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L.1.

PHYSIOLOGY AND PHARMACOLOGY OF THE CRYSTALLINE LENS: IMPLICATIONS IN CATARACTOGENESIS

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The ocular lens is an avascular, quasi-spherical transparent organ comprised entirely of epithelial cells at various stages of differentiation. An epithelial monolayer, which covers the anterior face up to the equatorial region, overlays the elongated, mature, enucleated cells, commonly referred to as the "lens fibers," that fill the bulk of the organ. The whole lens is surrounded by a collagen capsule. The majority of homeostatic functions are mediated by the epithelium, the transport activities of which, affect the overall lens status due to the syncytial nature of the organ. Because it is a syncytium, the crystalline lens resembles a giant cell due to its gross appearance, high intralenticular [K], and low [Na]. An electrogenic Na-K pump located in the surface, basolateral membrane of the epithelium maintains the [K] and [Na] levels. The epithelial monolayer covers about 2/3 of the anterior surface and is absent on the posterior polar region.. In addition, Na-K pump activity within the epithelium is non-uniform. This is due to the absence of the Na-K pump activity at the anterior polar region, where passive inflow of Na occurs, and a high pump density at the equatorial surface. Thus, the lens is an asymmetrical organ, both structurally and functionally, with localized transport properties. Presently, the identification and localization of specific transporters and ion channels within the epithelium and lens fibers are among topics of contemporary studies. Nevertheless, the recognized asymmetrical nature of the lens provided the underpinning for a widely held microcirculation model for fluid movement within the lens. It is proposed that epithelial electrolyte transport leads not only to currents that have been shown to circulate around the lens, but that such ionic transport provides the driving force for the intralenticular circulation of nutrients and metabolites due to a hypothetical fluid entry across the polar regions, followed by fluid circulation within the lens and equatorial exit. Such fluid movement would follow the Na currents that would develop due to the asymmetrical distribution of Na-K pumps on the lens surface. Any disturbance to this transport system leads to localized lens swelling and cataracts. For example, endogenous digitalis-like compounds, which inhibit the Na-K pump activity of the epithelium, were recently identified in human cataractous lenses.

The lens epithelium also expresses various G-protein receptors that are coupled to the release of intracellular calcium. These include members of the muscarinic, adrenergic and purinergic families. Some of these receptors regulate the transport activities of the epithelium, while others may have roles in lens development. What is presently clear is that disruptions of lens Ca homeostasis also leads to cataracts. A prolonged increase in intracellular calcium would be expected to activate proteases such as calpain and induce irreversible breakdown of important structural proteins, as well as, inhibit the Na-K pump. In addition to external and metabolic factors, pharmacological interventions can result in the formation of cataract.

Thus, an understanding of these mechanisms is important in preventing or delaying cataract formation.

L. 2.

RATIONAL USE OF DRUGS AND PRESCRIPTIONS USING GENERIC NAME OR THE INTERNATIONAL NONPROPRIETARY NAME

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Prescribing drugs using the International Nonproprietary Name (INN) or Pharmacological Generic Name, is now obligatory in Argentina since the law Nº 25.649 (09/18/2002) is in force. The procedure to give an INN to drugs was adopted by the World Health Organization in the Assembly of 1993 and it is accepted by all Member States. The need to identify each drug by a unique, globally recognized pharmacological generic name, it is of critical importance in facilitating communication of drug performance in medical practice. The INN is the accepted scientific name used in academic teaching, in reference books and medical journals. Until the mentioned law in Argentina, all drugs were compulsorily prescribed using a brand, label, mark or proprietary name. As a consequence, in the pharmaceutical market each drug have numerous mark names. Under these circumstances, the mark name is the central element in marketing and promotion which are directed to a specific target group (prescribers health professionals). Biased promotions and false information's are also frequent practice in marketing of a drug. Moreover, under the point of view of the therapeutic quality, there are also in the market numerous drugs of no proven efficacy and other that are irrational combinations of several drugs in a same pharmaceutical form, all sold with varied mark names. As a consequence of the irrational use of drugs, the economic cost of the pharmaceutical services in Argentina rise continuously in the last 10- 15 years, being the 30 % or more, of total health expenditures.

In Argentina there are not true generic drugs until now. These are drugs with expired patent, without a proprietary name and are bioequivalents with the reference product. In the other hand, there are countless pharmaceutical equivalents. These are mark names containing the same drug, in the same pharmaceutical form, the same salt, identical dose for the same medical indication and are interchangeable, in accordance with the law. Prices of these pharmaceutical equivalents are usually greatly different. Same marks may cost 300-400 % more than the least expensive. Prescriptions using the INN allow patients to choose the price more appropriate.

Rational use of drugs can be defined as the use of medicines with adequate data on efficacy and safety available from clinical trials, prescribed in accordance with the physiopathology of the patient illness, in proper dosage only for the necessary time, with patient agreement. Between drugs of the same family, the cost/benefit ratio is also a major consideration in rational use of drugs. Health care is an essential human right and a population with good health is the more important capital of a nation. To meet this objective it is indispensable to have access to medicines. In this situation prescriptions made by the use of the INN promote interchangeability and accessibility to drug therapy which is the base of the rational use of drugs.

L. 3.

ROLE OF ANGIOTENSIN II RECEPTORS IN DEVELOPMENT

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Development and organogenesis involve cellular processes, which are precisely controlled by different mechanisms, one of them is the by regulating the level of receptor expression. Angiotensin II (Ang II), the effector peptide of the renin-angiotensin system (RAS) acts on its target tissues via two main types of membrane receptors: AT_1 y AT_2. Besides, the classical actions of Ang II on blood pressure and homeostasis control, a role of these receptors has recently been described on growth and development. AT_2 receptors are expressed at a very high level in fetal tissues, like fetal kidney, aorta, connective tissue among others and rapidly disappear after birth. Binding autoradiography, allows us to demonstrate the localization and distribution of Ang II receptors in cerebellum and brainstem of rats at different stages of development. We also analyzed the expression of Ang II AT_2 receptors by multiplex RT-PCR at different developmental stages: 8, 12, 15, 30, 40 and 60 postnatal days. Co-amplification of AT2 receptors together with the GAPDH ubiquitous gene allows us to quantify AT_2 receptors on cerebellum and brainstem. The specificity of the fragment amplified was determined by using restriction enzymes. We observed a variation on the expression level of AT_2 receptors with development in cerebellum, while the level was comparable on brainstem. Autoradiographic analysis agree with the results from RT-PCR. We identified nuclei expressing differentially either AT_1 or AT_2 receptors. Our results suggest an important regulation on the expression of these receptors with development, in a time curse which accompanies maturation of the cerebellar function. These results agree with previous observations about an alteration of mobility behavior of AT_2- receptors disrupted mouse.

Treatment with Ang II competitors during late pregnancy affects kidney development and the pattern of expression of Ang II receptors on new born rats. The transduction mechanisms as well as the pattern of expression of Ang II receptors strongly suggest a role of these receptors on growth control and development.

S. 1.

EFFECT OF EPIDERMAL GROWTH FACTOR (EGF) ON THE ONSET OF PARTURITION IN THE RAT

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Despite the remarkable increase in the knowledge of the mechanism underlying parturition, its precise regulation remains unknown. Many authors hypothesize that EGF participates in parturition. Also, prostaglandins (PGs), nitric oxide (NO) and progesterone (P_4), recognized modulators of pregnancy and labor, are modulated by EGF.

The aim of our work was to study the effect of the *in vivo* administration of EGF on the onset of parturition in the rat.

We observed that the intra-uterine (i/u) administration of EGF 500 ng on day 21 of gestation delayed delivery for 18±0.6 hs compared to the control rats. EGF action was mediated by diminished uterine cyclooxygenase expression and PGs level in the uterus and amniotic fluid. EGF augmented nitric oxide production and maintained high P_4 serum concentration. The overall effect was the reduction in uterine amplitude contractions, which in turn delayed delivery onset. This *in vivo* effect raises the question of whether EGF plays a physiological role in the initiation of parturition.

The results presented above, suggested that EGF might be exerting its effect on the onset of delivery through a protective effect on the corpus luteum, the tissue in charge of producing P_4 throughout pregnancy. Thus, we decided to study uterine cyclooxigenase-I expression and $PGF_{2\alpha}$ production along time after EGF administration. We found that both, cyclooxigenase-I and $PGF_{2\alpha}$ were minimum, 12 hs after the treatment. Also, P_4 serum concentration was maximum at that time. When we analysed the content of oxidative stress indicator molecules in the corpus luteum, we found that 12 hs after EGF – treatment the products of plasma membrane oxidative metabolism were lowered compared to day 21 of gestation. This suggested that the corpus luteum from EGF – treated rats were more functional than those becoming from control animals.

All in all, this work demonstrates that exogenous EGF might participate in the mechanisms underlying parturition, probably through an inhibitory effect over luteolysis.

S. 2.

ASSESSMENT OF 2,4-DICHLOROPHENOXYACETIC ACID AS RAT ENDOCRINE DISRUPTOR EXPOSED THROUGH DEVELOPMENT

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Exposure to toxicants during development is of particular concern because many feedback mechanisms functioning in the adult are absent and adverse effects may be noted at doses lower than those observed in the adult. Sexual maturation in the rats results from complex interactions of the hypothalamus, anterior pituitary and the sexual organs. There are growing concerns over the potential effects that may result from exposure to chemicals that have the potential to alter the normal functioning of the endocrine system and male/female reproductive function throughout the lifespan in laboratory animals, in wildlife and humans. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), its esters and salts has become a substantial environmental pollutant because of its extensive and intensive use since the early 1940s to the present. Although no environmental toxicity has been conclusively linked to 2,4-D, the literature shows that it is toxic not only for animals, but also for humans. Recently, we observed that 2,4-D administered daily to female Wistar rats from pregnancy day 16^{th} to the end of lactation period impaired neonates growth. This effect was not a consequence of maternal toxicity because dam body weight and food and water consumption were not modified during the treatment (Stürtz *et al*., 2000). In addition, we also demonstrated an induced long-term disruption in the male and female sexual behavior due to 2,4-D exposition (Stürtz *et al*., 2002). We propose that 2,4-D treatment during pregnancy and lactation caused several effects on the neonates by interfering either with hormonal mechanism involved in the masculinizing action of the CNS or by direct action of the herbicide on pups during development.

S. 3.

PITUITARY GABA B RECEPTOR SUBUNIT EXPRESSION: EFFECT OF SEXUAL DIFFERENTIATION

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In previous results we have shown a clear sexually dimorphic ontogenic expression of GABA B receptor subunits in rat pituitary membranes (Neuropharmacology 40:185-192:2001). In the present work the participation of sexual steroids in these events was investigated. Rat groups consisted of 8 day-old females (8F), 8F treated with 1 μg/day of testosterone propionate (TP) on days 1-4 of life(8F1TP), 8F treated with 100 μg TP on day 1 (8F100TP), 8F treated the anti-androgen flutamide [Flut: 2.5 mg/100g BW of pregnant mother in embryonic life (e17-e23) (8F-Flut)], 8 day-old males (8M), 8M castrated on day 1 (8MC), 8M treated with Fl (as above)(8M-Flut), 8M treated with Flut and castrated on day 1 (8MC-Flut), 15 day-old females (15F), 15F treated with 100 μg TP on day 1 (15F100TP), 15 day-old males (15M) and 15M castrated on day 1 (15MC). Animals were sacrificed on day 8 or 15 by decapitation, trunk blood and pituitaries were collected and frozen. GABA B receptor subunit expression was determined on pituitary membranes by Western Blot with antiserum Ab 174.1 which detects GABA B1a/b subunits, using α syntaxin expression to ensure comparable protein load. Hormones were determined by RIA.

As previously described, GABA B1a and GABA B1b expression was higher in 8F than in 15F or 8M [GABA B1a (AU): 8F: 0.98±0.08 vs 15F: 0.46±0.6 vs 8M: 0.56±0.02, $p<0.01$]. Treatment of females with 100 μg TP decreased GABA B subunit expression to male levels in 8 day-old females ($p<0.05$) but did not modify them in 15 day-old females [GABA B1a (AU): 8F100TP: 0.58±0.07, 15F100TP: 0.55±0.08]. Treatment of 8F with 1μg TP or Fl did not alter GABA B subunit expression. In 8M, Fl treatment, both alone or in combination with castration, increased subunit expression to 8F levels, $p<0.05$, though castration alone was not effective [GABA B1a (AU): 8MC: 0.67±0.06, 8M-Flut: 0.81±0.05, 8MC-Flut: 0.97±0.03). GABA B1b subunit expression followed the same pattern as GABA B1a. While 100 μg TP profoundly altered serum gonadotropins at 8 days of life, no such effect was observed with 1μg TP, though these last animals were also effectively adrogenized, as evidenced by lack of cycling and LH surge at adulthood.

We conclude that androgens acting both during late embryonic and early postnatal life modify pituitary GABA B receptor subunit expression, inducing sexually dimorphic patterns, and that only certain protocols of female androgenization are effective in inducing the male pattern.

S. 4.

EFFECT OF DISRUPTION OF THE DOPAMINERGIC D2 RECEPTOR ON THE HYPOTHALAMIC-PITUITARY AXIS

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We determined the consequences of the loss of D2 receptors (D2R) on the prolactin and GH-IGF-I axis using mice deficient in functional dopamine D2 receptors by targeted mutagenesis (D2R-/-). Body weight was similar at birth, but somatic growth was lower in male D2R-/- mice from 1-8 months of age and in D2R-/- females during the first 2 months. The rate of skeletal maturation, as indexed by femur length, and the weight of the liver and white adipose tissue was decreased in knockout male mice even though food intake and serum leptin levels were not altered. The serum GH concentration was significantly decreased during the first 2 months in knockout female and male mice, and IGF-I and IGF-binding protein-3 were also lower in knockout mice. Prolactin was significantly higher in knockout mice, and females attained higher levels than males. Pituitary cells from adult knockout mice had impaired basal GH release and a lower response to GHRH *in vitro,* while response to somatostatin was unaltered. We propose that the D2R participates in GHRH/GH release in the first month of life. In accordance, the D2R antagonist sulpiride lowered GH levels in 1-month-old wildtype mice. Our results indicate that lack of D2R alters the GHRH-GH-IGF-I axis, and impairs body growth and the fate of somatotrope population.

S. 5.

MOLECULAR BASES OF ACTIVATION AND MODULATION OF NICOTINIC RECEPTORS

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Ligand-gated ion channels allow rapid responses in the nervous system. Their strategic positions in the pathway of information flow makes them molecular targets for drugs and neurological diseases. The nicotinic acetylcholine receptor (AChR) has been the model for structure-function relationship studies on this superfamily. AChRs are composed of five homologous subunits. Each subunit contains an amino terminal extracellular domain, which carries the binding sites, and four transmembrane domains (M1-M4). ACh interacts with a ligand-binding site triggering a conformational change in the protein that results in the opening of an ion channel. The detailed structural mechanism of this process, which is known as gating, remains a mystery. Changes in gating kinetics due to mutations in the primary sequence lead to pathological processes, such as congenital myasthenic syndromes. The existing equilibrium among the different conformational states of the AChR (closed, open and desensitized) are perturbed by endogenous compounds and pharmacological agents. The relationship between their chemical scaffolds and mechanisms of action has not been yet identified. Our approach has been to combine genetically-modified AChR subunits with macroscopic current recordings and single-channel kinetic analysis to understand the molecular arrengements underlying channel gating and to elucidate the mechanistic bases for the modulatory action of clinically relevant drugs at AChRs. We identified residues in the less studied M1 and M3 domains as well as in the lipid-exposed M4 domain of the nicotinic receptor (AChR) that are involved in channel gating and described their mechanistic contributions. Our results revealed that these domains govern opening and closing rates in a subunit-selective manner. We have also clarified the fundamental mechanistic steps that are altered in several myasthenic syndromes associated with mutations in the AChR. Given that tricyclic antidepressants (TCAs) modulate LGIC and that hypercholinergic neurotransmission has been shown to be associated with depressed mood states, we investigated their actions at AChRs. Our results revealed that TCAs interact with resting (closed), open, and desensitized channels, do not affect significantly channel opening and closing rates, inhibit activation of resting AChRs, and that they may increase the rate of long-lived desensitization from the open. AChRs in nematode muscles are targets for anthelmintic chemotherapy. To gain new insights into structure-function relationships of the AChR and to contribute to the development of more selective therapies we studied the action of pyrantel and levamisole on mammalian AChRs. We showed that these drugs are weak agonists and open-channel blockers of mammalian AChRs. By acting at the binding site, they activate mammalian AChRs with low efficacy; the activated channels are then rapidly inhibited by sterical blockade of the pore.

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S. 6.

EFFERENT FEEDBACK CONTROL OF VESTIBULAR AND COCHLEAR SENSORY SYSTEMS: FROM MOLECULES TO PHYSIOLOGY

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The sensory epithelia of organs responsible for hearing (the cochlea) and balance (vestibular labyrinth) share a unique subset of hair cells which transduce mechanical stimuli into electrical signals. These cells are under the influence of efferent fibers originating in the brain, which modulate the dynamic range of vestibular and cochlear afferent fibers. Acetylcholine is the principal neurotransmitter released by efferent axons. The existent data suggest a central role for an atypical, nicotinic subtype of receptors (nAChRs) located at the synapse between efferent fibers and vertebrate hair cells. Over the recent years we have cloned two novel nAChR genes involved in hair cell physiology: α9 and α10. We demonstrate the existence of two functional nAChRs: homomeric α9 and heteromeric α9 α10. While both α9 and α9α10 nAChRs exhibit similar pharmacological profiles, the presence of α10 modifies key biophysical characteristics of α9 nAChRs. Thus, coinjection of *Xenopus* oocytes with α9 and α10 cRNAs results in acetylcholine-gated currents which are ~100-fold larger than those observed with homomeric α9 receptors. Moreover, α9 and α9 α10 receptors exhibit distinct current-voltage relationships, desensitization kinetics and responses to extracellular Ca^{2+} ions. Both α9 and α10 transcripts are observed in adult cochlear outer hair cells and sensory epithelia of the otolithic organs and the semicircular canals. However, while cochlear inner hair cells express α9 from embryonic through adult stages, α10 transcripts are only observed during early development, before the onset of hearing. Our results indicate that efferent modulation of vestibular and outer hair cell function occurs via heteromeric nAChRs assembled from both α9 and α10 subunits. While α9α10 receptors are also responsible for mediating synaptic transmission between transient efferent fibers and inner hair cells at early developmental stages, it is possible that any direct, lateral olivocochlear innervation of adult inner hair cells is mediated via homomeric α9 nAChRs.

S. 7.

FLUID TRANSPORT ACROSS THE ISOLATED BOVINE CILIARY BODY

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The contribution of secretion and ultrafiltration to aqueous humor production remains controversial. Previously, we have determined that the Cl concentration in the bovine aqueous humor (AH) is higher than in plasma (P). Similar results were reported for the human AH/P Cl ratio. According to these results, the possible existence of an active Cl transport mechanism between P and AH in man, was postulated. In addition, it has been reported that the predominant ion being transported in the isolated iris-ciliary body (ICB) of bovine is Cl; while in the isolated ICB of rabbit is bicarbonate. These findings suggest that the bovine ICB may be a better model to study ionic and fluid transport for its implications in the physiopathology of glaucoma. In order to determine the contribution of active transport (secretion) to the total production of AH, we designed a system in which fluid transport could be measured without the influence of the intra-ocular pressure gradient. The isolated ICB is clamped between 2 pairs of O rings, exposing most of the ciliary processes to the Tyrode's bathing solutions.. The fluid transport was measured by the change in the height of a capillary tube connected to one of the hemi-chambers. Our objective was to determine the aqueous humor formation in control conditions, after changes in the ionic concentration of the solutions and after the addition of some pharmacological agents to the bating solutions. Our results show than with normal Cl concentrations (113.5 mM) on both sides of the ICB, fluid secretion was 4.8 ± 1.5 µl/hr. When Cl was omitted from the solution on the blood side of the ICB, the fluid secretion was reduced by 60%. Similarly, addition of Furosemide (10^{-4} M) to the blood side of the ICB, reduced fluid secretion to 0.3 µl/hr. Fluid transport was reduced by ouabain (10^{-4} M) by 60%, indicating its dependence on the Na:K ATPase. When the in-chamber-measured control values are corrected for the exposed surface of the ICB, they represent about 14% of the *in vivo* rate of 180 µl/hr. These measurements show for the first time that the isolate bovine ICB can transport fluid. Thus, by pharmacologically inhibiting the secretion component of AH production, it is possible to reduce the intra-ocular pressure.

