µg/mL.

According to these antecedents we decide to assay other concentrations of sauroine in order to determine the minimum concentration at which the alkaloid is active. Thus, the present study reports on the step-down test developed with 0.5 µg/mL and 1 µg/mL. Male rats of Wistar strain were used and the administered volume was 1 µL by intrahippocampal via.

The latency times (t) observed for animals treated with sauroine were 25,00±3,22 seg and 83,46±2,60 seg for 0.5 and 1 µg/mL, respectively whereas t for control animals was 15,00±2,38 seg. This results confirm the facilitation of memory retention by sauroine from 1 µg/mL onwards.

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Modulation of the intestinal P-glycoprotein activity on the ivermectin absorption is affected by the administration route in sheep

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Ivermectin (IVM), a broad-spectrum antiparasitic drug used in veterinary and human medicine, has been shown to be a substrate of the drug transport P-glycoprotein (P-gp). The aim of the study was to assess the comparative impact of the itraconazole (ITZ)- mediated modulation of the intestinal P-gp activity on the kinetic behaviour of IVM administered by the intravenous (IV) and intraruminal (IR) routes to sheep. Adult female Corriedale sheep were allocated into four groups. Animals (n=20) received IVM (50µg/Kg) by IV route either alone (Group A) or co-administered with ITZ (100 mg, 3 doses, orally) (Group B). Animals in Groups C and D (n=24) were IR-treated with IVM alone or with ITZ, respectively. Plasma was collected up to 15 days post-treatment. Twelve (12) IVM IR-treated sheep, with and without ITZ, were sacrificed to collect the mucosal tissue and luminal content of different gastrointestinal tract sections. IVM concentrations were measured by HPLC. The plasma disposition kinetics of IVM given IV was unaffected by the presence of ITZ. However, the IR administration of IVM+ITZ resulted in markedly higher IVM bioavailability compared to the IVM alone treatment. Likewise, the IVM concentration was enhance in different gastrointestinal mucosal tissues in the presence of the P-gp modulator agent.

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Obestatin, a peptide derived from the same prohormone that Ghrelin, opposes Ghrelin´s effects.

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Ghrelin (GR), a circulating appetite-inducing hormone, is derived from a prohormone by posttranslational processing. Another peptide, named obestatin (O) also derived from proghrelin has been recently isolated. In a previous work we have demonstrated that GR induces anxiety-like behavior and increases memory retention. In the present series of experiments the effects of icv O administration (0.3 and 3nmol/rat) upon memory retention, anxiety-like behavior and food intake were studied, using step down (SDT), object recognition test (ORT) and elevated plus maze test (EPMT). O icv injection exerts an anxiolytic effect as suggest the increased % of entries (61.7 ± 2.7 vs. 33.3 ± 1.5) and time spent in the open arms in EPMT (72.1 ± 4.2 vs. 25.8 ± 1.7) for the O 3nmol/ rat vs. control respectively. The O injection during the light or dark period resulted in a decrease on food intake during the periods 0-1, 1-2 and 4-24 h after administration. The rats also exhibit an increased performance for memory retention. O increases in a dose dependent manner the latency time in the SDT (O 3nmol/ rat 115 vs. Ctrl 24) and increases the % of time of exploring novel object (ORT) (O 3 nmol/rat 78.9 ± 2.74 vs Ctrl 61.5 ± 4.0). In conclusion, these results suggest that the O effects on anxiety-like behavior and feeding are opposite to those of GR, but are similar on memory retention.

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Comparative pharmacokinetics of two formulations of flubendazole in sheep

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Flubendazole (FLBZ), anthelmintic compound, has been shown a high distribution volume in sheep. Cyclodextrins (CDs) are used to enhance solubility of poor hydrosoluble compounds. The aim of this work was to compare the PK behaviour of FLBZ and its metabolites after its intraruminally (i.r) administration to sheep as a CD-solution or conventional suspension. In a Phase I of a crossover study, six male Corriedale sheep received either a CD-FLBZ solution (HPβCD 10%, FLBZ 2%) (Group 1, n= 3) or a carboximethylcellulose (CMC)-FLBZ suspension (CMC 0.5%, FLBZ 2%) (Group 2, n= 3) at the same dose (3.8 mg/kg). After a 21-days washout period treatment were reversed and the study repeated as Phase II. Blood samples were collected between 0 and 72 h post-treatment and analysed by HPLC. The administration of FLBZ as a CDs-solution in sheep (AUC=1.83± 0.12) did not induced a relevant enhancement on the plasma availability of FLBZ parent drug/metabolites compared to the CMC-suspension (AUC=1.53±0.40). It is likely that the CD-FLBZ complex, is hydrolised by the ruminal microflora-mediated metabolic process.

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Effects of an inhibitor of nitric oxide production on memory consolidation and reconsolidation

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The immediate post-training administration of L-NAME (3-100 mg/kg, ip), a specific inhibitor of the nitric oxide synthase, impaired retention test performance of a one-trial step-through inhibitory avoidance response in adult male CF-1 mice. Mice that were over-reinforced (1.2 mA, 50 Hz, 1 s) on the learning trial, exhibited a high retention performance 48 h after training. The immediate injection of L-NAME (3-100 mg/kg, ip) after the retention test, that is, after memory reactivation, significantly impaired retention performance over 5 consecutive days. We did not find spontaneous recovery 21 days after training when memory was retrieved 2 days after training and L-NAME was given immediately after it. Additionally, a saving protocol was used in order to examine a potential effect of L-NAME on memory extinction. Although we cannot definitively discard a retrieval deficit, the results obtained are in accordance with the storage deficit interpretation. Retention performance was unchanged in L-NAME - treated mice not undergoing memory reactivation session. These results, taken together, indicate that L-NAME, not only impaired consolidation, but also reconsolidation of an inhibitory avoidance task in mice, suggesting a critical participation of the nitric oxide pathway in both memory processes.