S. 8.

CELL VOLUME REGULATION IN MAMMALIAN COLLECTING DUCT: ROLE OF WATER CHANNELS

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The mammalian collecting ducts (CD) play a central role in the final regulation of urine volume and composition, as well as, salt and water balance. To accomplish this task it is crucial the presence of external osmotic gradients and arginine-vasopressin hormone modulation (AVP). Increases in external osmolality induces AVP liberation which acts increasing apical membrane water permeability, via cAMP cascade, through the insertion of specific water channels called aquaporin-2 (AQP2). In this case, water will move following the transepithelial osmotic gradient through the apical AQP2 and basolateral resident AQP3 and AQP4. Recently it was proposed that in addition to the AVP-cAMP signaling cascade, a further pathway activated by elevated effective osmolality is crucial for the expression of AQP2 and this response would be mediated via the tonicity-responsive elements. These osmotic water movements through epithelial cells would result in changes, at least transients, in cell volume. In order to maintain volume, cells require important volume-regulatory mechanisms generally linked to ion transport activity and to water movements. Although the main ion transporters have been identified, both their activation and the mechanisms by which cells sense these changes are poorly understood. Moreover, until today no studies were performed in order to identify water pathways during cell volume regulation and its consequences in transepithelial water transport. Consequently, the central aim of our work is to clarify the molecular mechanisms involved in osmotic water transport either at cellular and transepithelial level. For this purpose we have used the cortical collecting duct cell line ($RCCD_1$) wild-type and transfected with AQPs. Our cellular studies showed that hyper-osmotic shocks induces cellular shrinkage and activation of RVI mechanisms (regulatory volume increase) in both cells lines. However, AQPs expression clearly increased cell volume recovery. During exposition to hypo-osmotic shocks cell volume is rapidly increased in AQP expressing cells but no regulatory volume decrease mechanisms were activated (RVD). Transepithelial water movement studies showed that in the absence of AQPs osmotic water movement is dependent of cellular metabolism and RVI mechanism would be crucial. Cells transfection with AQPs included an additional via to water pathway independently of cellular metabolism. Moreover CCD cells show an asymmetrical behavior to osmotic gradients: Hypertonic shocks induced J_V rectification independently of AQPs expression. Hypotonic shocks only induced J_V rectification in the presence of AQPs. We conclude that cell volume osmotic changes may initiate a signaling mechanism that would modulate apical or basolateral membrane permeability to protect cells from osmotic shocks

S. 9.
ROLE OF ENDOTELIUM IN THE VASCULAR PHYSIOPATOLOGY
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During the last two decades, it has become evident that the vascular endothelium is an active paracrine, endocrine and autocrine organ. It is indispensable for the regulation of the vascular tone and maintenance of vascular homeostasis by modulating the production of vasodilator and vasoconstrictor agents. Endothelium dysfunction is characterized by a reduction of the bioavailability of vasodilators, particularly nitric oxide (NO), whereas endothelium-contracting factors are increased, denoting impairment of endothelium-dependent vasodilation. A large body of evidences described that reactive oxygen species (ROS), in particular superoxide anion radicals ($\bullet O_2^-$), are involved in the control of vascular tone. Under normal physiological conditions, the antioxidant defense system is able to minimize the levels of ROS, thus preserving NO bioavailability and maintaining a normal vascular tone. However, in vascular pathology the production of ROS from both endothelial and smooth muscle cells is excessive and outstrips the endogenous antioxidant defense mechanism inducing oxidative stress. Then, NO inactivation is accelerated inducing endothelial dysfunction and increased vascular constriction.
It has been reported that either the increased oxidative stress or reduced antioxidant defenses critically contribute to the pathogenesis of vascular diseases such as diabetes, dyslipidemia, hypertension and heart failure. On the other hand, endothelium dysfunction also involves a specific state of "endothelial activation", which is characterized by a proinflammatory, proliferative, and procoagulatory milieu that favors all stages of atherogenesis. Therefore, the presence of endothelial dysfunction represents the earlier vascular modification, preceding the appearance of atherosclerotic lesions and may serve as a predictive marker of unfavorable cardiovascular prognosis.
Taken together, current evidences suggest that endothelial status may be regarded as an integrated index of all atherogenic and atheroprotective factors reflecting their propensity to develop atherosclerotic disease.
Interventions, like risk factor modification and treatment with different drugs, including statins, angiotensin-converting enzyme inhibitors, anti-inflammatory and antioxidants, may improve endothelial function and thereby, potentially improve the prognosis of cardiovascular disease. Hence, given its reversibility, endothelial dysfunction may be an attractive primary target in the effort to optimize individualized therapeutic strategies to reduce cardiovascular morbidity and mortality.

S. 10.
ENDOTHELIAL DISFUNCTIONS AND DIABETES MELLITUS
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During the early stages of Diabetes Mellitus, as well as in previous periods of diagnosis (TAG and GAA), endothelium disfunction signs were detected (DE) such as: ↓ ON (nitric oxide), ↓ PC (prostaciclin), ↑ ET (endothelin), ↑ Adhesion molecules, ↑ procoagulant factors (PAI-1, TxA2, FVW, Fibrinógen).
It was proposed that hyperglycemia intensity and duration, where strongly related to the illness macrovascular development. But genetic factors and independent risks factors associated to them, where the response determinants to the damage and the responsible for the clinical course, the extension and the progression of the vascular disease.
Hyperglycemia, primary metabolic defect, leads to a second metabolic alteration (increase on sorbitol from the non-enzymatic glication of the proteins, etc.) that generates reversible initial complications (thanks to the metabolic cellular alteration); which expresses itself in an increase on vascular permeability and decrease on vascular dilatation with a morphologically intact endothelium. Glycemia normalization reverts this alterations.
Irreversible complications come from structural modifications in stable molecules that produce a definitive damage of the cellular function.
Hyperglycemia is related to the DE through the: stimulation of the polyol pathway, increase on the advanced products of the intracellular glication, kinase C protein activation, increase of the oxidative stress. This mechanisms are capable of interfering with the vascular cellular functions modifying the production patents of factors like paracrine and autocrine (growth factors, vasoactive factors, coagulant factors and those involving molecular adhesion) which interfere with the physiological refill of the vascular walls leading to an abnormal vascular remodel with tone and permeability alterations; also with coagulant disruptions.
Therefore, endothelial disfunction was displayed on patients with diabetes type 1 and 2, and it´s implied in the development of vascular complications of the illness. Hyperglycemia participation in its etiopatogenia is certain, however, it doesn't mean it´s the only implied factor because in patients with insulinoresistencia, the DE was detected before the diabetes diagnosis. The hypothesis that proposes that the DE could be the cause of the insulinoresistencia syndrome wasn't fully revealed. As regards treatment, suitable glycemic control have proved to diminished the incidence and progression of the microvascular and macrovascular complications in both types of diabetes. The analyzed drugs impacts in the endothelial function deserve even greater investigations.

S. 11.
ENDOTHELIAL DYSFUNCTION AS A PREDICTOR OF CORONARY HEART DISEASE
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Recent evidence clearly establishes the endothelium as the body's largest endocrine organ. Its effect regulate vascula smooth muscle tone, platelet adhesion and aggregation, local clotting and vascular growth.
The endothelium serves a dual role in the control of vascular tone: its secretes relaxing and constricting factors and the interaction between them provides a local mechanism that regulates vascular tone. The endothelium also exerts a dual effect on vascular growth. Endothelial cells can iniciate both angiogenesis and abnormal growth of smooth muscle.
Normal endothelial function is an inhibitory mode: its inhibits smooth muscle contraction, platelet aggregation, vascular smooth muscle growth, trombosis and monocyte adhesion, which is an early abnormality in the generation of an atherosclerotic plaque.
Evidence suggests that in established atherosclerosis there is a characteristic defect in vessel vasomotion. We know now about the interaction of endothelial dysfunction with coronary risk factors, that determine vasocontriction instead of vasodilation with different stimulus.
A lot of studies have demostrate a useful ralation between brachial and coronary artery endothelial function. This observation allows for the noninvasive stdy of brachial artery function as a surrogate measure of coronary function.
Numerous factors have been identified playing a role in the endothelial dysfunction: dyslipidemia, hypertension, cigarette smoking, menopause, diabetes, diet, sedentary life, aging.
The endothelial evaluation in a non invasive way, could be a possible tool to evaluate individuals in the primary prevention setting, in order to identify people in whom we need to be more aggressive with medical strategies.

S. 12.
ENDOTHELIUM, NITRIC OXIDE AND ARTERIAL HYPERTENSION
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Increased dietary sodium is considered to be one of the major environmental factors inducing hypertension in many populations. By this way, the acute and large reductions in salt intake have shown to cause a significant fall in blood pressure only in hypertensive patients while no significant changes were observed in normotensive individuals. More recently, it was shown in essential hypertensive subjects under an acute sodium restriction, a significant fall in blood pressure accompanied by small rises in plasma renin activity and aldosterone but not in normotensive controls. In addition, a subgroup of salt sensitive individuals was recently identified and named non-modulators since, when moved from a low to a high sodium intake, they failed to increase the effective renal plasma flow and decrease filtration fraction by a percentual ≥ 30%. In these subjects, the renin angiotensin system was overacting and the treatment with angiotensin converting enzime inhibitors reverted the non-modulation of the renal sodium handling. The renin-angiotensin-aldosterone system, in addition to its endocrine role in maintaining systemic perfusion and renal function, also exerts local effects on the heart, vasculature and kidneys. In addition, it is able to interrelate with kinins to regulate renal perfusion and sodium balance. In this way, we have previously shown, in non-modulating salt sensitive hypertensives, that urinary kallikrein like activity was lower than in normotensive controls and associated to higher plasma renin activity, thus suggesting that both systems are implicated in this model of hypertension.
On the other hand, in subjects with a positive family history of hypertension it is well documented that they are able to develop hypertension throughout their life span. Possible mechanisms involved are, among others, the salt sensitivity and non-modulation of the renin angiotensin system. In this regard, it has been shown in young normotensive men with family history of hypertension, an inadequate suppression of aldosterone secretion after high salt intake and angiotensin II stimulation.
Furthermore, recent observations postulate a physiologic equilibrium between the vasoconstrictor effect of angiotensin II and the vasodilator action of NO, the prostaglandins Na^+ reabsorptive effects and the natriuretic action of kinins to the control of the vascular tone and sodium homeostasis in the kidney. By this way, one of the main focuses of interest is the activity and regulation of the renin-angiotensin system and the influence of renal vasodilator systems, such as prostaglandins, kinins and NO generation, in the non-modulating condition. By this way, data, taken from essential hypertensives and offsprings of hypertensive parents, will be presented to show how modulating and non-modulating individuals are identified and to confirm the presence of an hormonal inbalance between the renin-angiotensin-aldosterone and the kallikrein-kinin systems and the involvement of NO and oxidative stress. Finally, data will also be provided showing the positive effects of ACEI to normalize renal hemodynamics and NO bioavailability.

S. 13.

DIAGNOSTIC AND PROGNOSTIC VALUE OF OXIDANT STRESS MARKERS IN THE PREVENTION AND FOLLOW-UP OF THE CARDIOVASCULAR DISEASE

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The vascular pathology is still one of the major causes of mortality in western countries and the great majority of subjects with illness such as hypertension, diabetes, dyslipidemic and/or heart failure still remain without diagnosis. This data gave support for the need to adopt preventive diagnostic measures and to improve therapeutic strategies for cardiovascular pathologies.
The term endothelial dysfunction has been used to refer to several pathological conditions, including altered anticoagulant and anti-inflammatory properties of the endothelium, impaired modulation of vascular growth, and disregulation of vascular remodeling. All this findings were specially associated to a decline in nitric oxide (NO) bioactivity in the vessel wall, occurs early in vascular disease and may be caused by a decreased production, a less sensitivity to nitric oxide or accelerated NO degradation by reactive oxygen substances (ROS). The oxidative stress is an early event leading to endothelial dysfunction and the consequent cardiovascular illness. Several experimental and clinical studies pointed out that oxidant stress is responsible of a quick NO inactivation promoting peroxynitrite ($OONO^-$) formation which has oxidative properties and promotes endothelium dysfunction increasing its sensitivity to vasoconstriction.
Clinical assessment of endothelial function is based on invasive or non-invasive procedures. Between invasives, it has been reported that both coronary and forearm endothelial dysfunction may predict long-term atherosclerotic disease progression and cardiovascular events rate. Initially, endothelial function in humans was assessed by intracoronary study and by strain-gauge plethysmography, for direct measurement of forearm blood flow (FBF) during intra-arterial infusion of acetylcholine (ACh) or other vasodilating agents. Celermajer and coworkers proposed a non-invasive detection of endothelial dysfunction based on the assessment of flow-mediated dilation (FMD) after reactive hyperemia, in the brachial or femoral artery, using high resolution ultrasound. Even if this last method involves a non invasive technique and has other methodological advantages, it shares with the formers the low sensibility in earlier states of vascular disease, being unable to denote an impaired vascular response. Near the beginning of vascular disease, the endothelium is able to compensate for the oxidant stress and preserves the normal vascular function, but only for a short period of time. After that, this capability is exceeded and functional failure expressed. Thus, the functional study of endothelial function not always reveals the actual endothelium status. Therefore, a complete evaluation of the oxidant stress and cellular antioxidant condition must been taken into account as a useful tools not only to promote a complementary preventive strategy in the cardiovascular pathology evaluation, but also for reversion or control of endothelial dysfunction.

S. 14.

ROLE OF MELATONIN IN REGULATION OF IMMUNOLOGICAL AND INFLAMMATORY PROCESSES

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This presentation discusses experimental evidence indicating that arthritis disrupts circadian organization, mainly derived from animal studies employing Freund's complete mycobacterial adjuvant (FCA). The defense response to antigenic challenge, mediated in part by cytokines, includes changes in chronobiological central nervous system (CNS) function, like depressed daily activity, superficial sleep or anorexia. Interferon (IFN)-gamma receptors are detectable in the central circadian pacemaker, the hypothalamic suprachiasmatic nuclei (SCN), at a time when capacity for photic entrainment of the pacemaker became established. Disruptive effects of systemic injection of IFN on circadian rhythms of locomotor activity, body temperature and clock-gene mRNA expression have been documented. In a study from our Laboratory on the effect of the intracerebroventricular (i.c.v.) injection of IFN-gamma in hamsters, animals received IFN-gamma or saline at "Zeitgeber" time (ZT) 6 or ZT 18, with ZT12 defined as the time of light off. I.c.v. administration of IFN-gamma at ZT 6 produced a significant phase advance in acrophase of rhythm, an effect not seen with injection at ZT 18. IFN-gamma depressed mesor value of rhythm significantly; the effect was seen both with ZT 6 and ZT 18 injections. The results support the view that the circadian sequels arising during the immune reaction can rely partly on central effects of IFN-gamma. In the last years we have also examined a number of immune and neuroendocrine circadian rhythms in Freund´s complete adjuvant (FCA)-injected rats, both in the preclinical phase of arthritis (2-3 days after FCA injection) as well as in the acute phase of the disease (18 days after FCA injection). In arthritic rats, the 24-h organization of immune and neuroendocrine responses becomes altered. A hormonal pathway involving the circadian secretion of melatonin and a purely neural pathway including as a motor leg the autonomic nervous system innervating the lymph nodes were identified. Significant effects of immune-mediated inflammatory response on diurnal rhythmicity of adenohypophysial and hypophysiotropic hormones occurred in arthritic rats. Melatonin treatment prevented alteration of 24-h rhythms of serum ACTH, prolactin and LH in rats injected with FCA. In addition, melatonin treatment prevented alteration of the 24-h variation in hypothalamic serotonin and dopamine turnover during the preclinical phase of Freund's adjuvant arthritis in rats. A comparison between the inflammatory and immune responses elicited by physiological and pharmacological doses of melatonin in FCA arthritis was performed. Pinealectomized rats exhibited a significantly less pronounced inflammatory response, which was restored to normal by a low melatonin dose (0.3 μg/ml of drinking water) whereas a high melatonin dose (30 μg/ml), that resulted in a 50-60-fold increase in plasma melatonin, augmented the inflammatory and immune response. These results should be considered in the light of recent reports that rheumatoid arthritis patients have increased nocturnal plasma levels of melatonin and that their synovial macrophages respond to melatonin with an increased cytokine production. In our studies on submaxillary lymph nodes of rats, melatonin augments $CD4^+$ lymphocytes and decreased $CD8^+$ lymphocytes. It it is not yet clear whether melatonin acts only on Th-1 responses or also on Th-2 responses

S. 15.
CHANGES IN CATECHOLAMINES AND GLUCOCORTOCOIDS IMMUNOREGULATION INDUCED BY STRESS EXPOSURE

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Stress has long been associated with altered homeostatic state of the organism including behavioral, endocrine and immunological changes. The consequences of the physiological stress response are generally adaptive in the short run, but can be damaging when stress is chronic and long-lasting. Activation of both the hypothalamic-pituitary-adrenal axis and the sympathetic-adrenal-medullar axis plays a key role in the response to psychological stress. In this context, we investigate the effect of acute and chronic stress exposure on the antibody production and the participation of catecholamines and glucocorticoids as mediators of stress on the immune response. A 2-h restraint treatment was used as an acute stress model. For chronic stress, we choose the chronic mild stress (CMS) model. This is a heterotypic model that implicates a chronic low-grade stress offering a reasonable approximation to the diverse stresses of daily life. The results indicated that humoral response to a T-cell dependent antigen. but not to a T-cell-independent antigen, is increased after acute stress exposure. In contrast, chronic stress had an immunosuppressive effect on IgG production without affecting IgM production, indicating an impaired isotype switching. This impairment was not only due to a delay in the kinetics of IgG production, since a rise in the antibody titer was not observed afterwards. Regarding the role of catecholamines and corticosterone in stress effects, we found that their levels were increased in acute situations but they were not modified after prolonged stress periods. These data suggest that there might be an adrenal (cortical or medullar) activation after acute stress but hormonal levels were adapted after long-lasting stress exposure. In fact, the habituation of the HPA axis after prolonged stress situations has been described. Indeed, we have observed an increase in these stress hormones during the first 2 weeks of CMS exposure that return to basal values after 3 weeks. On the other hand, lymphocyte sensitivity to the stress hormone effect is also altered. Catecholamines and corticosterone exert either a stimulatory or an inhibitory effect on lymphocyte reactivity depending on the concentration tested. Acute stress induced an enhancement of lymphocyte's sensitivity to the stimulatory effect of stress hormones, whereas chronic stress increased lymphocyte's sensitivity to the inhibitory effect of these hormones. These findings suggest an important role for adrenal hormone receptors on lymphocytes and indicate a physiological and adaptive mechanism through which epinephrine and corticosterone could act as mediators for the differential effects of stress on the immune system. Since stress is a common aspect of modern life and it participates in the etiology of many diseases, the emerging results of these studies will be helpful to improve and develop new and more efficient biomedical treatments.

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S. 16.
DIFFERENTIAL EFFECT OF THYROID HORMONES ON PROTEIN KINASE C (PKC) AND NITRIC OXIDE SYNTHASE (NOS) ISOENZYME EXPRESSION IN NORMAL AND TUMOR T LYMPHOCYTES

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Thyroid hormones exert a broad range of effects on development, growth and metabolism including the regulation of immune responses. Findings on the modulation of immune function by thyroid hormones are mainly based on the existence of thyroid hormone receptors on normal lymphocytes and lymphoblastoid cells and on the immune alterations observed in physiological and pathological fluctuations of thyroid hormones. However, the direct role that these hormones exert on lymphocyte activity as well as the mechanisms underlying their effects were not studied. By other hand, protein kinase C (PKC) is crucial for T lymphocyte activation and proliferation, while nitric oxide synthase (NOS) may function both as an activator or inhibitor of T cell apoptosis. Recently, we have studied both enzymatic activities in normal and tumor T lymphocytes and found differential participation of PKC and NOS isoenzymes in normal versus tumor cell growth.

On this basis and to further assess thyroid hormone direct actions on the function of normal and tumor T lymphocytes, their effect on cell proliferation was evaluated *in vitro* on resting and mitogen-stimulated normal T lymphocytes and on the T lymphoma BW5147. Also, L-thyroxine (T4)–mediated actions on PKC and NOS activities were studied. Thyroid hormones increased tumor, but not normal, cell proliferation and potentiated mitogen-stimulation of T lymphocytes. Twenty-four hours incubation with T4 induced a rise in total and membrane-associated PKC activities on both cell types. Also, T4 potentiated mitogen-induced PKC translocation in normal T lymphocytes and lead to a rapid non-genomic effect on tumor cells. T4 enhanced atypical PKC ζ expression on BW5147, but increased classical PKC isoenzymes on mitogen-stimulated normal T cells. Additionally, T4 augmented NOS activity only on tumor cells. This effect was downstream PKC, as both PKC inhibitors as well as the intracellular delivery of an anti-PKC ζ antibody inhibited NOS activation. These results show, for the first time, differential intracellular signals involved in T4 modulation of normal and tumor lymphocyte physiology. Thyroid hormone administration in selected cases of congenital hypothyroidism that result in severe immunodeficiency has been indicated. Also thyroid hormone deficiency may significantly alter the balance between malignant tumor viability/growth versus cell death. The best understanding of the biochemical mechanisms induced by thyroid hormones in the control of lymphocyte proliferation would be useful to characterize their physiological role on immune processes and give basis for their use in cases on immune dysfunction and neoplastic disorders.

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1.

BIOGUIDED ISOLATION OF ANTITUMORAL COMPOUNDS PRESENT IN *Tilia cordata Mill.* FLOWERS

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Extracts (E) obtained from flowers of ***Tilia*** species have been used along centuries as antiaxient, psicological depressor and in colds, chills and bronchitis. In a previous investigation we demonstrated that aqueous, dichloromethanic (CM) and ethanolic E obtained from ***Tilia c*** flowers have antiproliferative action on BW 5147 lymphoma. The aim of this work was to: isolate and identify the active compounds present in CME, to analyze the effect of purified fractions and isolated compounds on normal T lymphocytes and on lymphoma BW 5147 proliferation. The effect on cells proliferation was determined by tritiated thymidine uptake on microculture. TLC analysis of CME shown the presence of phenyl propanoids among them, scopoletin (SC) was identified. By sephadex purification of CME a fraction (F6) rich in terpenes was also obtained. On tumoral cells: CME, SC, F6 exerted antiproliferative action (CME EC_{50}: 4.84 ± 0.3 µg/ml, SC EC_{50}: 94.62 ± 5.0 µg/ml, F6 EC_{50}: 3,8 ± 0.2), CME and SC induced apoptosis without affecting cell viability. On normal cells: CME, SC, F6 exerted antiproliferative action (CME EC_{50}: 14.12 ± 0.8 µg/ml, SC EC_{50}: 8.70 ± 0.5, F6 EC_{50}: 205 ± 18). CME and SC did not affect cell viability and induced apoptosis at high concentrations. CME present different antiproliferative compounds being the terpenes more selective on tumoral cell than scopoletin.

2.

ANTIFUNGAL AND ANTIBACTERIAL PROPERTIES OF *Ibicella lutea* VAN ESELTINE

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Ibicella lutea Van Eselt., is a quasi-carnivorous plant known in South America as "Cuerno del Diablo" (Devil´s horn) which grows freely in sandy places and is used in Uruguay in popular medicine as an antiseptic for the treatment of eye and skin infections. In previous studies made in Uruguay a stearic acid glycoside: 11-*O*-(6'-*O*-acetyl-β-D-glucopyranosyl)stearic acid] was the only constituyent isolated and identified. In view of the pharmaceutical interest of this species, we evaluated the antifungal and antibacterial properties of the main constituent isolated from the acetone extract of a sample of this species collected in the province of San Luis, Argentina. The triterpene 20 *S*, 24 *R*-epoxy-3α-acetoxy-1β, 12 β, 25- trihydroxydammarane was tested *in vitro* against several Gram-positive and Gram-negative bacteria and 10 fungi in the agar dilution method and showed antibacterial activity (MIC: 100 µg/ml) against *Staphylococcus aureus* and antifungal properties against dermathophytes (MIC: 100 µg/ml against *Trichophyton rubrum*, 125 µg/ml against *T. mentaagrophytes* and 125 µg/ml against *Microsporum gypseum*). The antibacterial and antifungal activities exhibited by the main active principle isolated from *I. Lutea* provides the pharmacological basis for the traditional use of this plant. The antibacterial and antifungal activities exhibited by the main active principle isolated from *I. Lutea* provides the pharmacological basis for the traditional use of this plant.

3.

INHIBITION OF *Trypanosoma cruzi* BY PLANT EXTRACTS USED IN CHINESE MEDICINE

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All the drugs recomended for treatment of Chagas disease have serious limitations including effectiveness and important drug-related side effects. New drugs are urgently needed. In previous SAFE meetings we have presented an screening method for potential antiprotozoan drug. We assessed in this work the effect of extracts obtained from 17 plants used in traditional chinese medicine. These extracts were tested *in vitro* with epimastigote form of *T. cruzi*, clone Bra C_{15} C_2, at 27`C in F-29 medium at the concentration of 100 µg/ml in axenic cultures. Allopurinol was used as reference drug. Seven plant extracts showed inhibitory activity of less than 25%. *Pueraria lobata, Mahonia bealei, Dictamus dasycarpus, Kochia scoparia, Sophora flavescens* and *Ligustrum lucidum* showed effects with inhibition between 25% and 60%, mean while *Lithospermum erythrorhizum, Saussurea lappa, Melia toxadan* and *Cinnamomum cassia* showed the greatest activity of 100% of inhibition. The IC_{50} of these extracts were: 0.4µg/ml, 2.4µg/ml, 1.8µg/ml and 3.9µg/ml respectively. These activities are greater than Benznidazole (IC_{50}≅50 µg/ml), the current drug used for treatment of Chagas disease. The MTT assay was made and did not show citotoxical activity. These results allowed us to suggest that *L. erythrorhizum, S. lappa, M. toxadan* and *C. cassia* could be a source of new compounds clinically active against *T. cruzi*.

4.

STRUCTURE-ACTIVITY RELATIONSHIP OF FLAVONOIDS ON CHRONIC INFLAMMATION

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Flavonoids are important constituents of the human diet, and there are found in fruits, vegetables and in several medicinal plants. Some reports showed that they had anti-inflammatory activity. The ability of certain flavonoids to inhibit the cyclooxigenase or promotion of scavenging activity may contribute to the anti-inflammatory activity. It seems to be directly related to the number of hydroxyl group at the ring B of their structure (Husain *et al*, 1987). The aim of this study was examine the structure-activity relationship of flavonoids on chronic inflammation model. **Material and Method:** Wistar rats (130-150 g) were implanted with a sterile cotton pellet into the dorsal area. They were divided in 30 groups and were administered during six days subcutaneously with: vehicle (control), dexamethasone 7 mg/kg (standard) and 30 flavonoids (25 mg/kg). At seven days granulomas were dissected out and weighed. The results were compared against with the control group. **Results:** Only 20 flavonoids inhibited induced granuloma and we observed that compounds carrying a cathechol or guaiacol-like B ring (3', 4'-dihydroxy or 3'hydroxy-4'methoxy or 3'methoxy-4'hydroxy) showed a significative statistical activity; 3', 4'-dimethoxy derivatives were less potent. **Conclusion:** Flavonoids are capable of inhibiting the development of granuloma induced by cotton pellet, especially when a cathechol or guaiacol-like B ring is present, while the involvement of ring A is still unclear.

5.

ANTINUTRIENTS IN *ACACIA VISCO* LEAVES AND EFFECT OF BLOCKING SULFHYDRYL GROUPS ON METHANOLIC EXTRACT ANTI-ULCEROUS ACTIVITY

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Antinutrients in *Acacia visco* leaves and the effect of blocking sulfhydryl groups with N-ethylmaleimide (NEM) on the gastric anti-ulcerous activity of their methanolic extracts (*A.v.*m.ex.), were studied. Antinutrients: hemagglutinins and hemolysins according to C. Do Prado (1980) and saponins to WHO/PHARM (1992). The gastric anti-ulcerous activity was evaluated according to Robert *et al* (1979): male Wistar rats, 200-220 g, deprived of food for 24 h, were given: saline solution (*p.o.*) (normal and ulcer control groups), 200 mg/Kg *A.v.*m.ex. (*p.o.*) (experimental group), 20 mg/Kg omeprazol (*p. o.*) (reference group) and NEM group: 10 mg/Kg NEM (s. c.) and after 30 min. 200mg/Kg *A.v.*m.ex. (*p.o.*). One hour later, absolute ethanol was administered (*p.o.*) to all groups (except normal group). The ulcer grade was evaluated after 1 hour according to Marazzi, Uberti and Turba scale. Statistical analysis was performed according to ANOVA-Tukey-Kramer. Results: hemagglutinins were not detected, foam index 200, hemolysis title 1/8. Anti-ulcerous activity: *A.v.*m.ex.200mg/Kg ($p<0.001$), omeprazol ($p<0.001$), NEM group ($p<0.001$), vs. ulcer control. NEM group vs. 200 mg/Kg *L.d.*m.ex. ($p>0.05$). Conclusions: In *Acacia visco* leaves, the hemolytic activity and foam index could be attributed to the presence of saponins. Sulfhydryl groups would not be involved in the anti-ulcerous activity of Acacia *visco* leaves methanolic extracts.

7.

IN VITRO* ANTIBACTERIAL ACTIVITY OF DEHYDROLEUCODINE AGAINST *Helicobacter pylori

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Dehydroleucodine (DhL), was isolated from *Artemisia douglasiana* Besser, known as "matico" and commonly used for its digestive properties. The antibacterial activity of DhL was assayed on six strains of *H. pylori* isolated from gastric biopsies and the reference strain *H. pylori* NCTC 11638, and was evaluated by agar dilution method. Serial dilutions of the drugs ranging from 0.008 to 128 mg/liter were prepared in Mueller-Hinton agar supplemented with 7% horse blood. Plates were incubated for 3 to 5 days, and the MIC was recorded as the lowest concentration of the drugs inhibiting visible growth. DhL showed good activity against all strains tested with MICs ranging from 1 to 8 mg/L.
In other study, twenty four hours before the experiment, Wistar rats were fasted and the duodenum was ligated (Melchiorri *et al*., 1997). Absolute ethanol was employed as ulcerogenic agent. DhL reduced ethanol-induced gastroduodenal damage in rats ($p<0.05$). The results presented indicate that DhL prevents the formation of gastric and duodenal lesions induced by absolute ethanol in ligated rats, and this compound has significant antimicrobial properties against *H. pylori*. DhL could represent an useful tool in relieving digestive disorders.

6.

GASTRIC ANTI-ULCEROUS ACTIVITY OF METHANOLIC EXTRACTS OF *LARREA DIVARICATA* LEAVES. EFFECT OF BLOCKING ENDOGENOUS SULFHYDRYL GROUPS

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The gastric anti-ulcerous activity of *Larrea divaricata* leaves methanolic extracts (*L.d.*m.ex.) and effect on the anti-ulcerous activity of blocking endogenous sulfhydryl groups with N-ethylmaleimide (NEM), were investigated. Wistar rats, both sexes, 200-230 g, deprived of food for 24 h., were used. The gastric anti-ulcerous activity was evaluated according to Robert *et al.* (1979). Saline solution (normal and ulcer control groups); 50, 100, 200, 300 and 400 mg/Kg *L.d.*m.ex. (experimental groups); 20 mg/Kg omeprazol (reference group) were administered (*p.o.*); NEM group:10 mg/Kg NEM (s.c.) and after 30 min. 300mg/Kg *L.d.*m.ex. was administered (*p.o.*). After one hour, absolute ethanol was administered *(p.o.*) to all groups (except normal group). The ulcer grade in each group was evaluated 60 min. later according to Marazzi, Uberti and Turba scale and was expressed as ulcer index. Statistical analysis was performed according to ANOVA with subsequent comparison by Tukey-Kramer.
Results: anti-ulcerous activity: *L.d.*m.ex. 50 and 100 mg/Kg ($p>0.05$), 200 mg/Kg ($p<0.05$), 300 and 400 mg/Kg ($p<0.001$), omeprazol ($p<0.001$), NEM group ($p<0.001$), vs. ulcer control. NEM group vs. 300 mg/Kg *L.d.*m.ex. ($p>0.05$). Conclusions: methanolic extracts of *Larrea divaricata* leaves exhibited a dose-dependent gastric anti-ulcerous effect, the sulfhydryl groups would not be involved in the anti-ulcerous action.

8.

NITRIC OXIDE AS MEDIATOR OF *Artemisia douglasiana* BESSER GASTRIC CYTOPROTECTION

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Artemisia douglasiana Besser, known as "matico", is commonly used for its digestive properties. Gastroduodenal cytoprotective activity and mechanism of action by *Artemisia douglasiana* Besser extract were investigated. Role of nitric oxide (NO) in the gastroprotection induced by *A. d*ouglasiana was evaluated.
Methods and Results: Twenty four hours before the experiments, Wistar rats were fasted and the duodenum was ligated (Melchiorri *et al*., 1997). Absolute ethanol was employed as ulcerogenic agent. *A. douglasiana* reduced ethanol-induced gastroduodenal damage. L-NNA, NO synthase inhibitor, antagonised gastroprotective activity of *A. douglasiana*. This effect was reversed by L-Arg, but not D-Arg.
Conclusion: *Artemisia douglasiana* Besser prevents the formation of gastric and duodenal lesions induced by absolute ethanol in ligated rats. These findings suggest that gastroprotective mechanism of *A. douglasiana* depends on NO.
These facts support the use in traditional medicine of *A. douglasiana* to treat digestive disorders. We conclude that the protection by *A. douglasiana* against ethanol-induced gastroduodenal injury is due, at least in part, to NO activity.

9.

EFFECT OF *Aristolochia argentina* ON INTESTINAL TRACT IN MICE

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Aristolochia argentina (family Aristolochiaceae) is popularly know as "charrúa". This species develops a strong underground system of subterranean stems and roots. The roots of this plant are used in folk medicine. Their infusions and tinctures are reputed to have antidiarrheic and astringent properties. The aim of this study was to assess the antidiarrheal effect by studying the inhibition of the effects of castrol oil and the effect on small intestinal transit in mice. Infusions (I) (10, 20%) and decoctions (D) (10, 20%) of the roots were prepared. Small intestinal transit in mice was tested using the charcoal method. In control mice, 20 min. after its intragastric administration, the charcoal meal travesed 54.9 ± 1.79% of the total length of the small intestinal transit. I and D significantly inhibed small intestinal transit: 10%I: 43.82 ± 2.9%; 20%I: 44.39 ± 1%; 10%D: 47.24 ± 2.1%; 20%D: 37.41 ± 2.24% (p<0.01). Antidiarrheal effect: Three hours after castrol oil administration all control mice produced copious diarrhea and the total score was 10. The treatment with I and D prevents diarrhea. The total scores of 10 and 20%I were both 3; and the total scores of 10 and 20%D were 3 and 4 respectively (p<0.05), showing that they produce an inhibitory action on intestinal functions. In conclusion, the results of the present study provide a scientific validation for the popular use of the medicinal plant studied.

10.

DIURETIC EFFECT OF *Cyclolepis genistoides* IN RATS

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Cyclolepis genistoides is a plant from the Asteraceae family commonly known as "palo azul" or " matorro negro" that grows close to saline soils in Argentina. The tradicional medicine use of *C.genistoides* infusion accounts for diuretic and antirheumatic actions and for treatment of renal and hepatic colics. The aim of this study was to achieve its diuretic properties in rats. **Materials and Methods:** Adult Wistar rats were employed and infusion of the aerial parts of the plant at 10% was prepared according to Pharmacopoea Argentina VI ed. and was administered orally according to Lipschitz *et al*. Control and Furosemide reference groups were run. Phytochemical assays and RMN studies were performed in order to dilucidate chemical structures. **Results and Conclusions:** Rats treated with *C. genistoides* showed a significative diuretic effect respect the control one, p< 0.05 (ANOVA). The chemical studies revealed the occurrence of two sesquiterpene lactones, one of them was deacylcynaropicrin. This isolated compounds are now being into assay for diuretic activity each one.

11.

EFFECTS OF *Lippia integrifolia* AQUEOUS EXTRACT ON RAT GASTROINTESTINAL TRACT

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Lippia integrifolia (Gris) Hieronymus (Verbenaceae) is a wild plant growing in NortWest of Argentina commonly known as "incayuyo", "poleo", "té del inca" or "pulco". The infusion is used in folk medicine for the treatment of human aliments particularly those of the gastrointestinal tract. It is also included in popular sour beverages. The aim of this study was to evaluate the effect of the infusion on rat isolated jejunum contractions and choleretic activity in rats. Sprague Dowley rats were used and segments of jejunum were removed by careful dissection. All the tissues were maintained in Tyrode´s solution and the contractions were recorded by an isometric force transducer. The extract in a concentration dependent manner (0.5 – 2 mg/ml) inhibited the jejunum contraction induced by 80 mM ClK. Moreover, infusion (0.5-3 mg/ml) had a significant inhibitory effect on acetylcholine concentration response curve, reducing the maximum induced contraction. Also the extract showed a significant increase of bile flow (55%) and bile acid content (30%) at the dose of 500 and 750 mg/Kg during the first 15 minutes. In conclusion, aqueous extract of *Lippia integrifolia* has spasmolytic and choleretic effects that could validate its traditional use in gastrointestinal disorders.

12.

CROSS-REACTIVITY BETWEEN *Philodryas patagoniensis* VENOM AND TETRAVALENT ANTIVENOM TO *Bothrops* SPECIES

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The aim of the present work was to detect if the antibothropic serum is able to neutralize *Philodryas patagoniensis* venom. This colubrid snake inhabits the northeast region of Argentina and it is considered as not poisonous, but it has caused serious lesions as described by the literature. According to this, previous studies have demonstrated that its venom exhibits a high hemorrhagic activity. Oucherlony double diffusion showed two immunoprecipitin lines when *P. patagoniensis* venom was tested with tetravalent antivenom to *Bothrops* species. Hemorrhagic activity of the colubrid snake venom was assayed in mice by Kondo method. This activity was neutralized when venom was pre-incubated with the antiserum. Results showed that there is cross-reactivity between antigens present in *P. patagoniensis* venom and antibothropic serum. It has relevance since both, antigens and antibodies, belong to different species.

These preliminary studies let us predict the potential use of the antibothropic serum, which is available in hospitals, for the treatment of *Philodryas patagoniensis* intoxication.

13.

HYPERHOMOCYSTEINEMIA: ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT OF ARTERIAL HYPERTENSION

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Objective:To evaluate whether hyperhomocysteinemia (HYC) induces arterial hypertension and if the antioxidant therapy may revert this effect. **Methods:** Control (C, n=24) and treated (T, n=24) male Wistar Kyoto rats received tap water or homocysteine-thiolactone (50 mg/kg/day), respectively during 8 weeks. After this period8 rats of each group were used to evaluate arterial blood pressure (BP), superoxide anion radical ($\bullet O_2^-$), thiobarbituric acid-reactive species (TBARS), total glutathione content (GSH), and nitrotyrosine levels. The remaining 16 rats of each group were further subdivided in two subgroups, receiving either vehicle (saline solution, n=8) or vitamin C (500 mg/kg/day, n=8) during three more weeks. At the 11th week, the involvement of oxidative stress in the development of HYC induced arterial hypertension was analyzed. **Results:** Compared to control group, HYC animals showed higher BP (156.0 ± 14.2 / 96.8 ± 6.6 mmHg versus 134.5 ± 5.2 / 76.5 ± 11.8 mmHg, $p<0.05$), $\bullet O_2^-$ (511.5 ± 173.7 versus 138.4 ± 94.1 cpm *103/ mg protein, $p<0.001$) and TBARS (113.0 ± 33.8 versus 20.8 ± 10.2 nmol malondialdehyde/mg protein, $p<0.001$). Western blot analyses showed a 48% higher mass of nitrotyrosinated proteins in the HYC group compared to the control one ($p<0.05$). Vitamin C reverted arterial hypertension, increased antioxidant defenses, reduced oxidative stress and NO breakdown.**Conclusion**: Our results suggested that HYC induces arterial hypertension by increasing the oxidative stress.