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The impairment on retention performance caused by repeated administration of gabapentin may not be explained by an anxiolytic effect on memory retrieval

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After more than a decade of clinical use, gabapentin (GBP) has been shown effective anticonvulsant, antineuropathic and anxiolytic effects, among others, but its mechanism of action remains unclear. Unless GBP was originally developed as a GABA analogue, it has not significant interactions with GABA receptors. The actions of GBP on learning and memory in rodents are practically unknown. Our laboratory demonstrated that post-training acute administration of GBP (50 mg/kg ip) enhanced memory consolidation of an inhibitory avoidance response in mice, whereas the repeated administration (50 mg/kg, ip, two daily doses, 12 h apart) impairs retention performance in the same task, and that both effects are correlated with changes on the activity of central cholinergic system. We report here that the repeated administration of GBP may also increase gabaergic activity, but that the impairment observed on retention performance of an inhibitory avoidance task may not be explained by an anxiolytic effect of the drug during memory retrieval

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Comparative diuretic effect of margyricarpus pinnatus and *tetraglochin alatum* (rosaceae) in rats.

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Margyricarpus pinnatus(M.P.) and *Tetraglochin alatum (TA)*are both plants from the Rosaceae family commonly known as "perlilla" and "espina de pescado" respectively, that grow in the central and west areas of Argentina. Infusions of these species are widely used in folk medicine principally as diuretic agents. The purpose of this study is to compare the diuretic activities of *M. P.* and *T.A.*.infusions in rats. Materials and methods: Adult Wistar rats, both sexes, and 10 % infusions of the aerial parts of the plants prepared according to Pharmacopea Argentina VI ed., phytochemical assays and urine Na and K determined by flame spectrophotometry were carried out. Saline solution(control) and Furosemide (reference drug) groups were established, all animal were administered orally according to Lipschitz *et al.* Results and Conclusions: Phytochemical assays showed the occurrence of flavonoids, genines, and saponins among others , both *T.A.* and *M.P.* infusions exhibited diuretic effect ($p<0,001$) while the *T.A.* infusion showed a greater diuretic effect against *M.P.* and an increased Na excretion Flavonoids are known to account for diuretic effect, therefore each compound isolated from these plants will be submitted for chemical identification and diuretic assay.

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***Tessaria absinthioides*: Attribution of anti-inflammatory properties. pharmacog-nostic and pharmacologic studies.**

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Tessaria absinthioides (*TA*)(Asteraceae), a plant from Cuyo region known as "pájaro bobo", is used in folk medicine for its hypocholesterolemic and balsamic effects. The aim was to incorporate it to plant studies with anti-inflammatory activity in order to attribute unknown pharmacologic activities. Methods. A) Pharmacognostic assays: aerial part of *TA* was recollected and an 20% infusion was prepared. A general screening was carried out to identify possible components. B) Pharmacological study: Paw edema was utilized to evaluate the anti-inflammatory activity. 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 20 % lyophilized infusion. 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. Results. A) Principal components found were glucids, flavonoids, tannins, saponins and alcaloids. B) Lyophilized infusions inhibited the paw edema at three doses evaluated, being 200 mg/kg the more effective one. The anti-inflammatory effect was observed from 3 h to the end of the experiment. Conclusion. We attribute anti-inflammatory properties to *TA*. Besides, we observed that the intermediate dose was the more effective, indicating the importance to determinate the optima concentration to possible phytomedicines.

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Alteration of neurotensin binding to cerebral cortex membranes by antipsychotic agents.

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Synaptosomal membrane Na^+, K^+-ATPase is inhibited by neurotensin (NT), an effect which involves high affinity neurotensin receptor (NTS1). In previous work, we studied NT effect on this enzyme activity of rats pretreated with antipsychotic agents. NT inhibitory effect on Na^+, K^+-ATPase activity was totally prevented with haloperidol whereas it was inverted after clozapine administration. In order to test whether these effects were related to alteration of peptide binding to its receptor, herein [^{3}H]-NT binding to cortical membranes after i.p administration of haloperidol (2 mg/kg) and clozapine (10 mg/kg) was analyzed. Rat cerebral cortex was removed and subjected to differential centrifugation in the presence of Tris-HCl buffer (pH=7.5) to obtain membrane fractions. It was observed that specific ligand binding increased to 198 ± 24% (n=3) whereas it decreased to 67.5 ± 9 (n= 4) with respect to control membranes after administration of haloperidol and clozapine, respectively. These changes in NT binding are not attributable to Na^+, K^+-ATPase alteration since basal enzyme activity (31 to 33 μmoles. mg $prot^{-1}$. min^{-1}) remained unaltered by haloperidol or clozapine treatments. It is postulated that opposite response of Na^+, K^+-ATPase to NT after antipsychotic administration may be due to alteration of peptide binding to its receptor.