14.

KINETIC AND DYNAMIC STUDY OF IRBESARTAN BY MICRODIALYSIS

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A kinetic-dynamic study of irbesartan (IRB) was made in anesthetized sham operated (SO) and aortic coarctated (ACo) rats. Wistar rats were used seven days after corresponding operation. A shunt probe was inserted into the carotid artery for the study of plasma kinetics. In a separated experiment a concentric probe was placed into anterior hypothalamus (AH) for the study of IRB distribution. IRB (10 $mg.kg^{-1}$ i.v.) induced a fast decrease of heart rate (HR) in ACo animals (ΔHR: -19.2 ± 2.0 bpm, $n = 6$, $P<0.05$ vs. basal HR) but not in SO rats (ΔHR: -6.7 ± 5.1 bpm, $n = 6$). Moreover, IRB reduced the blood pressure of both experimental groups, but the hypotensive effect was longer in ACo than in SO rats. Analysis of blood samples showed a lower constant of elimination of IRB in ACo rats (*Ke:* 0.67 ± 0.28 h^{-1}, $n = 5$, $P<0.05$) than in SO rats (*Ke:* 1.72 ± 0.30 h^{-1}, $n = 6$). Also, a greater distribution of IRB in AH were seen in ACo rats (*AUC:* 32 ± 4 $ng.ml^{-1}h^{-1}$, $n = 6$, $P<0.05$) than in SO rats(*AUC:* 12 ± 1 $ng.ml^{-1}h^{-1}$). In conclusion, the ACo reduces plasma elimination of IRB increasing distribution in the AH. The longer hypotensive effect of IRB observed in ACo rats could be explained by the slowest elimination of the drug. On the other hand, the effect of IRB on heart rate suggested that angiotensin II modulates heart rate in ACo rats.

15.

EFECT OF 5-HIDROXYDECANOATE ON HIGH-[K]o-CARDIOPLEGIA PROTECTION AGAINST ISCHEMIA-REPERFUSION FAILURE IN RAT VENTRICLES

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Cardioplegia (CPG) by 25 mM K-0.5 mM Ca protects heart against cardiac ischemia reperfusion failure CIRF (1). Now, we evaluated the mitochondrial K_{ATP} channel participation in CPG protection by blocking it with 100 μM 5-HD. Rat ventricles were perfused by aorta with Krebs-6mM K-2mM Ca (C) at 30°C, stimulated at 1 Hz Intraventricular pressure (P) of contractions was measured. Hearts were pretreated with C+5-HD, CPG or CPG+5-HD during 20 min, followed by 45 min ischemia (I) and 45 min reperfusion (R). At the end of I or R hearts were frozen to measuring energetic compounds by HPLC technique. In C-hearts, 5-HD did not significantly modify the increase in diastolic contracture (ΔLDVP) by I-R, decreased NS P recovery during R (49.2±6.0% vs 31.4± 4.8%, NS, n=5) and did not affect ATP (5.9±1.5 vs. 4.7±0.4 μmol/ g.d.w) nor PCr (21.4±2.6 vs. 12.3±3.8 μmol/g.d.w) contents. In CPG-pretreatment, 5-HD increased ΔLVDP during I (–8.6± 4.4 vs +25.5±10.7 mm Hg, $p<0.05$) and at the start of R (2.0±6.3 vs. +30.6±7.5 mmHg, $p<0.05$) but did not modify P recovery by R, while decreased ATP (from 7.8±0.4 to 3.8±1.7 μmol/g.d.w, $p<0.05$) and PCr (from 24.5±4.6 to 6.5±1.6 μmol/g.d.w, $p<0.05$) at the end of R. Results suggest that: a) 5-HD affects more the mitochondrial metabolic recovery under high-K-CPG pretreatment than under the C one, b) under CPG-I-R mitochondria would contribute to prevent diastolic contracture but not to regulate Ca peak for contraction.

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16.

ASPARTAME IMPAIRS VASCULAR REACTIVITY IN AORTIC RINGS OF RATS

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Aspartame (l-aspartyl-l-phenylalanine methyl ester) is the artificial sweetener most extensively used as a substitute for glucose or sucrose in the food industry. No information has been published up to date about the effect of this drug on vascular reactivity. The aim of the present study was to evaluate the "*in vitro*" response of rat aorta to aspartame. 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 aspartame (10^{-4} to 10^{-6} M), and then cumulative dose-response curves to phenylephrine (Phe) and acetylcholine (Ach) were performed. Aspartame 10^{-4} and 10^{-5} increased Phe contraction (Aspartame 10^{-4} M: 83±6.5% vs Control: 63.5±6.8%; Aspartame 10^{-5} M: 35.8 ± 5.9% vs Control: 19.5 ± 3.9% respectively, $p<0.05$). On acetylcholine-induced relaxation it was observed a disminished relaxation with aspartame 10^{-4} and 10^{-5} M (Aspartame: 34.6 ± 5.5% vs Control: 11 ± 2.8%, $p<0.01$ and Aspartame: 40.6 ± 5.4% vs Control: 23.3 ± 7.5%, $p<0.05$ respectively). Conclusions: The results previously shown support the hypothesis that aspartame (10^{-4} and 10^{-5}) M produces an impairment in vascular response that may be due to a reduction in NO production.

17.

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

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In a previous study we have shown that a decreased acetylcholine-induced relaxation, but not phenylephrine-induced vasocons-triction is obtained in aortic rings of rats with subtotal pancreatectomy (Ppx). This effect is amplified by pre-incubation in a high glucose solution -HG- (44mM/l). Based on these previous results, the aim of this study was to evaluate the mechanisms involved in the diminished relaxation observed. Rings of thoracic aorta were placed in organ chambers, and isometric tension was recorded. Cumulative dose-response curves to phenylephrine (Phe), in rings with denuded endothelium of sham-operated (SO) and Ppx rats, were performed. Then concentration-response curves to sodium nitroprusside (SNP) (a nitric oxide -NO- donor) were obtained (10^{-10}-10^{-5}M). No significant differences were observed in aortic rings of SO and Ppx rats during contraction (SO: 101.8±1,4 vs Ppx: 99.3±2.2 % NS) and relaxation (SO: 97.1±0.33 vs Ppx: 97.6±0.9 % NS), in rings incubated with HG. Similar results were obtained in rings pretreated with N-nitro-L-arginine (a NO synthase inhibitor). In other experiments, when SNP was added to pre-contracted aortic rings of Ppx and SO rats, relaxation was similar in both groups. Conclusions: These results support the hypothesis that in Ppx rats the decreased relaxation to acetylcholine may be due to a reduction in NO production.

19.

EFFECT OF ANDROSTENEDIONE ON PHYSIOLOGICAL LUTEOLYSIS INDUCED BY HYSTERECTOMY IN RAT

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It is known that androstenedione (A_2) is the principal substrate for the intraluteal estrogen biosynthesis in rats. We have demonstrated that A_2 exerts a direct luteotrophic effect in the normal pregnancy and in the luteolysis induced by hysterectomy (Hys) on day 15 of pregnancy in rat. In this study, we examinate the action of A_2 on the enzyme activities involved in the synthesis and degradation of progesterone (P), 3β-hydroxysteroid dehydrogenase (3β- HSD) and 20α-hydroxysteroid dehydrogenase (20α-HSD), respectively. Also, its effect on intraluteal levels of P and estradiol (E) in the luteolysis induced by Hys on day 18 of pregnancy, was studied. We work with female rats hysterectomized on day 18 of pregnancy, inyected with A_2 (10mg/0.2 ml in oil, via s.c.), 30 min. prior to surgery (Hys + A_2). They were compared with rats: 1) Sham (S), 2) S+A_2 y 3) Hys without A_2 treatment. All the rats were killed 48 h after surgery and the corpora lutea were collected. Luteal 3β-HSD and 20α-HSD activities were assayed by spectrophotometry. In addition, intraluteal P and E were determined by RIA. The changes in the enzyme activities, which were induced by Hys, are not modified by previous A_2 administration, however, A_2 reverses the decrease of intraluteal P and E. It is known that Hys on day 15 of pregnancy suppress the endogenous source of A. That effect is reversed by A treatment which exerts a direct luteotrophic action. In the Hys on day 18 of pregnancy the A effect could be due, at least, to its aromatization to estradiol. Probably, some other mechanism/s are involved.

18.

ADMINISTRATION OF ACETILCHOLINE IN COELIAC GANGLION MODIFIED THE OVARIAN NITRITE RELEASE FROM NORMAL PREPUBERTAL AND POLYCYSTIC RATS

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Previous studies in our laboratory using the *in vitro* system Coeliac Ganglion - Ovarian Superior Nerve -Ovary (CG-SON-O) had demonstrated its neural influence on the ovarian physiology during the estral cycle and pregnancy. Using that system, we study the effect of acetilcholine (Ach) (10^{-6}M) in the GC on the release of ovarian nitric oxide (NO) from prepubertal rats with polycystic ovary (PCO). As control (C), non PCO rats were used. PCO was induced by neonatal injection of estradiol valerato (100mg/rat). Nitrites were determined (soluble metabolite of the nitric oxide) in the ovarian cuvette at 60 and 120 minutes. The results were compared with those obtained in ovarian incubations in presence and absence of Ach. They were expressed as nmol/mg ovary. Statistical analysis: Test of Student. The same nitrite basal values were obtained in the GC-SON-O system of PCO and non PCO rats, but they were lower than those of ovarian incubations ($p<0.001$). The nitrite release changes only in the control rats when Ach was used to stimulated the CG, by increasing it ($p <0.005$). It is possible that the innervation acts in behalf of the ovarian physiology, by inhibition of the basal release of nitrites in C and PCO rats. This effect is higher in PCO rats where the hyperinnervation prevents the stimulatory action of Ach.

20.

EFFECT OF ZINC DEFICIENCY ON RAT EPIDIDYMAL MORPHOLOGY

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In previous studies we have analyzed the change in the concentration of different lipids, cholesterol:phospholipid ratio and antioxidant defense system such as TBAR's, protein carbonyls and enzymatic activities after two months of treatment undergoing a chronic zinc deficiency. In all cases we obtained differences in the Zinc deficiency (ZD) group.
This study aimed at investigating the effect of chronic Zinc deficiency as an essential factor for maintaining the integrity of epididymal epithelium.
Methods: Adult Wistar male rats were divided into two groups and fed respectively a moderately Zinc-deficient diet containing 5mg Zn/Kg and a Zn-adequate control diet supplemented with 30 mg Zinc/kg, in agreement with the (AIN 93-M) diet. Pieces of caput and cauda epididymis were processed for light microscopy. In epididymal caput; nucleus of principal cells were altered, they were anysocariotic and hypertrophic. In the cauda; epididymal epithelium was disorganized, nucleus of principal cells were altered and they were anysocariotic and hypertrophic. The epithelial cells showed vacuoles and infiltration in the perivascular zone as well as in the basal lamina. We conclude that chronic zinc deficiency modifies the lipid composition and defense system and these changes are reflected specially in the cauda epididymis histology.
Technical assistance: A. Bernardi.

21.

NEONATAL TREATMENT WITH A CONTROLLED RELEASE GNRH ANALOGUE. EFFECTS ON ADULT GONADAL FUNCTION

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Neonatal administration of sexual steroids elicits a derangement on the gonadal axis physiology in adulthood, probably interfering in the organizational process during development. The purpose of this study is to establish the long term consequences of the neonatal treatment with a GnRH analogue on reproductive function. Rats were injected s.c. with a single dose containing 0.170 mg of leuprolide acetate depot which was proved to block the pituitary GnRH receptor for 30 days. At puberty, female animals showed a delay in vaginal opening. Animals were sacrificed at 70, 84 and 91 days after treatment and the evolution of testis weight and plasmatic testosterone was considered. In the last group the pituitary response to natural GnRH was tested and treated male and female rats were submitted to fertility tests. Parameters such as the number and time of pregnancies, number of pups per litter and mortality were recorded. RESULTS: Except for testicular weight at all considered ages, no statistical difference was observed between treated and control animals. CONCLUSION: According to our results the administration of a GnRH analogue on the first days of life did not alter sexual function; all treated animals were fertile. Nonetheless, testicular weight was not recovered at 91 days of age.

22.

PRODUCTION OF A RABBIT ANTIBODY SUITABLE FOR THE IMMUNO LOCALIZATION OF THE SECRETORY PRODUCT OF THE *Pars tuberalis* CELLS IN THE BOVINE

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The *Pars tuberalis* (PT) it is a portion of the adenohypophysis whose cells secrete a product of unknown function. The aim of the present work was to immunostain the cells of bovine PT by means of a specific rabbit antibody (Ab). To rise the Ab the following steps were used: an extract of bovine PT fixed in 0.5% paraformaldehyde and homogenized in ammonium bicarbonate was employed as immunogen. One rabbit was immunized in 5 opportunities with a total of 25 mg of protein extract. The animal was bled five times. The best titles of Ab were obtain in the 4^{th} and 5^{th} bleeding, 125 and 150 days after immunization. Slices of bovine PT fixed in Bouin and embedded in paraffin were used for immunohistochemistry. The Ab reacted with a specific PT cellular population. These cells were located mainly near the EM.

This work was partially conducted at the Institute of Histology and Pathology, Fac. of Medicine, Univ. Austral of Chile. Valdivia. Chile.

23.

POPULATION PHARMACOKINETICS OF GENTAMICIN IN FULL TERM NEONATES FROM ICU: STANDARD VS ONCE-DAILY DOSING REGIMEN

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The aim of this work was to estimate population pharmacokinetics parameters of gentamicin in full term newborns (FTN) in an intensive care unit and compare standard dosing regimen with once-daily gentamicin dosage. A prospective/randomize study was conducted in 35 (FTN). Patients were divided in two groups according to initial dosing: A: 2.5mg/kg/doses every 12 h in patients with postnatal age (PE) <7 days (d), or every 8 h in patients with PE≥7d; B: 4 mg/kg/doses every 24 h. Serum gentamicin concentrations were determined by FPIA. Population data of elimination half-life ($t^1/_2$) and distribution volume (VD) were calculated by bayesian non-linear analysis, or by the population pharmacokinetic package NPEM2. In all cases a good clinical responses were observed. $t^1/_2$ and VD mean global data were 5.95 (±3.00) and 0.56L/Kg (±0.17), respectively. Values out of therapeutic range: group A: 16.40% and group B: 11.10%, showed no significant difference. $t^1/_2$ was significantly changed by EP. Mean $t^1/_2$ was 7.53h (±2.99) in EP<7d patients, and was 4.20 h (±1.79) [$p<0.001$] in EP≥7d patients. In FTN, efficacious dosages could be 4 mg/kg/dose once-daily in EP<7d patients and 3.5 mg/kg/dose twice-daily in EP≥7d. It is advised therapeutic drug monitoring after third day of treatment.

24.

APPROACH TO DISCRIMINATE BETWEEN OXIDATIVE ENZYMATIC PATHWAYS INVOLVED IN DRUG METABOLISM

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The metabolic activities of the flavin-containing monooxygenase (FMO) and cytochrome P450 (CYP) systems play a major role in determining the persistence of therapeutically used drugs in target tissues. The involvement of both enzymatic pathways on the liver microsomal sulphoxidation of different benzimidazole anthelmintics was characterised here. Sheep liver microsomes were incubated with 40 µM of either albendazole (ABZ), fenbendazole (FBZ), triclabendazole (TCBZ) or triclabendazole sulphoxide (TCBZSO). Inactivation of the FMO system was carried out by preincubation with methimazole (MTZ) either with or without heat pre-treatment (2 min at 50ºC). Incubation mixtures were cleaned up and analysed by HPLC. Liver microsomes were able to oxidise all the substrates assayed. The relative contribution of both enzymatic systems was estimated on the assumption that inactivation of FMO leaves the CYP system able to metabolise a given substrate. Both enzymatic systems were found to be involved in ABZ (FMO/CYP_{ratio} = 60/40), FBZ (81/19), TCBZ (62/38) and TCBZSO (48/52) oxidation in sheep liver. Such biotransformation pattern correlates with the pharmacokinetic behaviour of the main unconjugated metabolic products of these anthelmintics recovered in plasma and excreted in the bile of treated sheep. The metabolic approach tested here contributes to the identification of the oxidative pathways involved in the biotransformation of drugs extensively used in ruminant animal species.

25.

TRICLABENDAZOLE OXIDATION BY THE LIVER FLUKE *Fasciola hepatica*: IDENTIFICATION OF THE METABOLIC PATHWAYS

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Triclabendazole (TCBZ) is an halogenated benzimidazole flukicidal compound, whose mode of action has not been fully elucidated. Differences in the plasma and target tissue availabilities of benzimidazole sulphoxide metabolites have been attributed to the relative contribution of the flavin-monooxygensase (FMO) and cytochrome P450-dependent oxygenases to hepatic sulphoxidation. The current work was aimed to investigate the metabolism of TCBZ by adult stages of *F. hepatica*, following different strategies to identify the oxidative pathways involved in the formation of the sulphoxide metabolite (TCBZSO). The pattern of TCBZ biotransformation by the microsomal fraction of *F. hepatica*, in absence or presence of two metabolic inhibitors, methimazole (MTZ) and piperonyle butoxide (PB), was characterised. Incubation mixtures were cleaned up and analysed by HPLC. The parasite microsomal fraction oxydised TCBZ to their sulphoxide metabolite (TCBZSO). This TCBZ sulphoxidation was inhibited 47% for MTZ, a FMO substrate, and 4% for PB, a P450 substrate. These results are a further step to understand the differential pharmacological activity of this drugs against helminth parasites. The effect of metabolic inhibitors in the oxidation of TCBZ into less active TCBZSO, may have a relevant impact on drug activity. These findings are currently being applied to investigate the comparative metabolic patterns in liver flukes susceptible and resistant to TCBZ.

27.

METABOLIC STABILITY AND SOLID-FLUID PARTITIONING OF MACROCYCLIC LACTONES IN SHEEP DIGESTIVE CONTENTS

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A reduced systemic availability of macrocyclic lactones (ML), a broad-spectrum antiparasitic compounds, has been described after oral administration to ruminants. Different pharmacokinetic-based elucidations have been proposed to explain this reduced drug absorption. The goals of this work were to investigate the *in vitro* metabolic stability and the relative partitioning of the ML between the fluid phase (FP) and solid phase (SP) of the digestive tract in sheep. Three (3) adult sheep were slaughtered and samples of ruminal and abomasal content collected and immediately processed. Aliquots of either ivermectin (IVM) or moxidectin (MXD) were added to 2 ml of gastrointestinal (GI) content and incubated at 37ºC under anaerobic conditions over 2, 6 and 24 h. After each incubation, 1 ml of sample was centrifuged at 18000 *g* to separate the FP and SP and frozen at –20 ºC until HPLC analysis. No metabolic conversion was observed. IVM and MXD remained stable in the GI contents during the different incubation periods. There was a marked partitioning of the ML in the gastrointestinal contents. Higher concentrations ($P<0.05$) of IVM and MXD were recovered from the SP in both GI contents. The binding of IVM and MXD to the SP of the digestive contents may drastically affect the amount of drug available for intestinal absorption (oral treatment) and/or for enterohepatic recycling (re-absorption after oral and parenteral treatments).

26.

COMPARATIVE TRANSTEGUMENTAL DIFFUSION OF THE SULPHO- AND HYDROXY- METABOLITES OF TRICLABENDAZOLE IN THE TREMATODE PARASITE *Fasciola hepatica*

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Triclabendazole (TCBZ) is a benzimidazole compound active against immature and adult stages of the liver fluke *Fasciola hepatica*. Several TCBZ biliary metabolites have been identified in treated sheep. The experimental goal was to compare the *ex vivo* diffusion of TCBZ parent drug and its sulpho- and hydroxylated-metabolites into *F. hepatica.* The work was designed as part of the research approach oriented to investigate the relative contribution of the parent drug and its different metabolites to the potent flukicidal activity of this drug. *F. hepatica* were incubated in a KRT buffer containing 5 nmol/ml of either TCBZ, TCBZ-sulphoxide (TCBZSO), TCBZ-sulphone ($TCBZSO_2$), hydroxy-TCBZ (OH-TCBZ), hydroxy-TCBZSO (OH-TCBZSO) or hydroxy-$TCBZSO_2$ (OH-$TCBZSO_2$). After incubation, the amount of drug/metabolites recovered within the parasite was measured by HPLC. Unlike the uptake pattern observed for albendazole, the parent TCBZ and its sulphoxide and sulphone derivatives showed a similar ability to reach the parasite. Significantly lower concentrations of the hydroxylated-metabolites were recovered from *F. hepatica*. Understanding the relationship among the relative potency of each metabolic product and their ability to reach the target parasite, may be critical to optimise TCBZ flukicidal activity.

28.

POTENTIATION OF ENROFLOXACIN COMBINED WITH A NON-SPECIFIC IMMUNOMODULATOR IN THE TREATMENT OF ENDOMETRITIS IN MARES

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A more novel approach to thinking of the beneficial aspects of the antimicrobial's intracellular accumulation is to view the cell as a "drug delivery device". Mobile phagocytes are accumulated at the site of infections and seem to be a practical vehicle to deliver concentrations of fluorquinolones and macrolides into the infected tissue. The main goal of this trial was to evaluate the efficacy of the combination of enrofloxacin (EFX) and a non-specific immunomodulator of *Mycobacterium phlei* cell wall complex (Equimune®) on the clinical treatment of mares' endometritis. Fifteen natural infected cross-breed mares were allocated in three groups (n=5), according bacteriological and clinical scores and treated as follows: Group (G) I received one intravenous (IV) dose of Equimune® at 7am and 5 mg/kg of EFX IV at 4 h pm (time 0), the G II received one IV dose of Equimune® and 5 mg/kg of EFX IV given at time 0 and the G III received an IV dose of 5 mg/kg of EFX at time 0. Samples for bacteriological culture and cytology studies were taken pre (0h) and 48 h post-treatment. Bacterial cultures were blood agar and Eeosin Blue. Data were analysed by a contingence table using Fisher Exact Test. The treatment assayed for the GI shown over 80% of efficacy with bacteriological cure in comparison with results obtained for the GII and III (20% and 40% of efficacy, respectively). This treatment combination could be a new option for treatment of mares' endometritis in terms of clinical practice.

29.

PHARMACOKINETICS OF ENROFLOXACIN AFTER INTRAVENOUS AND INTRAUTERINE ADMINISTRATONS TO MARES WITH ENDOMETRITIS

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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 and new therapeutic alternatives need to be investigated The aim of the present work was to evaluate the pharmacokinetic (PK) behaviour of enrofloxacin (EFX) and its active metabolite ciprofloxacin (CFX) after the intravenous (IV) and intrauterine (IU) administration of EFX parent drug in diseased mares. Clinical endometritis were diagnosed in 10 mares. The mares were allocated into two groups (n=5) according clinical scores. The Group I was treated with EFX 2.5 mg/kg via IV (systemic treatment). The Group II received identical treatment that Group I by IU route (local treatment). Plasma samples were taken over 48h post-administration and analysed by HPLC with fluorescence detection. EFX and its metabolite CPX were detected in plasma over 48 h after both treatments. Fast absorption/distribution from the uterus and 100 % bioavailability characterised the PK behaviour of EFX given as a local treatment. EFX was metabolised to CFX in similar proportion (~7 %) after both treatments. These preliminary results are encouraging for further research on the optimisation of endometritis treatment in mares.

30.

PHARMACOKINETIC OF ERYTHROMYCIN IN DOMESTIC CATS

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Introduction: Erythromycin (ERY) is a macrolide antibiotic widely used and studied in human beings as well as in many domestic animals. However, its pharmacokinetics is poorly characterized in cats. The aim of this study was to analyze the serum disposition of ERY after its intravenous (IV), intramuscular (IM) and, also after two oral (PO) different formulations to cats.

Materials and Methods: 5 adult cats weighted 6.04 ± 2.11 kg received ERY 5 mg/kg IV (lactobionate), 10 mg/kg IM (base) and 15 mg/kg PO (ethylsuccinate, tablets or suspension). Blood samples were withdrawn at pre-determined times over a 12 h period. ERY serum concentration was determined by microbiological assay using *Micrococcus luteus* (ATCC 9341) as test micro-organism. Plasma disposition curves were analyzed by non linear methods using PcNonlin software.

Results: After IV administration ERY distribution was fast ($t_{½(d)}$ 0.21±0.16 h) and wide ($V_{(d(ss))}$ of 3.63±2.93 L/kg). Elimination half-life was 1.41±0.30 h and Cl_B 2.71±1.54 L/h.kg. After IM route ERY was totally available with an F value around 100 %. T_{max} was 1.17±0.50 h, and C_{max} 2.33±0.50 µg/ml. After PO administration serum concentrations were extremely low and unpredictable for both ethylsuccinate formulations in all the animals. In summary, ERY showed, after IV administration, a wide distribution and relatively long half-life. Also, based on the present results IM administration of ERY base is advisable due to its good bioavailability, long-lasting serum concentration and high Cmax. Contrarily, oral administration of ERY ethylsuccinate can not be advised due to its very low bioavailability in cats.

31.

PROSTATIC FLUID/PLASMA RATIO FOR CIPROFLOXACIN AND NORFLOXACIN IN DOGS

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Ciprofloxacin (CIP) and norfloxacin (NOR) are widely used in antimicrobial veterinary therapeutics. The purpose of this study was to determine CIP and NOR distribution into prostatic fluid (PF) in healthy dogs after multiple dosing. Eight adult male dogs (12.3 ± 4.6 kg) received 7 twice daily doses of CIP (15 mg/kg) and NOR (20 mg/kg) orally with a 15-day washout period between administrations. Serial venous blood samples were taken at predetermined times. PF samples were manually obtained at predetermined times after the first and seventh drug administration. CIP and NOR plasma and PF concentrations were determined by microbiological assay with *Klebsiella pneumoniae* ATCC 10031 as the test organism. CIP and NOR plasma concentrations were higher than PF concentrations. PF pH ranges were within normal (5.00-7.25). Median (25-75% percentile) of CIP and NOR penetration ratio (PF/ plasma concentrations) were 0.30 (0.22-0.44) and 0.62 (0.43-1.07), respectively. Mean (SD) of CIP and NOR PF concentrations were 0.21 (0.18) and 0.17 (0.13) µg/ml, respectively. Our results showed that penetration into PF was higher for NOR than for CIP.

32.

PHARMACOKINETICS OF AMIKACIN ADMINISTERED IN TRAINED MICE

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Amikacin, an aminoglycoside antibiotic, is a semisynthetic derivate of kanamycin. The aims of the present paper were to determine the pharmacokinetic behavior of amikacin administered in mice with and without training. Seventy-two male mice were taken at random for the two experiments (E1 and E2). During E1, the mice were trained swimming five days a week during seven weeks. Amikacin (10 mg/kg, im) was administered to those animals of E1. Mice of E2 remained without training. Amikacin administration in E2 was carried out by the same way and dose of E1. One blood sample per animal was taken postadministration of the antibiotic. The data for each time point was averaged and these values were used to calculate the pharmacokinetic parameters. Results: K_a (E1)= 9.4 and (E2)= 6.1 h^{-1}, λ_z(E1)= 0.31 and (E2)= 0.54 h^{-1}, AUC (E1)= 13.9 and (E2)= 15.6 µg/ml/h. *In conclusion*: Long-term physically training did not show significant differences in the pharmacokinetic profile of amikacin in comparison with untrained mice.

33.

IMPORTANCE OF P-GLYCOPROTEIN-MEDIATED SALIVARY SECRETION OF PARACETAMOL IN RATS

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Paracetamol pharmacokinetics can be determined from plasma or salivary levels. The relationship between salivary and plasma levels of this particular drug is almost constant once the distribution phase is established. Several ATP-dependent efflux transporters, including P-glycoprotein (P-gp), have recently been evidenced in salivary glands of healthy volunteers. To evaluate the influence Paracetamol P-gp-mediated salivary secretion in the rat submaxilar glands involved in the S/P constant values, we compared the kinetic parameters of the drug calculated from salivary and plasma levels obtained from control animals with those obtained from animals pretreated with erythromycin as a P-gp inhibitor. The results showed no significant difference between the parameters calculated from salivary or plasma levels when comparing between groups ($p<0.05$? The S/P ratios calculated with data from the elimination phase were not modified after inhibiting P-gp-mediated transport. The values obtained for the kinetic parameters were in correspondence with those previously published and no difference could be found between the parameters calculated from both fluids ($p>0.05$). We propose the absence of P-gp-mediated transport of paracetamol in the submaxilar glands of the rat and claim for the usefulness of salivary levels in the kinetic study of paracetamol.

34.

INFLUENCE OF NUTRITIONAL STATUS ON THE PHARMACOKINETICS OF CEPHALOTIN ADMINISTERED IN LACTATING GOATS

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The general aim of this study was to determine the pharmacokinetics behavior of cephalotin administered in lactating goats exposed to restricted and normal diets. Twenty two healthy creole pregnants goats, during the 4th and 5th month of pregnancy, with a body weigth from 25 to 50 kg were used. They were randomized to 3 groups: Experiment 1 (E1) (n=8), Experiment 2 (E2) (n=8) and Experiment 3 (E3) (n=6). Animals of E1 grazing in a restricted way. E2 animals were fed equal to E1 with the addition of an excess of calories in their diets. E3 group received grass plus an addition of equilibritated supplement. The effects of diets upon body weigth and metabolic profiles were under control until the administration of cephalotin by intravenous route (20 mg/kg). Blood and milk samples were obtained at selected times. Serum and milk pharmacokinetic parameters were significantly different ($p< 0.05$) in goats exposed to hypercaloric and restricted diets compared with normal ones.

35.

PHARMACOKINETICS OF AMIKACIN ADMINISTERED TO RABBITS WITH NORMAL AND LOW PROTEIN DIETS

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Objective: The aim of this paper was to assess the pharmacokinetics behavior of amikacin administered to rabbits fed with normal and low protein diets. **Design:** Twelve rabbits, 15 days after weaning, were randomly asigned to two trials (T1 and T2). These animals were fed ad libitum through 100 days with normal and low protein diets, respectively. After that time rabbits were injected with 10 mg/kg of amikacin by intramuscular route. Blood samples were drawn before and after amikacin administration. Amikacin was measured in serum (microbiological method). **Results:** Rabbits of T2 showed lower weigth (1550.0 ± 316.0 g) compared with T1 (3042.0 ± 113.4 g). The amikacin maximum serum concentration (C_{max} (T1)= 168.3 ± 25.6 and (T2)= 108.2 ± 46.0 µg/ml), area under the curve (AUC (T1)= 977.7 ± 289.5 and (T2)= 368.4 ± 266.4 $\mu g.ml^{-1}.h$) and peak time (t_{max} (T1)= 0.79 ± 0.3 and (T2)= 0.35 ± 0.17 h) were significantly lower in rabbits with low protein diets.

36.

USE OF AN ADENOVIRAL VECTOR TO EXPRESS TRANSGENIC INSULIN-LIKE GROWTH FACTOR I IN THE ARCUATE NUCLEUS AND SUBSTANTIA NIGRA OF RATS

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Insulin-like growth factor-I (IGF-I), is present in both the developing and adult CNS, and specific receptors for this peptide are widely distributed in the brain. IGF-I possesses important neurotrophic actions and its synthesis as well as its type 1 brain receptor numbers are known to decline during aging. Interestingly, replacement therapy with IGF-I in aged rats ameliorate certain memory capacities, possibly through a restorative effect of IGF-I on the dopaminergic D2 receptor levels in the brain**.** As an initial step to implement IGF-I gene therapy in central DA neurons of the senile rat brain, we constructed a recombinant adenoviral vector (RAd-IGF1) harboring the gene for IGF-I. This replication-defective vector was constructed by the two-plasmid method using the FLP recombinase for efficient vector rescue. The IGF-I transgene was placed under the control of a potent mouse cytomegalovirus promoter and its expression in transduced cells or brain tissue was assessed by radioimmunoassay. To reach the arcuate nucleus (ARC) and the substantia nigra (SN), the vector was stereotaxically injected at 10^{12} plaque forming units (pfu)/ml in a volume of 10 :l saline per side. Two days after surgery the rats were sacrificed and the target areas were dissected by punching out cylindrical specimens that were homogenized and measured. In ARC, control (saline injected) and experimental (vector injected) tissue IGF-I levels were (pg/sample ± SE) 3.77 ±0.96 and 6.70 ±0.79, P=0.045,whereas the corresponding values for SN were 2.60 ± 0.79 and 6.64 ± 0.79, P=0.011.The present results demonstrate that RAd-IGF-I administration can significantly increase IGF-I tissue levels in two important DA brain areas, thus constituting a suitably tool for experimental gene therapy in the aging CNS.

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37.

BENZODIAZEPINE WITHDRAWAL-RELATED ANXIETY AND C-FOS EXPRESSION IN MEDIAL PREFRONTAL CORTEX

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The interruption of chronic benzodiazepine (BZD) administration results in signs and symptoms characteristics of BZD withdrawal syndrome. Anxiety is a prominent symptom that has been reported in abstinent humans and in animal models of BZD withdrawal. The objectives of the present study were: 1) to investigate the effects of BZD withdrawal on c-Fos expression in medial prefrontal cortex and, 2) to asses the behavior in a model of anxiety (elevated plus maze) at different times after the last BZD administration. Male Wistar rats were chronically treated with diazepam (DZM) (2 mg/kg, i.p.) for 21 days and were tested in the elevated plus maze at 24, 48, 72 and 96 h after the last DZM administration. A clear reduction of open arm activity (increased anxiogenic behavior) was only observed in animals at 24-48 h after diazepam withdrawal. A prominent induction of c-Fos protein in medial prefrontal cortex (cingulate, prelimbic and infralimbic cortex) was evident one day after diazepam withdrawal. Thus, it seems likely that the enhanced emotional behavior could be functionally associated with the induction of c-Fos protein in medial prefrontal cortex.

38.

EFFECT OF ZINC DEFICIENCY ON THE EXPRESSION OF eNOS AND COX_2

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Zinc is an essential component of biomembranes and it is necessary for the maintenance of membrane structure and function. On the other hand nitric oxide derived from the endothelium is an important regulator of vessel homeostasis. NO has been implicated in the regulation of other processes including coagulation, inflammation, etc. These studies aimed at investigating the effect of chronic Zinc deficiency as an essential factor for maintaining the integrity of lung vessels and for protecting against inflammatory situations. Adult Wistar male rats were divided into two groups and fed respectively a moderately Zinc-deficient diet containing 5mg Zn/Kg and a Zn-adequate control diet supplemented with 30 mg Zinc/kg, in agreement with the (AIN 93-M) diet. The content of NO was quantified by Griess reaction. eNOS and COX-2 expression were analyzed by Western blot and the bands were quantified with Scion Image Software from NIH. NO production increased in lung ($p<0.01$) and serum ($p<0.01$), associated with the higher expression of eNOS ($p<0.05$) and COX-2 ($p<0.05$).
It would be important to use the knowledge of zinc deficiency implications in order to design therapies and public health interventions for high-risk groups of certain diseases, such as asthma, that could increase their life quality with zinc supplementation.

39.

CROSSTALK BETWEEN ANG II AND INSULIN RECEPTORS IN RAT LIVER PREPARATIONS

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Introduction: Ang II receptors and Insulin receptors are both present in rat liver. Besides the well-known effects of these effectors, it is now recognized that Ang II and Ins can control growth and differentiation. We examined the modulation of the phosphorylation induced by Ins due to Ang II. **Methods:** Phosphorylation assays were conducted in membranes or homogenates from adult rat livers. Receptors were stimulated with Ins and Ang II alone or combined, under different conditions. Phosphorylated proteins were analyzed by Western blot and immunoprecipitation. **Results:** Ins induces Tyr-phosphorylation of the 160 kDa and Ang II reduces significantly the phosphorylation level, a dose-dependent effect. Western blots reproved with either IRS-1 and anti-pTyr antibodies showed that pp160 was different from the IRS-1 protein. Losartan (10^{-6}M) and Sarile (10^{-6}M) completely blocked the effect of Ang II. Inhibitors for different tyrosine phosphatases and kinases were assayed: Dephostatin, completely reverts the Ang II effect. A similar effect was observed for Wortmaninn. **Discussion:** In the present study we found an opposite effect of Ins and Ang II on protein tyrphosphorylation. We identified a pp160 kDa protein which do not correspond to the classical Ins receptor substrate, IRS-1. The response is dose-dependent and completely blocked by the AT_1 selective competitor Losartan. The present study constitutes a first report about cross-talk between Ang II and Ins receptors in liver membrane preparations.

40.

VASORELAXANT EFFECTS OF SODIUM KAURENATE. A POSSIBLE NITRIC OXIDE-RELEASING AGENT

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Preliminary studies determined the anti-hipertensive activity of kaurenic sodium salt (KSS). In the present study the vascular response *in vitro* and *in vivo* of KSS was investigated: **(1)** Isolated male rabbits aortas were used with endothelium and denuded previous phenylephrine-induced contraction, mounted under 1 g isometric tension in an organ bath with Krebs-Henseleit solution. Cumulative curves to KSS and L-NAME were obtained **(2)** The *in vivo* response on spontaneous hypertensive rats (SHR) and Sprague Dawley (S-D) rats was measured using pletysmographic methods at basal, 30 minutes, 1, 6, 12 hours post KSS i.p. (20mg/Kg) alone or in combination with L-NAME (3mg/Kg) using sodium nitroprusside (NO) (0.1 mg/Kg) as reference. **(1)** Addition of phenylephrine (Pe) (10 μM/ml) induced a contraction of the rabbit aorta with endothelium 0.68 ± 0.13 g that was reduced 0.29 ± 0.12 g (42.67%) by KSS (0.16 μM/ml) ($p<0.05$); this effect was reversed by L-NAME (0.1 μM/ml) ($p<0.001$). On denuded endothelium the vasorelaxation by KSS was not appreciated, neither was observed the reversion by L-NAME. **(2)** In SHR/N, KSS and NO produced a decrease of medium arterial pressure (MAP) from 16.8% and 12.9% in relation to basal at six hours ($p<0.05$), these effects were reversed with L-NAME ($p<0.01$); in S-D rats L-NAME caused increase in MAP, lightly modified by KSS and NO. These results suggested that vasorelaxant effects caused by KSS could be endothelium-dependent; furthermore, an indirect mechanism could be inferred.
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41.

STUDY OF THE HYPOTHALAMIC ANGIOTENSIN SYSTEM IN AORTIC COARCTATED RATS

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The pressor activity of angiotensin II (AII) and the effect of angiotensin [1-7] (A17) on AII pressor response were studied in aortic coartated (ACo) rats with an acute (7 days) and chronic stage (42 days) of hypertension. Wistar urethane-chloralose anesthetized rats were used. A cannula was inserted in the carotid artery for mean arterial pressure (MAP) monitoring. A stainless-steel wire was inserted into anterior hypothalamus for the injection of AII, A17 and AII+A17. The pressor response to AII was enhanced in acute (SO rats: ΔMAP: 7±1 mm Hg; ACo rats: ΔMAP: 16±2 mm Hg, $P < 0.05$) and chronic (SO rats: ΔMAP: 8±1 mm Hg; ACo rats: ΔMAP: 18±1 mm Hg, $P < 0.05$) ACo rats. The injection of A17 did not modify blood pressure in all experimental groups. The coadministration of A17 with AII produced a blunting of AII pressor response in acute (A17+AII: ΔMAP: 4±2 mm Hg, $P < 0.05$ vs. AII) and chronic (A17+AII: ΔMAP: 10±4 mm Hg, $P < 0.05$ vs. AII) ACo rats but not in SO animals.

Our findings suggest a greater pressor activity of AII in both acute and chronic ACo rats, which is reduced by the application of A17. This would imply that A17 modulate the AII pressor activity when the same one is exacerbated.