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Enhancer effect on Quercetin delivery through pig skin

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Skin is an excellent barrier of many compounds. In order to increase therapeutic effectiveness of topical formulations, it is necessary include permeation enhancers in these formulations. These agents are substances which rapidly and reversibly promote percutaneous penetration of drugs, being the most frequently used alcohols, fatty acids, terpenes and different solvents. In the present work flux (J_m) and permeation (P) and diffusion (D) coefficients were determined. Systems formed by quercetin (Q) in carbopol gel (CG) and enhancers (2,52%) such as dimethyl formamide (DMF), l-menthol (M), propylene glycol (PG) and isopropyl alcohol (IP) through pig ear were studied. Experiments using an automatic sampler Microette with Franz-type diffusion cells at temperature and magnetical stirring constant (32±0.5°C, 180 rpm) were performed. Skin section was mounted on the receptor compartment of diffusion cells with stratum corneum facing donor phase. Skin was pre-treated with phosphate buffer saline pH=7.4 (PBS) which was used as receptor phase. Then 0.0225 g of gel formulation were uniformly spread on the skin stratum corneum. At predetermined intervals 50 μl of receptor phase were removed keeping the "sink conditions". Quercetin permeated was quantified by UV-vis spectrophotometry at 255 nm. Experiments were performed in triplicate. Cumulative corrections to determine total amount of drug permeated at each time interval were made. The results obtained suggest that, in quercetin transdermal permeation, the best enhancer was l-menthol. Values of physicochemical parameters for this system were: J_m= 6.91 x 10^{-7} $g.cm^{-2}.s^{-1}$, P = 2.13 x 10^{-5} $cm.s^{-1}$, D = 8.52 x 10^{-5} $cm^{-2}.s^{-1}$.

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Dopamine from nucleus accumbens mediates the amphetamine-induced decrease in the lymphoproliferative response.

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There is evidence that psychostimulant drugs can influence the immune response. We previously demonstrated that a single exposure to amphetamine (AMPH) decreases the lymphoproliferative response in rats, and that dopaminergic antagonist pretreatments can suppress this AMPH's effect. The main goal of this work was to study the participation of central dopaminergic system in this effect by a selective destruction of mesolimbic dopamine (DA)-containing terminals produced by bilateral injection of 2 μg of 6-hydroxy-DA (6OHDA) into the nucleus accumbens of Wistar rats pretreated with desipramine. Two weeks following the lesion, the animals received an acute AMPH dose (5mg/kg IP) and the proliferative response was assessed 4 days after. In 6OHDA pretreated rats we obtained a reduction of DA levels of a 51.4% (+/- 4.6) related to control. AMPH caused a decrease (73% +/- 8) in the concanavalin-A-induced lymphocyte proliferation that was completely abrogated in 6OHDA pretrated animals. Our results clearly indicate that mesolimbic DA is primarily involved in the AMPH's effect on lymphoproliferative response.

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Stress- and drug-induced sensitization to cocaine: common neuroadaptations in AGS3 in nucleus accumbens shell but not core and ventral prefrontal cortex. Cancela L.M., Melendez, R and Kalivas P.W. Department of Neuroscience, Medical University of South Carolina, Charleston, SC, 29425 USA.

The main goal of this work was to study the neurobiological substrates underlying the restraint stress-induced sensitization to stimulating properties of cocaine, by looking into those neuroadaptations previously identified in a model of cocaine-induced sensitization, in specific brain nuclei relevant for this process (e.g. nucleus accumbens (NA) core and shell, dorsal and ventral prefrontal cortex (dPfC and vPfC) and dorsal striatum (STR)). Since the expression of the Activator of G-protein signaling 3 (AGS-3), GluR1 and NMDA NR2A in the NAc and/or dorsal PfC have been seen to be affected by either repeated non-contingent or self-administered cocaine, we measured the levels of this protein in the model of chronic restraint stress 24 hours and 21 days after restraint. Immunoblotting techniques were used to this end. Three weeks after of the last restraint stress we found a significant increase (43.4 % above the control) of AGS3 in NAcShell as compared with that observed in the no-stress group, while no difference was observed in NAcCore, vPfC, dPfC and STR. Chronic restraint stress did not modify GluR1 and NR2a 24 hours or 21 days following the last restraint. A sensitized behavioural response to cocaine was observed in these chronically stressed animals. Since the up-regulation in AGS-3 could be altering D1-stimulated Gsα signaling in NAcShell, it is likely that this molecular mechanism may be associated to the restraint stress-induced behavioral sensitization to cocaine observed in this study.

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GM1 ganglioside facilitates the rewarding properties of cocaine in rats

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Gangliosides, which are natural components of neuronal membranes, seem to play a relevant rol in neuronal plasticity phenomena. Several lines of evidence have demonstrated that pretreatment with gangliosides accelerates plastic neuronal changes induced by different pharmacological treatments. Using an unbiased place conditioning procedure (CPP paradigm), we have analyzed the influence of GM1 ganglioside pretreatment on cocaine induced conditioned rewarding effects. Previous reports from our Lab have demonstrated that four conditioning sessions employing 10mg/kg i.p. of cocaine are necessary to induce conditioning effects in control rats. Pretreatment with GM1 (30 mg/kg i.p.) two hours before cocaine administration (5 mg/kg) elicited conditioned place preference following four conditioning sessions. Furthermore, GM1 co-administration leads to place conditioning using 10 mg/kg of cocaine after two and three conditioning sessions, whereas no effect was found after 4 sessions. Thus, GM1 co-administration enhanced the rewarding properties of cocaine since it decreased the effective dose of cocaine necessary to induce conditioned place preference as well as the number of conditioning sessions. Since central dopaminergic system is critically involved in reward mechanism, these preliminary results suggest that GM1 ganglioside plays an important role in the events related to dopaminergic plasticity.

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Perinatal protein malnutrition enhances locomotor's morphine effects in adult rats. Sensitization and cross-sensitization study.