42.

ESTROGEN MODULATION OF ANANDAMIDE RELAXANT EFFECTS IN THE MESENTERIC BED OF SPRAGUE-DAWLEY RATS

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We previously observed that the vasorelaxant effects of the endocannabinoid anandamide (AEA) were greater in mesenteric beds from female than from male Sprague-Dawley rats and that 0.5 μM 17β-estradiol (E2) potentiated the AEA responses in mesenteric beds from male rats only. In the present work the effects of E2 were tested in either ovariectomized (OVX) or sham-operated (SHAM) adult female rats. Some OVX were treated with E2-benzoate (450 μg/Kg i.m., OVX-E2) or with vehicle (OVX-veh) once a week. Twenty-one days after surgery, mesenteric beds were excised and vascular responses to bolus injections of noradrenaline (NA) were measured as changes in perfusion pressure. AEA (0.01-10 μM) induced a concentration-dependent reduction of NA contractions that was lower in OVX than in SHAM rats ($p<0.01$). E2 treatment in OVX increased ($p<0.001$) the effect of AEA. Moreover, in OVX, AEA relaxations were potentiated by *in vitro* incubation with 0.5 μM E2 ($p<0.001$). In male rats the potentiation of AEA effects caused by *in vitro* exposure to 0.5 μM E2 was not modified by cicloheximide (10 μM). On the other hand, the E2 effect was not observed in male de-endothelized mesenterics beds. In conclusion, in tissues that were deprived of circulating estrogens, E2 potentiates AEA relaxations through an acute non-genomic mechanism that probably involves the participation of endothelium. Furthermore, the presence of circulating estrogen is a positive modulator of the AEA relaxant effects in mesenteric vasculature.

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43.

INFLUENCE OF ETHANOL WITHDRAWAL ON AVERSIVE LEARNING: EFFECT OF D-CYCLOSERINE ON EXTINCTION OF CONDITIONED FEAR

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The objectives of the present work were to study the influence of ethanol withdrawal on: 1) fear response, as an expression of associative and non-associative aversive learning; 2) the extinction process of conditioned freezing and 3) the effect of D-cycloserine (DCS, a partial NMDA agonist) on extinction of conditioned freezing.

Adults male Wistar rats were submitted to chronic treatment with an ethanol (6% v/v) liquid diet for 12 days. Rats were exposed to 3 inescapable electrical foot-shocks (0.7mA, duration 3 s; conditioning), on the third day of abstinence. Twenty-four, 48 ,72 and 96 h later animals were exposed to the context without shock delivery, for 10 min (extinction training). One group of animals was injected with DCS (5 mg/kg i.p) immediately after the first session of extinction training. Ethanol withdrawn animals showed an enhanced associative aversive learning and a marked resistance to extinguish such emotional response. A facilitatory effect of DCS on the extinction process was observed only in ethanol withdrawn animals.

44.

INCREASED FEAR LEARNING, NEURONAL DISINHIBITION AND FACILITATED LTP IN BASOLATERAL AMYGDALA FOLLOWING BENZODIAZEPINE WITHDRAWAL

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Animals chronically administered with diazepam (2 mg/Kg/day i.p.) or vehicle (VEH) during 21 days were tested in a contextual fear-conditioning paradigm 4 days after the last administration. Increased freezing behavior was observed in both associative and non-associative learning in the contextual fear conditioning paradigm in abstinent animals. Brain slices containing the basolateral amygdala were obtained from VEH and withdrawn rats and used to record field potentials evoked by stimulation of the external capsule. In slices from VEH rats single stimuli evoked a field potential including a population spike (PS), whereas in abstinent rats the same stimulus evoked multiple PSs. Perfusion with DZM of slices obtained from abstinent rats eliminated repetitive spiking and GABAergic blockade with PTX in slices from VEH rats resulted in the appearance of multiple secondary PSs. In slices from abstinent rats a single train of 100 Hz stimulation induced a highly significant long lasting potentiation (LTP). Depressed GABAergic activity (disinhibition) in basolateral amygdala resulting from abrupt discontinuation of chronic BDZ administration could explain neuronal hyperexcitability leading to burst firing and facilitated LTP. Increased synaptic plasticity may be the root of the increased fear learning observed in withdrawn rats.

45.

THE GABAergic NEUROTRANSMISSION IN THE BASOLATERAL AMYGDALA PLAYS A POTENTIAL ROLE IN THE EMOTIONAL SEQUELAE INDUCED BY STRESS

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The aims of this study were to: 1) evaluate the effect of a restraint experience (RES), with or without midazolam (MDZ) administration (0.5 mg/kg, i.p.), in fear conditioning; 2) asses the influence of bicucculline (BMI, $GABA_A$-R antagonist) into the basolateral complex of the amygdala (BLA), with or without MDZ, in fear conditioning; and 3) explore the influence of RES, with or without MDZ, on the LTP generation in BLA. Male Wistar rats were submitted to 30 min of RES or bilaterally injected with BMI (10 pmol) and 24 h later were exposed to one session of three foot-shocks. The freezing response, as an index of fear, was evaluated 24 h later in the context without shock delivery for 10 min. Both RES and BMI induced an exaggerated fear response. These effects were reverted by MDZ administration 15 min before RES or BMI injection.
Field potential evoked by the stimulation of external capsule were recorded in BLA. In the control group single stimuli evoked a field potential including a population spike (PS), whereas in RES rats the same stimuli evoked multiple PSs and facilitated LTP generation. We suggest that GABAergic neurotransmission in BLA could be involved in the modulation of the emotional response to aversive stimulation.

46.

NEW ANTIULCER AGENTS INHIBIT RAT PERITONEAL MAST CELL DEGRANULATION INDUCED BY COMPOUND 48/80

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A lactone isolated from *Artemisia douglasiana* Besser (DhL), a xanthanolide isolated from *Xanthium cavanillesii* Schouw (Xt) and a synthetic butenolide (But), prevent gastrointestinal damage elicited by necrosis-inducing agents. The present work examines the effect of these molecules on compound 48/80-induced degranulation from mast cells, to determine whether our compounds may act as mast cell stabilizers. Rat peritoneal mast cells were purified in Percoll and incubated with: 1) PBS or 2) compound 48/80 or 3) DhL+48/80 or 4) Xt+48/80 or 5) But+48/80. Serotonin release studies by HPLC, evaluation of mast cell morphology by light and electron microscopy, dose-response and time-response studies, cell viability evaluation, and comparative studies with ketotifen (Ket), were carried out. Compound 48/80 increased serotonin release from mast cells and elicited evident granule ultraestructural changes. These effects were inhibited by DhL, Xt and But in a dose-dependent manner. The inhibition percentages were 110% (200 μM DhL), 67% (200 μM Xt), 33% (200 μM But) and 33% (200 μM Ket). In conclusion, the present study demonstrates that DhL, Xt and But inhibit compound 48/80-induced mast cell activation, acting thus as mast cell stabilizers. Our findings also show that the inhibitory effects exhibited by DhL and Xt are stronger than that of ketotifen, a classical mast cell stabilizer.

47.

AN ENDOGENOUS NA^+, K^+-ATPASE INHIBITOR (ENDOBAIN E) AND ASCORBIC ACID ENHANCE NEUROTRANSMITTER RELEASE FROM CEREBRAL CORTEX SYNAPTOSOMES

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Inhibition of sodium pump or Na^+, K^+-ATPase (its enzymatic version) increases neurotransmitter release in several experimental models. The isolation of a soluble brain fraction which behaves as an endogenous ouabain-like substance, termed *endobain E*, has been described. Endobain E contains two Na^+, K^+-ATPase inhibitors, one of them identical to ascorbic acid. In this study neurotransmitter release in the presence of endobain E and ascorbic acid was analyzed. Synaptosomes were isolated from cerebral cortex of male Wistar rats by differential centrifugation and Percoll gradient. Synaptosomes were preincubated in HEPES-saline buffer with 1 mM D-[^{3}H]aspartate (15 min at 37º), centrifuged, washed, incubated in presence of additions (60 sec at 37º) and spun down; radioactivity in the supernatants was quantified. D-[^{3}H]aspartate release was enhanced up to 100% with 0.5-5.0 mM ascorbic acid in the presence of 40 mM KCl. Endobain E concentration dependently enhanced D-[^{3}H]aspartate release (7-8 times). Ouabain at 1 mM concentration roughly doubled the release. It is concluded that endobain E as well as ascorbic acid, one of its components, due to their ability to inhibit Na^+, K^+-ATPase, are candidates to modulate neurotransmitter release at synapses.

48.

A FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY OF THE DOPAMINE RECEPTOR HYPERSENSITIVITY CORRELATES WITH DRUG INDUCED DYSKINESIAS (DID)

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Functional Magnetic Resonance Imaging (fMRI) is a non-invasive tool to study neuronal correlates of central nervous system dysfunction as the technique allows mapping changes in regional cerebral blood flow (BOLD) in response to task activation. A well characterized rat model of Parkinson Disease (PD) is the unilateral 6-hydroxydopamine (6-OHDA) lesioning of the nigrostriatal dopamine tract. fMRI studies in this model demonstrated an asymmetric increase in cerebral blood volume in the lesioned striatum after stimulation with apomorphine consistent with the concept of functional receptor supersensitivity. To assess the role of fMRI signal changes as a measure of local receptor sensitivity for DID, striatal and cortical fMRI signal time courses in response to apomorphine (D1/D2 agonist) or quinpirole (D2 agonist) was studied in a animal model of DID by repeated administration of the respective drugs. We measured abnormal involuntary movements (AIM) and rotational behavior. These results confirm that BOLD fMRI is a useful tool to map dopamine receptor-sensitivity. Moreover, rotational behavior and dyskinesias were shown to be correlated with the BOLD signal in the striatum and/or motor cortex suggesting that D1/D2 receptor hypersensitivity may play a contributing role for the development of DID.

49.

PHARMACOLOGICAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF LEAVES FROM *CHILIOTRICHUM DIFFUSUM* (ASTERACEAE)

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In this work we studied the phytochemistry and the cardiovascular effects of leaves from *Chiliotrichum diffusum*. The leaves were air-dried after its collection and powdered leaves were extracted separately with ethanol and by decoction. These extracts were characterized by chemical reactions and by paper chromatography. Antibacterial activity was analyzed by agar diffusion assays. For cardiovascular studies, urethane-cloralose anesthetized Wistar rats were used. Aqueous extract was dissolved in saline solution for iv administration. The femoral artery was cannulated to register mean arterial pressure (MAP). Flavonoids, carbohydrates, proteins, steroids and anthraquinones were identified. *In vitro* screening has demonstrated an inhibitory activity against *S. aureus*. *In vivo*, decoction had a dose-dependent depressor effect, with a maximal response at 10 $mg.kg^{-1}$ (ΔMAP: -34±3 mmHg; n=6). This depressor effect was partially blocked by the β-adrenergic antagonist propranol (ΔMAP: -16±3 mmHg; n=6) and by atropine muscarinic blockade (ΔMAP: -9±1 mmHg; n=6). In conclusion, the decoction shows antibacterial activity and a dual muscarinic and β-adrenergic depressor effect.

50.

BEHAVIORAL ATTENUATION INDUCED BY D_1 AND D_2 RECEPTORS BLOCKADE DURING OPIATE WITHDRAWAL

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In previous studies, we have found that a significant decrease in striatum and cortical dopamine (DA) levels takes place during MOR withdrawal in male mice but not in female. In order to examine sex differences related to the dopaminergic system, in the present study we evaluated the behavioral effect of two DA selective antagonists SCH 23390 (D_1) and raclopride (RAC) (D_2) during MOR withdrawal. Swiss-Webster mice (24-27g) were rendered dependent by i.p. injection of MOR (2 mg/kg), twice daily for 9 days. On the 10th day, dependent mice were divided into three groups: *withdrawal* group received naloxone (NAL, 6 mg/kg, i.p.) after the last dose of MOR in order to precipitate the abstinence syndrome and the other 2 groups received SCH 23390 (0.2 mg/kg, i.p.) or RAC (0.3 mg/kg, i.p.) before NAL injection. After NAL injection, behavioral signs have been recorded for each mice in the open field for 30 minutes. Results have shown that during MOR withdrawal, significant increases in liquid feces, wet dog-shakes and sniffing either male or female mice. When mice were pretreated with SCH 23390 or with RAC, the amounts of those three behavioral signs significantly decreased in both sex.
The modification of the MOR withdrawal signs by selective DA antagonists, supports the idea that both D_1 and D_2 receptors play a critical role in the expression of MOR abstinence signs. This finding could be related to the known influence of dopaminergic system on the opiate reward.

51.

RELATION BETWEEN METALLOPROTEINASE-1 AND ADHESIVE IMMUNOGLOBULIN INTERCELLULAR IN OSTHEOARTHRITIS

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The mechanism involved in the destruction of joint in ostheoarthitis (OA) is still poorly understood. The metalloproteinase collagenase (MMP-1) play important roles in the connective tissue destruction seen in OA.
The expression of proteinases is regulated also by interactions mediated by adhesive molecules.
Synovial fluid and cartilage were obtained from 24 joints with OA or posttraumatic knee injury after total endoprosthesic surgery. MMP-1 and s I-CAM were measured in the cartilage and synovial fluid , using specific double-antibody sandwich ELISA.
Results: MMP-1 in cartilage was 1967.6±619.2 ng/ml and 667.6 ±124.93 ng/ml, $p<0.001$. sICAM in synovoial fluid was 243.8±47.1 and 123.2±28.1 ng/ml , $p <0.001$.
Conclusions: The production of MMPs from cartilage can be aumented by costimulation through adhesion molecule. Even if alterations in ICAM expression is not indicative of ostheoarthritic, they still represent very attractiv therapeutic targets.

52.

IN VIVO IRON AND ZINC DEFICIENCY DIMINISHED T- AND B- SELECTIVE MITOGENIC STIMULATION OF MURINE LYMPHOID CELLS THROUGH PROTEIN KINASE C (PKC)-MEDIATED MECHANISMS

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Iron (Fe) and zinc (Zn) are crucial mineral components of human diet as their deficiency lead to several disorders including alterations of immune function. The aim of this work was to analyze if Fe or Zn imbalances regulate lymphocyte activity and the intracellular signals involved in this effect. BALB/c mice were fed with Fe- (IR) or Zn (ZN) deficient diets or received mineral supplementation (control, C). All diets were prepared according to the American Institute of Nutrition Rodent Diets. Levels of Fe and Zn were assessed in blood and liver samples by atomic absorbance. Selective mitogen stimulation of T and B lymphocytes was performed on lymphoid cell purified from lymphoid organs of all animals. Cell proliferation was evaluated by [^{3}H]-thymidine uptake. We found a diminished proliferative response in T and B lymphocytes from ZN and IR animals respect to controls, with the lowest responses related to ZN animals. These effects were related to a decrease in mitogen-induced translocation of PKC activity to cell membranes on both cell types, as evaluated by enzymatic phosphorylation of a PKC specific substrate. Our results demonstrate that iron and zinc deficiencies affect both T and B lymphocyte function by altering PKC activation. This could explain some immune alterations already described in Fe- or Zn-deficient individuals.

53.

ACUTE STRESS INCREASE T-CELL ANTIBODY PRODUCTION. ROLE OF ENDOGENOUS GLUCOCORTICOIDS AND CATECHOLAMINES

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Stress has long been associated with altered immune function. It was proposed that acute stress increase immune surveillance having a protective effect to health. The aim of this work was to analyze the effect of acute stress exposure and stress hormone involvement on antibody production after antigenic challenge.
For this purpose acute stress was administered by placing animals in well-ventilated restrainers for 2 h. Normal and stressed animals were inoculated with sheep red blood cells (SRBC) or dextran B-512 (Dx) to determine T cell-dependent and independent humoral response, respectively. We found that anti-SBRC IgG primary response was higher in acute animals whereas no differences were observed to anti-SRBC IgM production. Similarly, titers of anti-SRBC IgG were higher in stressed animals than in controls for secondary response. On the contrary, no significant differences were found in the anti-Dx titers. As expected, acute stress induced a significant increase in serum corticosterone levels and in the splenic catecholamine content. On the other hand, a stimulatory effect on normal T-cell reactivity was observed for physiological concentrations of epinephrine and corticosterone. This effect was higher for T-lymphocytes from acute animals. These results indicate that corticosterone and epinephrine may be important mediators of enhancing effects of acute stress on T-cell dependent antibody production.

55.

THROMBOLYTIC EFFECT OF DERMATAN SULFATE SUBPOPULATIONS. STUDIES *IN VITRO* AND *IN VIVO*

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The glycosaminoglycan dermatan sulfate (DS) stimulates thrombin inhibition by heparin cofactor II. We have previously described the experimental conditions for the specific interaction between DS and the first protein complex of the complement system (C1) that makes possible the isolation of a very small fraction of DS which has an almost four-time increment of its biological activity as compared with starting material in *in vitro* studies. In the present work, we report the structural characterization of these DS subpopulations, and the thrombolytic effect in animal models with an experimental venous thrombosis. The molecular masses and the sulfate concentration of DS subpopulations were estimated. The thrombus weight (TW) and the thrombin time (TT), were measured after an intravenous administration of DS subpopulations to animals with surgical induced thrombosis. The TW was significantly reduced by the administration of DS original material compared to that obtained with the vehicle (2.40±0.48 vs 7.23±1.47 mg mg; n=3), while the DS of the supernatant obtained in the interaction was less effective (4.35±0.17 mg, n=3). In all cases, no differences of the TT could be detected (45.50±5.5 sec). The sulfate content of the DS subpopulation recovered from the precipitate was significantly increased. The thrombolytic effect, detected *in vivo*, of the very small fraction of the DS isolated by precipitation with C1 correlates with the degree of sulfation.

54.

ACUTE AND CHRONIC ADMINISTRATION OF *D*-AMPHETAMINE RESULT IN DOSE-DEPENDENT EFFECTS ON LYMPHOCYTE PROLIFERATIVE RESPONSES IN RATS

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Drug addicts are known to be highly susceptible to bacterial, viral and fungal infections, and to have important deficits in the immune function. We previously demonstrated that chronic *d*-amphetamine (AMPH) treatment facilitates the immunosuppression following the exposure to an aversive stimulus. The main goal of this study was to determine the influence of AMPH acute and chronic treatment on mitogen-induced lymphocyte proliferation in rats. Wistar rats were treated with AMPH acutely (2.5mg/kg IP or 5mg/kg IP) or chronically (1mg/kg/day IP or 2mg/kg/day IP during 5 days). On day 4 of abstinence the spleens were removed and the proliferative response was assessed. Acute AMPH at doses of 2.5 and 5 mg/kg inhibited splenic proliferative responses by 50 and 65%, respectively, and chronic AMPH at doses of 1 and 2 mg/kg inhibited by 45 and 65%, respectively. This study demonstrates that either a single injection of AMPH or a chronic AMPH treatment results in a dose-dependent decrease in the proliferative response of splenocytes. The present findings extend our previous evidence on AMPH- and stress-induced immunosuppressive effects on lymphocyte subpopulations. Considering that drugs of abuse, stress, and psychiatric disorders are associated with deficits in the immune system, this study might contribute to understand the abnormal immunologic function frequently observed in human addicts.

56.

IMMUNE RESPONSE AND AUTONOMIC NERVOUS SYSTEM (ANS) RELATIONSHIP IN TYPE I AND II DIABETIC STATE

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It has been suggested the existence of an immunosuppressive state in diabetic disease. ANS have an important role in the control of immune responses and we have previously studied its relation with immune response during the first three-month experimental type I diabetes induction. We have reported suppression in T-cell dependent humoral response after one month of diabetic induction. With respect to ANS influence, norepinephrine (NE) shows a biphasic effect on normal T cell proliferation, stimulating and inhibiting at low and high concentrations respectively. After one month of diabetic induction, only the inhibitory effect was observed. We here studied immune response and the involvement of ANS in mice, with type II and I diabetes, after 6-month induction. Results show suppression in T-cell dependent humoral responses in both diabetic type mice and lower mitogen induced T-cell proliferation only in type I. Concerning neurotransmitter influence on proliferative responses, only NE-mediated inhibitory effect was observed in normal 6-8 month old mice. Experiments performed with specific agonists and antagonist for α_2 and β_2 adrenergic receptors indicate suppression for α_2 subtype. No differences were observed in NE response between 6-8 month old normal and type I and II diabetic animals. The cAMP response after adrenergic stimulation was according with these findings. These results indicate that immune response in type I and II of diabetes is diminished which is not correlated to an alteration in neurotransmitter response on lymphocytes at this age.