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The influence of neuronal alterations induced by early undernutrition on the locomotor effect of morphine was evaluated in adult rats submitted to a protein malnutrition schedule at perinatal age. To assess the sensitization phenomenon induced by repeated morphine administration, different groups of control (C) and deprived (D) received five intermittent injections (every 48 hours) of morphine (7.5 or 10 mg/kg, i.p.) or saline (1ml/kg, i.p.). Following each administration behavioral parameters were assessed in a locomotor activity cage. Our data revealed a shift to the left in the activity curves of the D rats compare to controls. Thus, D animals showed a clear behavioral sensitization to the lower dose of morphine, whereas this phenomenon was only observed in C rats for the higher dose used. Moreover, only D animals expressed cross-sensitization to locomotor activity after a challenge with cocaine (10 mg/kg, i.p., 48 hours after the final morphine administration) in morphine pre-exposed animals (lower dose). These results suggest that D rats had a lower threshold for developing a progressive behavioral sensitization to morphine as well as cross-sensitization to cocaine.

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Effects of glibenclamide on transport processes in epithelia isolated from the toad *Bufo arenarum*

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The urinary bladder (TB) and skin (TS) isolated from toads exhibit great similarity to mammalian transport epithelia, particularly those of the distal kidney tubule, and are extensively used in studying Cl^- and water transport processes. We measured the effects of glibenclamide (Glib) on water passage across the TB exposed to an osmotic gradient (Jw) by a gravimetric technique, and the electrical parameters of the TS of *Bufo arenarum* using the technique of Ussing. Glib significantly increased the Jw in TB exposed to oxytocin (which increases the intracellular generation of cyclic AMP [cAMP]) or to theophylline (THEO, which reduces intracellular hydrolysis of cAMP), in agreement with its action as inhibitor of the membrane permeability to cyclic AMP, previously described in the literature. Glib has also been known to inhibit the flow of Cl^- across the cystic fibrosis transmembrane conductance regulator (CFTR) channel, blocking the passage of Cl^- involved in the glandular secretory response to catecholamines in the TS. In contrast, Glib exerted no effect on the short-circuit current generated by a chloride concentration gradient in the TS exposed to amiloride and THEO (SCCg, gradient generated SCC), described by us and shown to be a measure of the transepithelial passage of Cl^-. The present data, together with previous results from our laboratory demonstrating that exposure of the TS to catecholamines does not alter SCCg, strongly suggest that the CFTR channel is not involved in the generation of the SCCg.

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Modulation of NMDA receptor subunit expression by administration of a Na^+, K^+-ATPase inhibitor.

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Endobain E is a soluble brain factor isolated from cerebral cortex which shares biological properties with ouabain, including the inhibition of Na^+, K^+-ATPase activity. Endobain E decreases [^{3}H] dizocilpine binding to NMDA receptor. In the search of an interplay between NMDA receptor and Na^+, K^+-ATPase, herein we analyzed the expression of NMDA subunits after endobain E treatment. Endobain E was isolated by gel filtration and anionic exchange HPLC from a rat brain soluble fraction. Rats were administered i.c.v. with endobain E or saline solutions; 3 days later, animals were decapitated, cerebral cortex and hippocampus removed and crude membrane fractions isolated. NR1, NR2A, NR2B and NR2C subunits were quantified by Western blot. Results were expressed as the ratio between treated *versus* control. After administration of 10 µl endobain E (1 µl = 28 mg tissue) NR1 expression enhanced 5 fold and 2.5 fold in cerebral cortex and hippocampus, respectively. NR2A expression increased 2 fold in cerebral cortex and 1.5 fold in hippocampus. NR2B expression rised 3 fold in cerebral cortex but remained unaltered in hippocampus. NR2C expression was unaffected in either area. Lower changes were found with 1 µl of endobain E. Results indicate that endogenous Na^+, K^+-ATPase inhibitor endobain E modifies the expression of NMDA receptor subunits, supporting the hypothesis of a relationship between this receptor and Na^+, K^+-ATPase at synaptic region.

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Functional relevance of aminopeptidase M (APM) and neutral endopeptidase (NEP) in the biological inactivation of des-Arg^{10}-KD (DAKD) in human umbilical vein (HUV).

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Introduction and Goals: APM and NEP are metallopeptidases that inactivate BKB_1 receptor agonist, DAKD, in isolated HUV. The aim of the study was to evaluate the functional relevance of APM and NEP in the modulation of the DAKD responses in intact (E+) and deendothelized (E-) HUV. **Methods and Results:** E+ and E- HUV rings were mounted under isometric tension in Krebs solution at 37ºC. After 5h, concentration-response curves (CRCs) were obtained to DAKD. Amastatin (A) 10μM, an APM inhibitor, enhanced contractile responses elicited by DAKD in E+ and E- HUV (pCE_{50} E+:9.16±0.02, pCE_{50} A E+:9.51±0.03, $p<0.001$; pCE_{50} E-: 9.50±0.01, pCE_{50} A E-:9.70±0.03, $p<0.001$). Phosphoramidon (P) 10μM, a NEP inhibitor, did not produce any potentiation of DAKD responses neither in E+ nor E- HUV. However, concomitant inhibition of APM and NEP potentiated DAKD responses in E+ and E- HUV and this effect was considerably increased compared with the CRCs obtained under single APM inhibition (pCE_{50}A+P E+:9.87±0.04; pCE_{50}A+P E-:9.82±0.02; $p<0.01$). No differences were observed in maximal responses. The state of endothelium was confirmed by histology. **Conclusion:** These results indicate that APM, localized in endothelial and smooth muscle HUV cells, is functionally relevant in modulating DAKD responses in HUV. Also, APM's inhibition unmasks NEP's activity, acting synergistically in regulating DAKD responses in HUV.