57.

CHOLINERGIC REGULATION OF NA^+-K^+-ATPASE ACTIVITY IN RAT PAROTID GLAND. CHANGES AFTER CASTRATION

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In this study we investigated the different signalling pathway involved in M_3 muscarinic acethylcholine receptor (mAChR) dependent modulation of Na^+-K^+-ATPase in parotid glands from control and castrated rats. Carbachol-stimulation of M_3 mAChR down regulates the enzyme activity of parotid slices from control rats. The inhibition of calcium-calmodulin (CaM) by trifluoperazine (TFP) abolished the inhibitory effect of the agonist while the inhibition of protein kinase C (PKC) by staurosporine induced an up-regulation of the Na^+-K^+-ATPase. An up-regulation of the enzyme was observed with carbachol in parotid slices from castrated rats and TFP inhibited this stimulant effect while staurosporine lack of effect. Our results indicate that in control glands the activation of a phospholipid-calcium-calmodulin dependent PKC is the responsible of the inhibitory effect of carbachol on Na^+-K^+-ATPase activity while in castrated rats, the carbachol stimulatory effect on the enzyme activity is regulated by the CaM-stimulating action, failing in activating PKC.
The up-regulation of the Na^+-K^+-ATPase observed in castrated rats resulted in a decrease of carbachol-induced K^+ release and thereby could decrease salivary fluid production. We suggest that such an effect could underlie the dry mouth that occurs in the elderly.

58.

DEHYDROEPIANDROSTERONE MODIFIES GH RELEASE FROM CULTURED PITUITARY CELLS. MECHANISM OF ACTION

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There has been a strong resurgence of interest in DHEA (the main steroid produced by the adrenal gland) because of its suggested antitumoral and anti-ageing effects. We recently showed that DHEA *in vivo* was able to increase GH release, and partially reverse ether-induced inhibition of GH. The aim of this study was to determine the effects of DHEA on basal or GHRH- and somatostatin (STT)-induced GH secretion in normal pituitary cells *in vitro.* Pituitary cells of 60-day old female rats were cultured and stimulated with DHEA, in combination with STT or GHRH. Then the GH, cAMP and $[Ca^{2+}]_i$ increase were measured by RIA or by the FURA-2 fluorometric method. DHEA ($1*10^{-5}$ to 10^{-7} M) did not modify basal GH secretion, but it increased GH secretion induced by GHRH (10^{-8} M) (227 $\pm$ 20 and 321 $\pm$15% of basal secretion for GHRH and DHEA+GHRH, respectively). This effect was paralleled by cAMP production in response to GHRH. But it was not related to the intracellular Ca^{2+} mobilization induced by GHRH, which was in fact reduced by DHEA pretreatment. On the other hand, DHEA partially reversed STT (10^{-8} M) -induced GH inhibition (45 $\pm$ 6 vs 74 $\pm$ 8% of basal secretion, for STT and DHEA+STT, respectively) without affecting STT-induced decrease in cAMP production. We conclude that GH release evoked by DHEA *in vivo*, may be related to its direct pituitary modulation of GHRH and STT action.
With the support of CONICET and and ANPCYT.

59.

CHRONIC TREATMENT WITH FLUOXETINE INCREASES HIPPOCAMPAL GLUTAMATE RELEASE AND DECREASES CONVULSIVE THRESHOLD

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Fluoxetine (Flx) is a frequently prescribed antidepressant whose effects in hippocampal glutamatergic neurotransmission remain controversial. The main goals of this work were to investigate two parameters of hippocampal glutamatergic neurotransmision and their possible consequences on two glutamate-associated behavioral responses. Male adult Wistar rats were acutely (single dose) or chronically (21 daily injections) treated with saline or 10 mg/kg (i.p.) of Flx. [^{3}H]MK-801 binding to hippocampal membranes and the glutamate release induced by a 60 mM K^+ stimulus were assessed. Convulsive threshold was evaluated by pentilenetetrazole test and the spatial learning was studied in a radial maze. With the acute treatment no changes were observed either in the binding parameters or in the ex vivo glutamate release. However, a significative increase (200%, $P < 0.001$) was observed in the basal glutamate outflow from hippocampi of chronically treated rats. Chronic treatment also reduced the latency to the first convulsive event ($P < 0.05$) and increased convulsive scores ($P < 0.001$). On the other hand, no differences were observed in the radial maze test. These results indicate that Flx induces a decrement in the convulsive threshold probably due to an increment in the basal extracellular glutamate level.

60.

PHARMACOLOGICAL MODULATION OF STRIATAL ANALGESIC EFFECTS

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The nigrostriatal dopaminergic projection has been involved in central pain modulation. In previous studies, we demonstrated that electrical or chemical striatal stimulation inhibit the nociceptive jaw opening reflex (JOR). The objetive of this study was to examine the role of dopamine on the analgesic striatal action. In anesthetized rats, the dental pulp of lower incisors was stimulated with implanted electrodes. The JOR was recorded in the digastric muscle. A dose-response curve to apomorphine (0, 3, 10, 30 nmol/ 0.5 µl) microinjected into the striatum produces an inhibition of the JOR at doses of 10 and 30 nmol as compared with control (44.1±10.5 and 73.6±9.9%, Neuman Keuls $p < 0.005$, n=11). Microinjections of the D_2 agonist quinpirole at doses of 10 and 30 nmol/0.5 µl produced similar inhibition of the JOR, but not by the agonist D_1 SKF 38393 (17 nmol/0.5 µl) and the antagonist D_2 haloperidol (7 nmol/ 0.5 µl). Naloxone (0.2 mg/kg i.v) partially reverted the effect of quinpirole. The nociceptive response of the neurons of the trigeminal caudal nucleus was also inhibited (49.6±9.5%, compared to control; NK $p < 0.05$, n=11) by intrastriatal microinjections of quinpirole (30 nmol/0.5 µl) and reverted by naloxone. The present study suggests that the striatal D_2 dopamine receptors are involved in the analgesic action of the nucleus, mediated by opiod receptors, and that this effect is not tonic since microinjection of haloperidol was uneffective.

61.

OVARIC HORMONES EFFECT ON GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN THE AMYGDALOID COMPLEX OF THE RAT

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In several brain regions the presence of GABA and glutamic acid decarboxylase (GAD) cycling modifications during the estrous cycle has been demonstrated. In this work, we have studied GAD activity during the estrous cycle in the rat central and medial area of the amygdaloid complex. We have found that throughout different times of the day, GAD activity presented diurnal fluctuations. This appeared constant during diestrus 1 period, while a daily afternoon increase was found from diestrus 2 to proestrus. On the other hand, during the proestrus and estrus night, GAD activity decreased significantly compared to the values measured in the afternoon. Since estrogen plasma levels increase from diestrus 2 to proestrus afternoon, the effect of ovaric hormones on GAD activity were studied in the afternoon for ovariectomized rats both untreated and treated with estrogen (5 ug/100 gr) or estrogen plus progesterone (2 mg/rat). Ovariectomy induced a decrease in enzymatic activity compared with that of proestrus rats. This fact contrasted with the increase promoted 3 days later by estrogen administration, while estrogen plus progesterone in the 3rd day of estrogen injection induced a decrease of GAD values similar to those found in ovariectomized rats.
These results point to a modulation by ovaric hormones of GAD fluctuations during the estrous cycle in the amygdaloid area studied.

63.

EFFECT OF LITHIUM ON THE MELATONIN SYNTHESIS IN THE PINEAL GLAND OF VIZCACHA (*Lagostomus maximus maximus*)

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Melatonin is a neurohormone synthesized in the pineal gland and it is also produced, but to lesser extent, by extrapineal tissues. Lithium (Li) is a drug that has been shown to affect different parameters of circadian rhythms, that may relate to its therapeutics effectiveness and may affect melatonin rhythmicity. In this study, we investigated the effect of lithium on the biochemical parameters involved in melatonin synthesis in the vizcacha pineal gland. Adult male vizcachas were divided in two groups and daily i.p. injected: a) LiCl (1 mEq/kg), b) vehicle (C) for five weeks. Serum Li was determined by atomic absortion spectrometry. Pineal glands were cut into 15-µm-thick cryostat sections. *In situ* hybridization for mRNA encoding β_1-adrenoceptor and arylalkylamine N-acetyltransferase (AA-NAT) enzyme, were quantified. Pineal glands were homogenized, AA-NAT activity and melatonin content were determined by radiochemical method and RIA, respectively. Results: Serum Li (µEq/ml): 0.61±0.071 (Li), 0.006±0.0025 (C), $p<0.005$, β_1-adrenoceptor mRNA (Dpm/ mg tissue):459.0±6.75 (Li), 471.9± 9.23 (C), n.s.. AA-NAT mRNA (Dpm/mg tissue): 311.15± 15.53 (Li), 455.96± 8.32 (C), $p<0.001$. AA-NAT Activity (pmoles/ mg tissue/10 min): 53.69±1.15 (Li), 40.20±7.87 (C), n.s.. Pineal melatonin (pg/mg tissue): 283±39.85 (Li), 449.4± 56.72 (C), $p<0.02$. Our data suggest that lithium treatment affect the expression of the mRNA encoding AA-NAT enzyme, and consequently the melatonin synthesis in the pineal gland of vizcacha.

62.

CYTOTOXIC ACTIVITY OF KAURENE DERIVATIVES

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Kaurenic acid sodium salt **(1)**, 15-ceto-16,17 epoxy-kaurenic acid **(2)**, 15-acetoxy-16,17 epoxy-kaurenic acid **(3)**, kaurenic acid rhamno-pyranosyil ester **(4)** and kaurenic acid gluco-pyranosyl ester **(5)** were tested at 10, 50, 100 and 200 µ/g/ml in MEM and examined for their *in vitro* cytotoxic effect against melanoma B16F1 cell line in comparison with taxol **(6)**. Tumour cells cultures were started from C57BL/6 mice at least passage and kaurene sugar derivatives were obtained from kaurenic acid according to Mitsunobu. Epoxy derivatives were obtained by treatment of 15-cetokaurenic acid and grandifloric acid with dimethyloxyrane. Structure and configuration of compounds were established by 2 D NMR analysis. Cellular viability was determined in the presence or absence of kaurenes and taxol. Compounds that produced 60% cell lethality were considered citotoxic. The citotoxic activity of kaurenes on B16F1 was **(1)** 60/40 (0.33 µM) and 67/33 (0.66 µM); **(2)** 33/67 (0.30 µM); **(3)** 55/45 (0.26 µM); **(4)** and **(5)** 10/90 (0.033 µM) in relation to **(6)** 37/67 (6.02 µM). These data confirm the citotocity of sodium kaurenate **(1)** and suggest the need of a complete *in vivo* study for kaurene derivatives.
Supported by Grant CDCHT 025-ME-1998, Medicine School-UCLA and Badan-Lara. The cells in vitro were recognised by Dr. Dolinar J and count in vivo by Univ. Linárez G.

64.

GLUTAMATE AND PROLINE UPTAKE BY CEREBRAL CORTEX SYNAPTOSOMES DURING POSTNATAL DEVELOPMENT

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During development, a number of reports demostrate the developmental change of the neuronal responses to excitatory and inhibitory transmitters. Here, we examined the influence of synaptic activity on Glutamate (Glu) and Proline (Pro) transporters responses in postnatal development. Both L-Pro and L-Glu are excitatory amino acids in the mammalian central nervous system. The fact that both amino acids are metabolically interconvertible in the brain, gives rise to an idea that the action of L-Pro could be partly mediated by processes associated with neurotransmission by L-Glu. Neonatal rat brain synaptosomes were freshly prepared for determination of incorporation of radiolabeled amino acids in Krebs Ringer buffer at 2°C and 30°C for different periods up to 30 min. The accumulation of [^{3}H]-Pro and [^{3}H]-Glu was almost abolished following replacement of NaCl by KCl in the incubation medium and reduction of incubation temperature from 30°C to 2°C, respectively. Neonatal rat brain synaptosomes could take up both radiolabeled amino acids in a fashion dependent on sodium ions and temperature. The data cited above suggest that temperature-dependent uptake may be at least in part involved in mechanisms associated with neuronal activities of both L-Pro and L-Glu during postnatal development.

65.

AN ACUTE RESTRAINT STRESS INDUCES SENSITIZATION TO STIMULATING EFFECTS OF AMPHETAMINE AND MORPHINE ON DOPAMINE RELEASE FROM THE SHELL AND CORE OF THE NUCLEUS ACCUMBENS: INVOLVEMENT OF NMDA RECEPTORS

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Acute restraint stress exposure induces sensitization to stimulating properties of amphetamine (AMPH) and morphine (MOR). The mesolimbic system innervating the nucleus accumbens (NAC) shell and core subregions have been specifically activated by drugs, stress and it is also implicated in the development and/or expression of sensitization. Our main goals were: 1) to study the acute restraint-induced sensitization to AMPH and MOR, on DA release by microdialysis from NAC shell and core, 2) the involvement of NMDA receptors in the stress-induced sensitization. Wistar male rats (250-320 g) were used. Two days after the surgery, we administered MK-801 (0.1 mg/kg i.p.) or vehicle 30 minutes before the restraint stress (2h). The following day we evaluated the effect of AMPH (0.5mg/kg i.p.) and MOR (2mg/kg i.p.) on DA release from NAC by microdialysis during 3 hours. Both drugs induced a significant higher increase in DA release from NAC core and shell in the restraint group, compared to that observed in the no restraint group. MK-801 blocked the restraint stress-induced effects of drug on DA release. These findings showed a restraint-induced sensitization to AMPH- and MOR-evoked DA releases from NAC core and shell, and an involvement of NMDA receptors on it. A glutamatergic-dopaminergic neurotransmission interaction in NAC can be underlying the stress-induced behavioural sensitization to abuse drugs.

66.

STRUCTURAL BASIS FOR INHIBITION OF CYCLOOXYGENASE (COX) BY MELATONIN

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Previous work of our group have shown that melatonin is an acute anti-inflammatory agent. COX is the key enzyme in the synthesis of prostaglandin, which is one of the mediators on the inflammation processes. Melatonin is an indole with a chemical structure similar to indomethacin, as known inhibitor of COX. Several three dimentional structures of COX 1 and COX 2 complexed with indomethacin and other inhibitors have been reported previously. The aim of this work was to investigate the interaction of melatonin with COX using computer graphic applications and docking algorithm. **Results:** Docking experiments of melatonin in the deep hydrophobic active site of COX showed that melatonin may interact with the enzyme in a similar manner such as known COX inhibitors like indomethacin, aspirin and others.

67.

POSSIBLE ADVERSE EFFECTS INDUCED BY 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) IN DEVELOPING RATS

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Histological and morphometrical studies of reproductive organs complement the determination of developmental and madurative parameters when reproductive toxicity must be analyzed. Virgin female 90 day-old Wistar rats were made pregnant, and exposed to 2,4 D (70 mg/kg/day, sprayed on food) from gestation day 16 onwards. On postnatal day 23, pups were weaned and the treated group continued to be fed with 2,4 D until sacrifice at 45 or 60 days of age. Histological studies were performed in ovaries and testes fixed and stained with H-E. In ovary, the number of corpora lutea and primordial, primary and secondary follicles were counted. In testes, mean tubular diameter and height of the seminiferous epithelium were measured.

Histological studies reveal no morphological alterations in these organs. The differential ovarian follicles counts showed diminution in number of corpora lutea and primordial and primary follicles in 60 days treated. The mean tubular diameter was increased in 45 days treated, without alterations in epithelium height.

These results, together with the others parameters already determinated, suggest an indirect testicular toxicity and a direct ovarian toxicity of 2,4-D.

68.

MORPHINE WITHDRAWAL INCREASED FREEZING AND c-fos EXPRESSION IN SELECTED BRAIN REGIONS AFTER EXPOSURE TO AN EMOTIONAL CUE

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Besides physical signs, the abrupt discontinuation from chronic morphine (MOR) administration results in a well known motivational withdrawal syndrome. Early-expressing genes activation may be the mechanism through which a short stimulus would cause long-term changes in genetic expression, and alter the neuronal response to subsequent events. In the present study, we assess the influence of MOR abstinence on: 1) the emotional fear response within a conditioned fear paradigm 2) c-Fos expression in selected brain areas associated with the modulation of emotional behavior in response to fear conditioning stimulus. Male Wistar rats were submitted to the implantation of 2 (50 mg/Kg) morphine pellets (s.c) or placebo, which were removed by the third day. One week following removal, animals were evaluated in a contextual conditioned fear paradigm and their brains treated to assess c-fos through immunohystochemistry. Abstinent MOR rats display an increased fear response (freezing) and a robust c-fos expression in brain regions involved in fear emotional response modulation after the exposure to the contextual cue.

69.

GABAPENTIN ADMINISTERED REPEATEDLY IMPAIRS RETENTION OF AN INHIBITORY AVOIDANCE RESPONSE IN MICE

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Gabapentin (GBP) has been shown in extensive preclinical and clinical studies to be an effective anticonvulsivant drug. Although designed as a GABA analogue it is clearly not a GABA-mimetic. The actions of GBP on learning and memory in rodents are practically unknown. We recently demonstrated that post-training acute administration of GBP (50 mg/kg ip) enhanced memory consolidation of an inhibitory avoidance response in mice, probably through a desinhibitory effect on the activity of central cholinergic mechanisms. We report here that GBP, when given following a repetitive schedule of administration (50 mg/kg ip, two doses at day, 12 hs apart, during 7 days), impared retention test performance of the avoidance response, and reduced significantly the activity of the high affinity choline uptake on the hippocampus. It is suggested that the impairment on retention due to repeated doses of GBP, might be a consequence of a possible neuromodulatory negative and indirect interaction between the cholinergic and other neuronal systems, perhaps GABAergic in nature.

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70.

POTENTIATED ADRENALINE VASOCONSTRICTOR RESPONSE BY SUBTHRESHOLD CONCENTRATIONS OF SEROTONIN, ENDOTHELIN-1 OR U-46619 IN HUMAN UMBILICAL VEIN (HUV): EVALUATION OF RECEPTORS INVOLVED

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Serotonin (5-HT), endothelin-1 (ET-1) and thromboxane A_2 mimetic, U-46619 (U), not only act directly on blood vessels but also may amplify adrenaline-mediated responses. The aim of this study was to asses whether subthreshold concentrations of mentioned agonists potentiate adrenaline-induced contraction in HUV rings and determine the receptor subtypes involved. Rings were mounted in isolated organ baths suspended in Krebs solution, 37ºC, pH 7,4. After 2 hs equilibration period rings were pretreated with 5-HT (1.7 nM), ET-1 (0.1 nM) or U (0.3 nM) during 10 min in the absence and presence (30 min) of the following antagonists: ketanserine 10 nM (5-HT_{2A}) and SB-216641 10nM (5-HT_{1B}), FR-139317 10 μM (ET_A and ET_B) or SQ-30741 100 nM (TP). Then, concentration responses curves (CRC) to adrenaline were constructed. 5-HT, ET-1 and U produced a leftward shift of CRC to adrenaline ($p<0.05$). The antagonists ketanserine, FR-139317 and SQ-30741 abolished potentiation whereas SB-216641 had no effect. We conclude that potenciated adrenaline vasoconstrictor effects of 5-HT, ET-1 and U are mediated by estimulation of 5-HT_{2A}, a mix population of ET_A and ET_B and TP receptors, respectively in HUV.

71.