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Effect of a new semisynthetic butenolide on mast cell activation induced by the calcium ionophore A23187

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In previous work we have demonstrated that a new semisynthetic butenolide with antiulcer properties (3-benzyloxymethyl-5*H*-furan-2-one; But), inhibits mast cell exocytosis induced by the G protein stimulant compound 48/80. The present work examines the effect of But on mast cell degranulation induced by the calcium ionophore A23187, to determine whether But acts upstream or downstream of cytosolic calcium increase. Rat peritoneal mast cells were purified in Percoll and incubated with: 1) Tyrode solution or 2) A23187 or 3) But+A23187. Serotonin release studies by high performance liquid chromatography (HPLC), evaluation of mast cell morphology by light microscopy, dose-response and time-response studies, cell viability evaluation by the tripan blue dye exclusion, comparative studies with ketotifen (Ket), and drug stability evaluation by thin layer chromatography (TLC) were carried out. Calcium ionophore increased serotonin release from mast cells and elicited evident morphological changes. These effects were inhibited by But in a dose- and time-dependent manner. The inhibitory effect exhibited by But was stronger than that of ketotifen, a classical mast cell stabilizer. In conclusion, the present study demonstrates that But inhibits A23187-induced mast cell activation, acting downstream of cytosolic calcium increase.

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Distribution of rat pars tuberalis secretory product is affected by albendazole in a dose dependant manner.

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Specific cells of *Pars tuberalis* (PT) release a secretory product (SP) not well characterized yet. Albendazole (ABZ) is a drug used in treatment of parasitosis; it depolymerizes microtubules (MT) causing alteration of functions of cells, including secretion. The aim of the present research was to establish the minimum concentration of ABZ that alters the distribution of the SP. To do this, six groups of adult rat (1 control and 5 experimentals [n=3]) were used. Experimental animals were treated orally with 0.5, 1.0, 1.5, 2.0, and 2.5 g/kg of ABZ; 48 h after that animals were sacrificed. Distribution of SP and MT were detected by means of immunocytochemical techniques; SP was labeled with a polyclonal antibody (FB12), and MT with monoclonal antibody against α tubulin. In control animals, FB12 immunostained SP in way that its distribution appears paranuclear. In experimental animals treated with 0.5 g/kg the distribution was similar to the control, but after 1.0 g/kg the immunolabel of SP distributed throughout evenly in the cytoplasm; the distribution of the SP, even at maximal concentration was not granulous as in the control. With antibody anti-α tubulin no polymer structures were observed; rather than, the label appears to correspond to the heterodimer correlating with the label of FB12. Results allow to conclude that depolymerization of cytoplasmic MT impairs distribution of the secretion of *Pars tubueralis* in a dose dependant way.

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Neutral endopeptidase up-regulation in isolated human umbilical artery: involvement in desensitization of bradykinin-induced vasoconstrictor effects.

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Introduction: Bradykinin (BK) prod- uces constriction of human umbilical artery (HUA) with high potency and efficacy. The objective of the present study was to evaluate the role of two known BK-inactivating enzymes, angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP), as a function of *in vitro* incubation time isolated HUA. **Methods and results:** Concentration response curves to bradykinin after a 2 h incubation failed to be modified by 1 μM captopril (ACE inhibitor) or 10 μM phosphoramidon (NEP inhibitor). However, BK-elicited responses at 5 h were potentiated when tissues were exposed to captopril or phosphoramidon. In addition, the potency of control responses elicited by BK at 5 h was significantly lower than that observed at 2 h. NEP activity determined in tissues incubated during 5 h showed an increase over activity in 2 h and non-incubated tissues. Moreover, tissue incubation with 10 μM cycloheximide reversed the activity increase observed at 5 h. Furthermore, Western blot experiments in HUA whole cell extracts showed a single 100 KDa band corresponding to NEP. Densitometric analysis revealed that NEP content in HUA at 5 h was higher than in 2 h-incubated and non-incubated tissues. **Conclusion:** The results suggest that NEP is up-regulated in HUA after prolonged *in vitro* incubation and is involved in desensitization of bradykinin-induced vasoconstrictor effects in HUA.

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Effect oxidative of albendazole in parasites cestodes

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The objective of this study was to evaluate the damage oxidative in tissues of Moniezia expansa experimentally subjected to albenzazole (ABZ) effect. Adult parasites obtained from steers were used. The cestodes(n= 4) were incubated with ABZ (5 nmol/ml Kreb´s Ringer Tris), 1g homogenate/10 ml, during 180 min. to 37 ºC) (Group Treated, GT). Control parasites (n= 4) in similar conditions but without ABZ were incubated (Group Control, (GC). Hence lipid peroxide levels were measured as thiobarbituric acid reactive substances (TBARS). 1,1,3,3-Tetramethoxypropane (Sigma) was used as an external standard, and the level of lipid peroxides was expressed as nmol of MDA/mg protein. The lipid peroxide level in GC was 23,8 ± 2,08 and in GT 34.1 2,38 nmol MDA/mg of protein ($p<0.01$), 30 % higher than that for the parasites GC Our results indicate that in adult parasites the generation of lipid peroxides exceeds the antioxidant capacity, so generating a situation of oxidative stress and lipid peroxidation .

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Ultrastructural study of *ECHINOCOCCUS GRANULOSUS* cysts treated *in vivo* with an abz-cyclodextrin solution

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Albendazole (ABZ), currently used for chemotherapeutic treatment of cystic hydatid disease, is usually formulated as a suspension. The aim of this work was to study the ultrastructural alterations produced *in vivo* by an ABZ-cyclodextrin solution on *E. granulosus* cysts using scanning and transmission electron microscopes (SEM and TEM). BalbC mice were intraperitoneally infected with protoscoleces (1500/animal). A year after infection, mice were divided in two groups (n= 10): 1) control, untreated; 2) orally treated with a ABZ-cyclodextrin solution (500 µM) for 50 days. After treatment, animals were euthanised and the cysts were weighed and subjected to ultrastructure study. No significant difference between control and treated group was found related to the weight of cyst masses ($P > 0.05$). All cysts removed from control mice appeared turgid and no alteration in ultrastructure was detected. Cysts from the treated group revealed at TEM alterations in the germinal layer with the presence of numerous vacuoles. At SEM, only cellular debris of the germinal layer was observed.