CHRONIC DIABETES MODIFIES VASCULAR PROSTAGLANDIN PRODUCTION IN THE RAT

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Vascular disease is a frequent complication of diabetes mellitus (DM) and prostanoids (PR), metabolites of arachidonic acid (AA) through the cyclooxigenase (COX) pathway, are vasoactive substances produced and released by the vessel wall. The aim of this work was to study the influence of chronic streptozotocin (STZ) DM in the production of PR in vascular tissues of rats. Type 1 DM was induced in Wistar rats of either sex by an i.p. injection of STZ, 55mg/kg in citrate buffer. Control rats received buffer alone. Animals with glycemia above 16 mM/l were considered as diabetic. 120 days after the induction of DM, the animals were sacrified and the thoracic aorta (A) and mesenteric vascular bed (MVB) were dissected and incubated in Krebs solution. After extraction, PR released to the incubation medium were measured by HPLC. In MVB, DM reduced the 6-keto prostaglandin $(PG)F_1\alpha$ (stable metabolite of prostacyclin or PGI_2) and PGE_2 release ($p<0.01$) as compared with the non-diabetic group, without modifications in the other PR assayed ($PGF_2\alpha$ and thromboxane (TX). On the other hand, in A, DM reduced only the 6-keto$PGF_1\alpha$ release ($p<0.01$). In both preparations, the PGI_2/TX ratio was diminished in the DM group ($p<0.01$). In conclusion, the reduction in vasodilator PR release without change in vasoconstrictor PR release induced by chronic DM favours the production of deleterious PR, impairing the functional status of the cardiovascular system. This could be related with the vascular complications of DM.

72.

ORAL EFFECT OF BENZOPHENONE-1 IN RATS

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Introduction and objectives: The ability of benzophenones to absorb and dissipate UV radiation is well-known. It can have important application in medicine and cosmetic industry for purposes of protecting the skin from UV rays. There 12 types of benzophenones described (BZ-1 through BZ-12), the BZ-3 being the most used sunscreen on the market today. In previous studies we studied comparatively the effect of BZ-1 topically administered in rats in order to evaluate toxic effects. The aim of this study is to assay different doses of BZ-1 by oral route for further toxicological comparative purposes. **Materials and methods:** Wistar rats of both sexes were housed individually with free access to food and water at constant temperature (25º) under a 12 h light-dark cycle. BZ-1 (Sigma Co.) was dissolved in corn oil and administered orally to rats at unique doses of 40, 100 and 500 mg/kg body weight, controls animals received corn oil only. Blood samples were withdrawn via cardiac puncture under light ether anesthesia before and 6 h after the drug administration for hematological and clinical chemistry studies. **Results and conclusions:** no deaths records were observed in the BZ-1 treated animals after 48 h of the administration and the clinical blood exams were not altered . This study suggests that BZ-1 in the conditions herein assayed were not toxic to rats.

73.

INVOLVEMENT OF HIGH AFFINITY NEUROTENSIN (NTS1) RECEPTORS IN PHOSPHOINOSITIDE HYDROLYSIS STIMULATION BY OUABAIN AND CARBACHOL IN NEONATAL RAT BRAIN

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Neurotensin (NT) is a neuropeptide involved in neurotransmission or neuromodulation which is widely distributed in the CNS. This peptide colocalizes with the cholinergic system and its effects are mediated by specific membrane receptors, which present high (NTS1) and low (NTS2) affinity binding sites. Phosphoinositide (PI) hydrolysis is stimulated in rat brain by the Na^+, K^+ -ATPase inhibitor ouabain and by the muscarinic agonist carbachol. To study the participation of NTS1 receptors in such effects, we tested [^{3}H]inositol phosphate (IP) accumulation in neonatal rat brain in the presence of ouabain, carbachol, with or without SR 48692, a specific non-peptidic antagonist for NTS1 receptor. Cerebral cortex miniprisms from Wistar neonatal rats were incubated with [^{3}H]*myo*inositol and PI turnover was quantified. IP accumulation was 1183 ± 338% (n = 6) and 258 ± 24% (n = 5) of basal value with 10^{-4}M ouabain and 10^{-3}M carbachol, respectively. Such effects were significantly decreased with 10^{-4} M SR 48692, which alone failed to alter PI hydrolysis.These results suggest that NTS1 receptors are involved in PI hydrolysis stimulation by ouabain and carbachol in neonatal rat brain cortex.

75.

NA^+, K^+-ATPASE ACTIVITY AND GLUTAMATE TRANSPORTERS: ARE THEY COUPLED?

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Glutamate transport (GluT) plays a crucial role in clearing glutamate from the synaptic cleft and in the regulation of metabolism and glutamate recycling, thus being absolutely essential for homeostasis. GluT is driven by Na^+ and K^+ transmembrane concentration gradients that are generated by Na^+,K^+-ATPase. Therefore, we decided to study the relationship between GluT and the Na^+,K^+-ATPase in rat brain prisms using uptake of Rb^+ as a measure of Na^+,K^+-ATPase activity. Prisms (0.1 mm x 0.1mm x thickness of cortex) were incubated at 37 °C, separated by rapid vacuum filtration, extracted and analysed for both Rb^+ and K^+ content by atomic absorption spectroscopy. Uptake of Rb^+ (125 mM) increased linearly with time for at least 12.5 min. Ouabain significantly decreased both Rb^+ and K^+ levels (IC50~17 mM), however, several typical substrates and/or inhibitors of Na+-dependent L-glutamate transport failed to produce any significant effects (up to 500 mM). In Mg^{2+}-free media, a decrease was seen in the Rb^+ and K^+ levels in the presence of NMDA receptor agonists. The present data indicate that at least a subset of glutamate transporters could be strongly dependent on the activity of Na^+,K^+-dependent ATPase but provide no evidence for any direct/molecular two-way link between the Na^+-dependent transport of L-glutamate and Na^+,K^+-dependent ATPase in brain tissue *in vitro.*

74.

CHANGES IN CORTICAL μ-OPIOID RECEPTOR BINDING IN MORPHINE WITHDRAWN MICE

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We have previously demonstrated sex differences in whole brain and striatal μ-opioid receptor binding parameters during the morphine (MOR) withdrawal syndrome in mice. In this study, we evaluated cortical μ-opioid receptor binding in male and female mice, during MOR withdrawal and its prevention with the $GABA_B$ agonist, baclofen (BAC). Swiss-Webster mice (24-27g) were rendered dependent by i.p. injection of MOR (2 mg/kg), twice daily for 9 days. On the 10th day, dependent mice were divided into two groups: *withdrawal* group received naloxone (NAL, 6 mg/kg, i.p.) after the last dose of MOR in order to precipitate the abstinence syndrome, while *prevention* group received BAC (2 mg/kg, i.p.) before NAL injection. After these treatments mice were killed, prefrontal cortex was dissected and binding of [^{3}H]-DAMGO to μ-opioid receptors was performed. A significant increase in cortical μ-opioid receptor density occurs during MOR withdrawal in either sex, but no alterations in affinity of μ-binding sites occur in males nor in females. In prevention groups, no differences were shown, neither in density nor in affinity, compared with the control groups. Our results indicate that cortical μ-opioid receptor upregulation observed during the NAL-precipitated withdrawal occurs in either sex. Considering that prefrontal cortex is involved in the reward system, a possible mechanism intending to compensate the blockade of μ-opioid receptors is developed similarly in males and females.

76.

LONG TERM EFFECTS OF PERIODIC PSYCHO-PHYSICS AGGRESSION (CHRONIC STRESS) ON METABOLIC AND PRODUCTIVE VARIABLES IN CHICKENS

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The preliminary results of the use of immobilisation, high-density and looking-up maneuvers, which induce psycho-physical stress, are presented here. Organic-induced modifications compatible with a chronic adaptation were performed to evaluate their metabolic-productive impact. Male chickens of autosexed crossing, New Hampshire x Barred Plymouth Rock (32 of 64 for each one of two blocks), were housed in twelve cages (experimental unit) during a period of 55 days. Maneuvers were made periodically, using one block in summertime and the other in wintertime. With a DBCA and analysis of repeated measures, there were significant differences in the response to fear and hematocrit variables; not being statistically different in body weight or carcass weight, neither in the relative weight of abdominal fat. In other experiences there have being reductions in the body weight in stressed broiler chickens that posses a genetic difference and higher susceptibility. Increments of the abdominal fat, relative weight and fat content of liver induced by exogenous ACTH or corticosterone, to 18 hs and 9 days of the respective treatment were reported as well.

77.

FAILURE OF OXYMETHOLONE (OX) TO ELICIT AN ERYTHROPOIETIN-HYPERSECRETORY STATE IN THE ORCHIDECTOMISED MOUSE

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Erythropoietin-hypersecretory state (EPO-HS) is a condition elicited by inducers that is easily observed in transfused-polycythaemic rodents, in which hypoxia-stimulated EPO secretion is higher than in polycythaemic controls. Steroids with different androgenic/anabolic ratios have probed to be potent inducers of EPO-HS. However, both steroid activities do not appear to have similar importance. The purpose of the present study was to evaluate the ability of OX, that shows the highest anabolic/androgenic ratio, to elicit an EPO-HS. Mice were orchidectomised when aged 30 days. One month later, groups of animals were injected with graded doses of OX during 4 weeks. All mice were hypertransfused at the end of the injection period and then exposed to 506.5 mbar for 6 h. Plasma EPO titre was determined by immunoanalysis and taken as a reflection of the EPO-production rate. Kidney, seminal vesicle and levator ani muscle weights were registered as index of renotrophic, androgenic and anabolic effects, respectively. The steroid brought out a significant anabolic and a poor androgenic response. Any of the doses of OX tested increased EPO production in orchidectomised polycythaemic mice and, therefore, an EPO-HS was not induced. This finding, coupled with previously reported ones, suggest that only steroids showing a certain degree of androgenic action have the capacity to evoke an EPO-HS.

78.

APOPTOSIS AND BCL-XL EXPRESSION DURING HEMATOPOIETIC RECOVERY AFTER CITOTOXIC INJURY

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Apoptosis (programmed cell death) play an important role in regulation of normal tisular homeostasis. It can be triggered by various stimuli, including chemotherapeutic drugs. On the other hand, the survival of hematopoietic cells is also controlled by the Bcl-2 family. Some members, such as Bcl-2 and Bcl-xl are considered as anti-apoptotic proteins whereas Bax and the alternatively Bcl-xs gene product, act as cell death promoter. However, there is limited understanding about how apoptosis is modulated in bone marrow (BM) cells, after Paclitaxel (Px) injury. The aim of this work was to study the *in vivo* recovery of murine hematopoiesis and to correlate changes expression of Bcl-xl (inmmunoblotting), apoptosis (TUNEL assay) and BM cellularity, following a single dose of Px (29 mg/kg; i.p.).Whole bone marrow lysates, was examined at 0; 6, 12 , 24 , 48 ; 72 ; 96 and 120 hours post-Px injury. Apoptosis (24 ± 0.81%, $p<0.01$) and decrease in BM cellularities (28 ± 4.2% of control) were maximal at 24 h, and returned to normal values (3.08 ± 0.61%) by day 3 post Px. The minimal expression of the antiapoptotic protein was noticed at 12 h ($p< 0.01$). Bcl-xl were increased significantly over control values between 24 - 48 h ($p< 0.05$ and $p< 0.01$ respectively). These results suggest that Bcl-xl is playing a key role in survival of bone marrow progenitors in order to restore size and composition of hematopoietic compartment.

79.

CD 44 AND MATRIX METALLOPROTEINASE MMP-2 EXPRESSIONS IN PACLITAXEL-INJURED HEMATOPOIETIC CELLS

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Normal hematopoiesis takes place in the bone marrow (BM) microenvironment. Cell-cell and cell-extracellular matrix interactions through adhesion molecules play a major role on its regulation. CD 44 and the matrix metalloproteinase MMP-2 are responsible for the homing and mobilization of hematopoietic cell progenitors. Moreover, these processes might be affected by cytoreductive drugs. The aim of this study was to evaluate CD44 and MMP-2 expressions in murine BM and splenic (S) hematopoietic progenitors after Paclitaxel (Px) injury (29 mg/Kg i.p). BM and S cells were collected at 1, 2, 4, 6, 8 and 10 days post-injection. Cells expressing CD44 and MMP-2 were identified by FITC immunocytochemistry. DAPI counterstaining was used to evaluate nuclear morphology. Percentages of viable cells that express the proteins under study were obtained from 10 random fields documented at each day from three different assays in triplicate. In physiological conditions, CD44 is expressed in BM but not in S, meanwhile MMP-2 failed to express in both tissues. BM CD 44 expression decreased until the 7 th day ($p<0.01$) and it was detectable in splenic tissue only at the 3rd day post Px injury. BM MMP-2 expression increased significantly ($p<0.01$) between 1-3 days of recovery. These data suggest that during the maximal injury occurs a reversal relationship between CD44 and MMP-2 in BM microenvironment. Moreover, the changes in those protein expressions would justify the mobilization of BM progenitors for their transitory splenic homing during Px recovery.

80.

HEMATOPOIETIC MICROENVIRONMENTAL INJURY INDUCED BY CADMIUM

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Cadmium (Cd) is an ubiquitous toxic that is capable of modulating hemopoietic response. The injury and toxic effect of Cd were examined on hematopoietic tissue.

The aim of this work was to study *in vivo* recovery of hematopoiesis after Cd injury, correlating apoptosis and necrosis, characterized by specific morphological and histopathology changes. Therefore mice were injected with 1 mg/kg i.p. of Cd, and their bone marrows (BM) and spleens (Sp) were removed at 6, 12, 21 days later. BM and Sp cellularities, and hematopoietic precursors were determined. Cd induced at 21 days, a significant decrease of BM and Sp cellularities (30%, $p<0.01$), and concomitant erythroid and myeloid precursor were reduced from day 6 until the end of the experience ($p<0.01$). The expression of apoptosis and necrotic markers were determined by light and electronic microscopy. Under these conditions, the Cd treatment induced both a time and dose-dependent increment on apoptotic index and necrosis severity. These data demonstrate that necrosis is a major mode of elimination of critically damaged cells in acute Cd injury in mice, and it precedes an active process of programmed cell death.

81.

EFFECTS OF CHRONIC TREATMENT WITH CORTICOSTERONE ON CYTOSKELETAL MARKERS OF HIPPOCAMPAL NEURONS

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Glucocorticoids overexposure induces CA3 pyramidal neurons atrophy and hippocampal dysfunction (i.e. impairment of inhibitory avoidance learning). The aim of our work was to study the following neuronal cytoskeletal markers in the hippocampus of rats chronically treated with corticosterone: light (NFL), medium (NFM) and heavy (NFH) neurofilaments together with microtubule associated protein 2 (MAP-2).

Adult male Wistar rats were subcutaneously implanted with corticosterone pellets (T1 200mg/animal, and T2 400 mg/animal) during 21 days. The control group was sham operated but received no implantation. Neurofilaments and MAP-2 were quantified by immunohistochemistry and image analysis.

A marked decrease in NFL (91%, $p<0.01$), NFM (38%, $p<0.05$) and NFH (60%, $p<0.001$) was found in CA3 with T2. Similar observations were obtained in dentate gyrus and CA2 with T2 and in the three areas with T1. On the other hand, while T1 treatment did not change MAP-2 immunoreactivity, T2 significantly reduced it in CA3 and dentate gyrus ($p<0.05$).

These results may underlie hippocampal atrophy previously reported and demonstrate a dissimilar sensitivity of cytoskeletal structures to corticosterone, as neurofilaments are affected by both doses of the hormone, while MAP-2 remains unchanged with the lower.

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82.

FLUOXETINE IMPROVES BEHAVIORAL PARAMETERS BUT FAILS TO REVERT NEURONAL CYTOSKELETAL ALTERATIONS PRODUCED IN AN EXPERIMENTAL MODEL OF DEPRESSION

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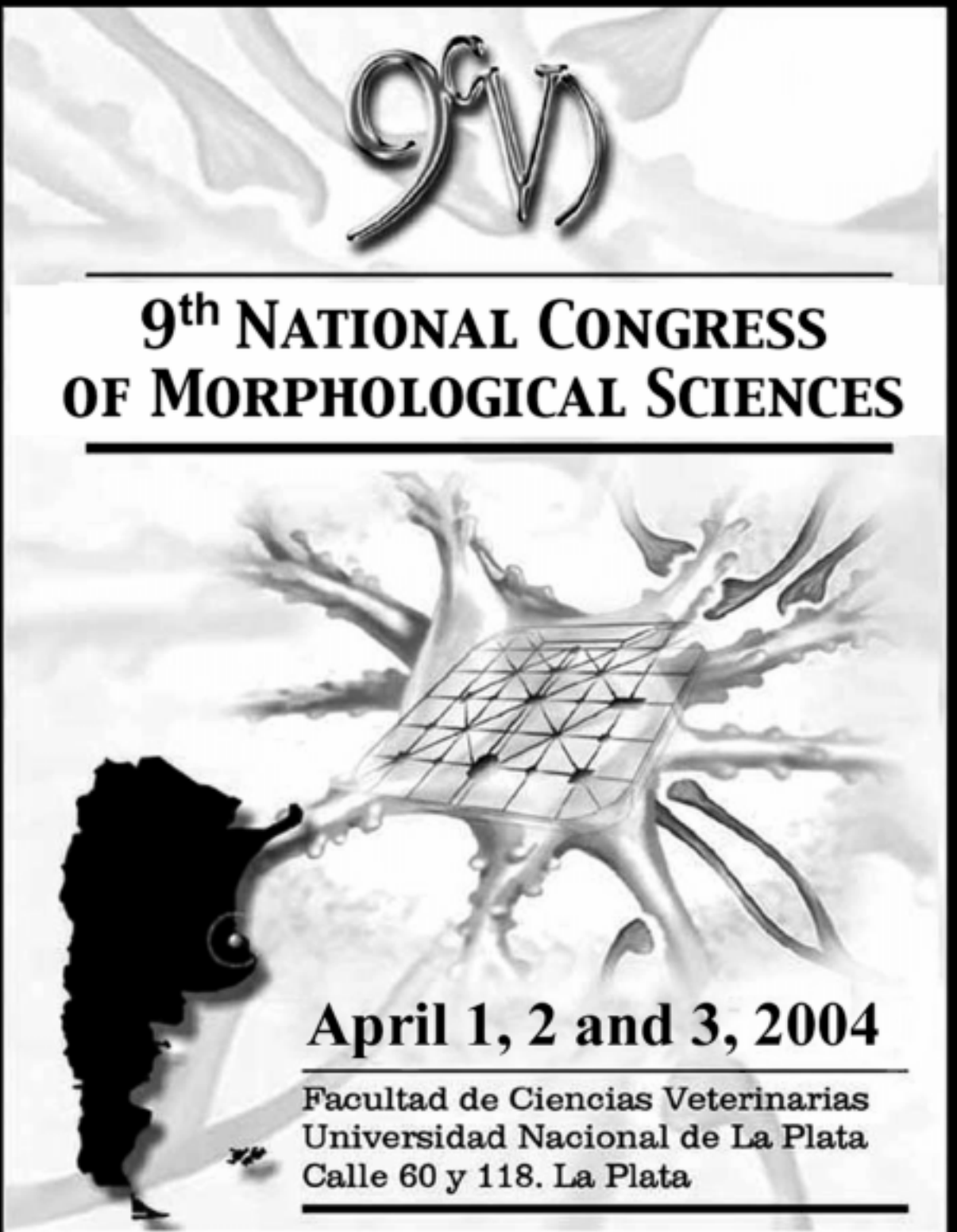
Apical dendrite atrophy of CA3 pyramidal neurons has been reported in experimental depression. We have previously studied different neuronal cytoskeletal markers in the hippocampus of rats exposed to a learned helplessness paradigm and observed a NF-68 decrease without changes neither NF-160 and NF-200 nor MAP-2 in CA3 and DG. The aim of the present study was to evaluate the effect of fluoxetine (FLX) administration on hippocampal morphological changes induced by the same experimental model of depression. Rats were trained with 60 inescapable foot shocks (0.6 mA / 15 sec) and escape latencies and failures were tested 4 days after training. Animals in which learned helplessness behavior was observed were treated with saline solution (SF) or 10 mg/Kg fluoxetine for 21 days. Control rats received no foot shocks and were treated with SF or 10 mg/Kg FLX. In depressed animals, FLX treatment recovered escape latencies and failures nearly to control values ($P>0.05$ *vs* control) but failed to revert NF-68 immunostaining decrease ($P<0.05$ *vs* control) in all studied areas. NF-160, NF-200 and MAP-2 immunostainings were similar in depressed and control animals and were unaltered by FLX treatment. Our results suggest that FLX antidepressant action is not related to hippocampal morphological recovery.



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