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Mouse skin as an *in vitro* model to predict the transdermal absorption of moxidectin in cattle.

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Endectocide parasitic compounds are extensively used for broad-spectrum parasite control and their topical administration to cattle is widespread in clinical practice. However, there is not a practical, rapid and easy method to determine the absorption of new topical formulations before clinical trials. The objective of the present work was to characterize the transdermal absorption of two topical formulation of moxidectin (MXD) using mouse skin as an *in vitro* model. Subcutaneous tissues were rubbed using an ethanol-wet cotton. Mouse skins were mounted in Franz-type diffusion cells and treated with two commercial formulations (1.7 mL) for use in cattle. The receptor medium was buffer phosphate (0.1 M), bovine albumin (4.5%) and ethanol (20%). MXD concentrations in the receptor medium were measured by HPLC with a fluorescence detector. MXD percutaneous absorption reached a steady state at 24 hours post-administration. There were no statistical differences between the penetration rates of both formulations (18.1 ± 11.4 vs. 22 ± 13.6 $ng.cm^{-2}.h^{-1}$, $P>0.05$). The correlation coefficient (r) between penetration rates and plasma $AUC_{partial}$ from previous *in vivo* works was 0.9329 (CI 95%: 0.6046 to 0.9903). This investigation contributes to the searching for an *in vitro* model to predict the percutaneous absorption of topical formulation of endectocides in cattle.

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Characterization of xenobiotic biotransformation enzymes in cattle duodenal mucosa.

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The intestinal mucosa is an absorptive barrier in the uptake of xenobiotics, which could be also metabolized in this tissue. The objective of this work was to evaluate the activity and the expression of xenobiotic metabolizing enzymes in cytosolic and microsomal fractions obtained from the duodenal mucosa of veal calves and adult cattle. Oxidative (CYP- and FMO-dependent), hydrolitic and conjugative enzyme activities were measured using known marker substrates. Proteins were identified by SDS-PAGE and Western blotting. Cattle intestinal microsomes showed CYP2C, 2B and 3A-mediated activities. Benzphetamine (CYP2B) and aminopiryne (CYP2C) N-demethylase activities were ~60% higher ($P<0.05$) in the duodenal mucosa of veal calves compared to adult cattle. Microsomes obtained from the duodenal mucosa of both cattle categories showed almost the same ethylmorphine N-demethylase (CYP3A), methimazole S-oxidase (FMO) and indophenol acetate esterase activities. Immunoblot analysis showed the presence of CYP2C, 3A4 and 3A1/2 immunoreacting proteins (but not CYP1A or CYP2B proteins). Intestinal microsomes were able to conjugate 1-naphthol, an UGT-mediated reaction. GST-mediated conjugation of 1-cloro,2,4-dinitrobenzene was 47% higher ($P<0.05$) in the duodenal mucosa of adult cattle compared to calves. A GSTα immunoreacting protein was detected in cytosols obtained from cattle duodenal mucosa

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Comparative Endometrial Concentrations Of Enrofloxacin After Intravenous And Intrauterine Administrations To Healthful And Endometritis Mares

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Bacterial endometritis is a common cause of infertility in mares. Rational use of antimicrobial drugs is an obvious option for controlling endometritis. The local therapy with "concentration dependant" antimicrobials is attractive for avoiding the bacterial re-growth and resistance. The aim of this trial was to evaluate the pharmacokinetic (PK) behaviour of enrofloxacin (EFX), formulated as injectable solution, after the intravenous (IV) and intrauterine (IU) administration in healthful and diseased mares. *Study I*: 10 mares with diagnosed clinical endometritis, were divided in two groups (n=5) according clinical scores. Group I was treated with EFX 2.5 mg/kg, solution formulation, via IV. Mares of the Group II received identical treatment that Group I by IU route (local treatment). *Study II*: same schedule work of *Study I* was used for healthful mares. Endometrial tissue samples were taken over 48h post-administration and analysed by HPLC with fluorescence detection. EFX was detected in endometrial tissue over 48 h after both treatments. The results of local treatment in terms of AUC (µg.h/mL), compared with the systemic treatment, showed an increase of 3830.7 % and 504 % for healthful and endometritis mares, respectively. High concentrations in endometrial tissue, characterised the PK behaviour of EFX given as a local treatment. These preliminary results are encouraging for further research on the optimisation of local treatment in mares.

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Effect Of The Mycotoxin Ochratoxin A On *In Vitro* And *In Vivo* Rat Brain Microtubule Dynamics

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The mechanism of toxicity of the mycotoxin ochratoxin A (OTA) is not fully understood for neither animals nor humans. Since some records suggest that OTA affects cytoskeleton, we used three different approaches to search for its action on the assembly of rat brain microtubules. Absorbance at 350 nm was recorded in the following systems: a) Increasing amounts of OTA were added to the medium containing rat brain supernatant and placed in a spectrophotometer thermostatized at 37° C; b) rat brain thick slices were incubated with and without OTA for certain amount of time; high speed extracts were obtained and set in conditions for polymerization under identical conditions; c) a group of rats were treated with OTA administered orally, other group was used as control, brains were handled similarly to thick slices. In all three systems, statistically significant decrease of the readings of optical density at 350 nm compared with control were obtained when OTA was present or administered. Results clearly indicate that OTA affects microtubule assembly or dynamics in all three systems; this would contribute to a better understanding of the molecular mechanisms of OTA-mediated toxicity as well as to help to answer questions relevant to microtubule dynamics.

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Endometrial Concentrations Of A Novel Gel And Conventional Solution Based- Enrofloxacin Formulation In Mares.

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Enrofloxacin (EFX) is used in horses more that other fluoroquinolones, being a worthy preference for gram negative infections which require expensive or potentially toxic injectable antimicrobials. Rational use of antimicrobial drugs and new therapeutic alternatives need to be investigated. The aim of the present study was to evaluate the endometrial concentrations of enrofloxacin (EFX) given as solution, after the intravenous (IV) and given as gel formulation, intrauterinelly (IU) using the healthful mares as experimental model. Ten (10) mares were allocated into two groups (n=5). Mares of Group I were treated with EFX/solution based-formulation, (5 mg/kg) by IV route. Animals of Group II received same treatment above but, with EFX/gel based-formulation by Intrauterine route. Endometrial tissue samples were taken over 48h post-administration and analysed by HPLC with fluorescence detection. The local treatment with gel showed a substantial increase in AUC (µg.h/mL) values of the 2275 % on the endometrial tissue, compared with the IV treatment. EFX/Gel formulation will be a potential advantageous option for its possible more permanency on endometrium, and thus, to simplify the treatment' schedule in diseased mares.

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Prostaglandin $F_{2\alpha}$ receptor (FP) expression in human umbilical vein (HUV).

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Introduction: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogs bimatoprost free acid and latanoprost free acid (selective FP receptor agonists), promote constriction of HUV. This effect is selectively blocked by FP receptor antagonist, AL-8810, suggesting that FP receptors are involved in the vasoconstrictor effect. Therefore, the aim of the present study was to analize the presence of FP receptor at mRNA and protein level in HUV by RT-PCR and Western blot, respectively. **Methods and results:** Total RNA (HUV) and proteins (HUV and mouse uterus) were extracted employing Trizol and RIPA lysis buffer, respectively. RNA was quantified at 260/280nm and proteins were measured with Bradford at 595 nm. PCR products were electrophoresed on 2% agarose gels with ethidium bromide and photographed under UV. Endonuclease digestion was used to confirm product identity. Proteins were electrophoresed on 10% SDS-PAGE and electrotransferred to PVDF membranes which were blocked in TTBS buffer with 5% milk; then incubated overnight with anti rabbit FP receptor (murine) polyclonal antibodies. Membranes were revealed with alkaline phosphatase-conjugated goat anti-rabbit IgG. Immunoreactive bands were detected by chemiluminescence and compared with those obtained in mouse uterus (control tissue). **Conclusion:** The results indicate that whole HUV express a FP receptor at mRNA level and a protein of similar molecular weight that one observed in mouse uterus, a rich source of FP receptors.

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Pharmacological characterization of Prostaglandin $F_{2\alpha}$ receptor (FP) in human umbilical vein (HUV).

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Introduction: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) promotes constriction of many tissues, with a "promiscuous" stimulation of prostanoid TP or EP receptors. We documented that the contractile response mediated by $PGF_{2\alpha}$ in HUV is not dependent of TP or EP receptors (*Daray y col., Br J Pharmacol, 2003, 139; 1409-1416*). The aim of this study was to investigate the effects of specific FP antagonism, using AL-8810 on contractions induced by $PGF_{2\alpha}$ and its analogs, bimatoprost and latanoprost free acids (selective FP receptor agonists).**Methods:** Umbilical veins were dissected out from Wharton's jelly and cutted into rings that were placed in organ baths with warm Krebs (37ºC) and bubbled constantly with 5%CO_2:95%O_2. After 1h, rings were tested with KCl 40mM to evaluate their viability. At the 2hs, concentration-response curves were performed for each agonist, and in the case of antagonist, it was added at the bath 30 min before the curve was performed. **Results and conclusions:** Agonists gave pEC_{50} and maximal responses of: $PGF_{2\alpha}$ 6.0±0.1, 11.8±1.1 g (n=9); latanoprost 5.8±0.1, 11.2±2.0 g (n=4); and bimatoprost 5.9±0.03, 10.7±1.6 g (n=4). AL-8810 antagonized $PGF_{2\alpha}$ with a pK_B=5.8 ±0.1 (n=9), in according to previous reports at the cloned human FP receptor. Similar results were obtained with latanoprost and bimatoprost (pK_B=5.3±0.4, n=2 and 5.7±0.2, n=3). Results employing FP receptor agonists and antagonist suggest the presence of FP receptors mediating vasoconstriction in HUV.

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Antidepressant treatment prevents chronic stress induced impairment of T-cell immunity and enhancement of tumor growth.

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Chronic stress is involved in the onset of specific psychiatric diseases such as major depression. Stress also affects the immune response. T-cell mediated immunity is a key component in solid tumor rejection. Depression of antitumoral immunity induced by stress could contribute to tumor growth, and antidepressant treatment could prevent this effect. We studied the effects of chronic stress and antidepressant treatment in the immune response as well as in the evolution of neoplasic pathology. BALB/c mice were subjected to chronic restraint stress (CRS), a well validated model of depression. Lymphocyte proliferation to a T selective mitogen was evaluated by [^{3}H]-thymidine incorporation. A significant reduction in T cell proliferation was observed in CRS animals. CRS and normal syngeneic mice were subcutaneously injected with $1x10^6$ LBC T lymphoma cells to generate a solid tumor. Measures of tumor volume indicated that growth is increased in CRS mice. To test if these effects are reversed by antidepressant treatment, CRS mice were concomitantly treated with 15 mg/kg fluoxetine. Fluoxetine prevented T cell impaired proliferation in CRS animals. Moreover, these animals showed the same lymphoma evolution than their normal counterparts. These results suggest that stress-related depressive state promotes tumor growth by depressing T-cell mediated immunity and that chronic antidepressant treatment prevents enhanced tumor evolution by reversing T-cell impairment.

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Physical chemistry study of enalapril transdermal liberation.

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Enalapril, enalaprilate pro-drug, an antihypertensive action drug, acts inhibiting angiotensin converter enzyme. It is used for treatment of essential arterial and renovascular hypertension, and other tie upheavals to the circulatory system. In order to reduce undesirable effects of oral administration as gastric irritation and to eliminate first hepatic step, *in vitro* enalapril transdermal permeation through dermatomized pig skin were studied. Carbopol gel as vehicle and l-menthol as permeation enhancer (up to 5 %) were used. The enhancer is necessary to obtain satisfactory systemic concentrations. Experiences were made by triplicate in vertical type Franz cells, using saline phosphate pH 7.4 buffer as receptor. Enalapril permeate quantification was made by UV-vis spectroscopy at 209.2 nm. Physical chemistry parameters were calculated (flux, diffusion and permeation coefficients). Graphical representation of diffusion coefficient versus percentage of menthol allowed to conclude that 4.03 % of the enhancer ($D = 5.374 \times 10^{-6}$ $cm^2.s^{-1}$) was the most convenirnt concentration for enalapril in carbopol gel transdermal formulation.

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Molecular Characterization Of Fasciola Hepatica Triclabendazole-Susceptible And Resistant By Rapd-Pcr Method

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Triclabendazole (TCBZ) is an halogenated benzimidazole antihelmintic widely used to control the fluke *Fasciola hepatica*. TCBZ has excellent activity against mature and immature stages, but its intensive use has resulted in the development of resistant liver flukes having a high economic significance in animal production. Previous work in our lab demonstrated that TCBZ metabolism into their sulphoxide metabolites was significantly higher in TCBZ-resistant flukes than in TCBZ-susceptible ones. Certain mechanisms of resistance appear as a result of mutations at the genomic level modifying the primary structure of the target molecule of the drug. The aim of the present work is to approach a molecular characterization of *F. hepatica* TCBZ-susceptible and TCBZ-resistant strains using the RAPD-PCR method. DNA extracted from two strains of *F. hepatica* was used, Sligo strain (TCBZ-resistant) and Cullompton strain (TCBZ-susceptible). Comparing the obtained pattern of bands with three different initiators (primers) we can infer that by this technique it is not possible to detect genomic differences. These results reinforce the importance of the enhanced metabolic activity in the resistant strain like a fundamental mechanism in the presentation of the resistance activity. These results are a further step to understand the differential pharmacological activity of this drugs against helminth parasites and contribute to understand the mechanisms of resistant to TCBZ in liver flukes.

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Role of endocannabinoid system in the memory consolidation and LTP induction.

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Long-term potentiation (LTP) is a strong candidate for memory storage mechanism. The cannabinoid receptor CB1 is abundantly present in the brain, with large concentration in the hippocampus, an structure essential for memory formation and extensively studied in LTP experiments. Activation of hippocampal CB1 receptors inhibits GABA release. Here, we studied the effect of the intrahipocampal administration of CB1 receptor selective antagonist AM251, and of the agonist anandamide upon the memory consolidation f the step-down inhibitory avoidance task (IA), and in the LTP induction in a electrophysiological hippocampal slice setup. Standard extracellular electrophysiology techniques were used to record field excitatory postsynaptic potentials from the dendritic region of CA1 neurons in response to high frequency stimulation of Schaffer's collaterals; a micropipette has ejected 0.2 mM of AM251 or 28.8 mM of anandamide (both in DMSO/PBS vehicle) 2 min before the stimulus; in the IA task, immediately after training (footshock, 0.5 mA), animals received a bilateral infusion of 0.55 or 5.5 ng/side of AM251 or of .05, 0.5 or 1ug/side of Anandamide, or their vehicles, in the CA1 region, and test was performed 24 h later. Our behavioral results show that AM251 has caused a deficit in memory consolidation while anandamide was ineffetive (in the studied doses). Consistently to this, electrophysiology shows that anandamide has had no measurable effect upon LTP induction, while AM251 has fully blocked the phenomenon.

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Role of oxidative stress in vessels from aortic coartation rats.

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Abominal aortic coarctation (Aco) above the renal arteries leads to severe hipertension. Elevation of systemic blood pressure is associated with increased of reactivy oxygen species activity and enhanced nitric oxide inactivation in various models of genetic and acquired hypertension. The aim of this study was to investigate the vascular anion superoxide (O_2^-) production, characterize the oxidase involved in this process and examine the effects of superoxide dismutase mimetics (tempol) and statins (simvastatin) on the vessels. Female Wistar rats were used to the 7 days of the Aco or sham operation (SO). The mean arterial pressure of the Aco rats was higher than that of the SO rats. Basal O_2^- generation, was higher in thoracic aortic rings from Aco than in those from normotensive rats (SO). Vascular homogenates from Aco rats suggested that the source of O_2^- activated is an NADPH oxidase (DPI treatment: 1137.6 ± 34.9, inhibition: 82%). In response to acetylcholine, the relaxation of thoracic aortic rings precontracted with 10^{-7} M of phenilephrine was lower in the Aco group (62.8 ±3.1%) than SO group (85.25 ± 3.1%). When tempol was administrated in the drinking water (1mM/day) during 7 days, aortic vascular O_2^- production disminished and vascular relaxations were normalizad, however simvastatine treatment (10 mg/kg/day) had not effect. In conclusion,the alteration of vascular relaxation was likely at least in part due to the increase in vascular O_2^- production. Besides, the findings from this study suggested that tempol but not simvastatin improves the vascular function inhibiting oxidative stress.

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