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L. 1.

CANNABINOIDS AND THE CARDIOVASCULAR FUNCTION

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Cannabinoids offer an attractive model to study the possible therapeutic applications of this substance of abuse, largely known for its recreational use. The possible pathophysiological role of endocannabinoids was analized in mesenteric beds isolated from control rats as well as from rats made hypertensive through long-term nitric oxide synthase (NOS) inhibition.The endocannabinod anandamide reduced in a concentration-dependent manner the contractile responses to noradrenaline in mesenteric beds isolated from control rats.This effect was enhanced in rats made hypertensive after long-term NOS inhibition but not in the hypertension induced by aortic coarctation. Moreover, whereas the anandamide-induced relaxations were unmodified by either endothelial removal or exposure to cannabinoid CB1 receptor antagonists, they were significantly attenuated by the potassium channel blocker tetraethylammonium as well by the vanilloid receptor antagonist capsazepine. In addition, relaxations caused by anandamide were higher in female compared to male rats and also enhanced in early stages of the endotoxemic shock induced by lipopolysaccharide administration. In the mesenteric bed, anandamide appeared to be taken up through a time-dependent and temperature-sensitive process. The relaxant effects of anandamide on noradrenaline-induced contractions were attenuated by inhibition of anandamide uptake as well as by inhibition of its enzimatic degradation. The intrathecal administration of anandamide to anaesthetized rats caused a dose-dependent reduction in the mean blood-pressure, apparently of central origin, as suggested by the antagonism caused by nicotinic ganglionic blockade through intravenous hexamethonium administration. It is concluded that anandamide could help to maintain the vascular homeostasis whenever the NO production is long-term impaired. On the contrary, anandamide could contribute to exacerbate the vascular hiporeactivity during the endotoxemic shock. Hence, pharmacological manipulations of endogenous anandamide levels could offer a new therapeutical approach for the management of the cardiovascular alterations linked to a reduction of NO production, as in the case of the essential hypertension or, alternatively, to the decreases in blood pressure refractories to conventional therapeutic approaches, as in the case of the septic shock.

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L. 2.

PHARMACOGENOMICS: POTENTIAL OF GENE THERAPY IN THE NEUROENDOCRINE SYSTEM

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Gene therapy, the transfer of genetic material for therapeutic purposes, has undergone an explosive development in the last few years and is part of what has come to be known as pharmacogenomics. Although significant technical problems remain to be solved before effective and safe interventive gene therapy strategies can be routinely applied at clinical level, the unrelenting pace of biotechnology and the growing interest in the clinical potential of gene therapy allow us to anticipate a relevant place for this methodology in the therapeutic arsenal of twenty-first century medicine. The development of gene therapy approaches for the neuroendocrine system, while incipient, has already generated a core of results which emerge as a promising area of research in the neuroendocrinology of aging. A major target for gene therapy is the treatment of neurodegenerative diseases, whose incidence increases progressively with age. In humans, Parkinson's disease, a degeneration of nigro-striatal dopaminergic (DA) neurons, which affects 0.1-1% of the population, is the most conspicuous reflection of the vulnerability of DA neurons to age. Currently, there is a growing interest in the use of neurotrophic factors that prevent the degeneration and enhance recovery of remaining DA neurons.

In rats, aging brings about a progressive degeneration and loss of hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons, which are involved in the tonic inhibitory control of prolactin (PRL) secretion and lactotrophic cell proliferation in the adeno-hypophysis. Excessive loss of TIDA neurons during normal aging is associated in the female rat, with progressive hyperprolactinemia and the development of pituitary prolactinomas. Therefore, we could say that the aging female rat develops a sort of "neuroendocrine Parkinson". In Sprague Dawley female rats we have shown that aging brings about a significant loss of DA neurons in different hypothalamic nuclei, a change that is correlated with a progressive increase serum PRL levels and pituitary tumors. In this model, we wish to implement two types of restorative gene therapy strategies. First, we want to use suicide genes, like the gene encoding the herpes simplex virus type-1thymidine kinase (HSV1-TK), to treat spontaneous (age-related) or experimental prolactinomas in rats. Second, we want to transfer, at hypothalamic level, genes for neurotrophic factors, in order to protect TIDA neurons from the deleterious actions of age or pharmacologic insults (used as experimental tools).

Concerning the first objective, we have successfully used an adenoviral vector harboring a suicide gene coding for HSV1-TK, for the treatment of the rodent pituitary tumor cell lines GH3 and AtT20. The same vector was subsequently used *in vivo* to treat estrogen-induced pituitary prolactinomas in rats. In this case we obtained a partial remission of the tumors. At hypothalamic level, we are trying to specifically lesion TIDA neurons in rats by means of hypothalamic estrogen implants. In this model we will implement the transfer of therapeutic genes like those coding for glial-derived neurotrophic factor (GDNF) and insulin-like growth factor-1 (IGF-1), both of which are known to possess neuroprotective actions on DA neurons.

L. 3.
NEUROENDOCRINE CONTROL OF THE THYMUS
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The thymus gland is a central lymphoid organ in which bone marrow-derived T cell precursors undergo differentiation, eventually leading to migration of positively selected lymphocytes to the peripheral lymphoid organs. This differentiation occurs along with cell migration in the context of the thymic microenvironment, formed of epithelial cells, macrophages, dendritic cells, fibroblasts, and extracellular matrix components (EMC). Various interactions occurring between microenvironmental cells and differentiating thymocytes are under neuroendocrine control. For example, thymic endocrine function, represented by thymulin production, is up-regulated, both *in vivo* and *in vitro*, by thyroid and pituitary hormones, including prolactin and growth hormone and interestingly in this case, a bidirectional circuitry seems to exist since thymic-derived peptides also modulate hormonal production. Hormones and neuropeptides also enhance the expression of ECM ligands and receptors, as well as the degree of TEC-thymocyte adhesion. T-cell migration is also hormonally regulated as ascertained by the thymocyte entrance into and exist from thymic nurse cell complexes used herein as an *in vitro* model for ECM-mediated intrathymic T-cell migration. In addition to their role in thymic cell proliferation and apoptosis, hormones and neuropeptides also modulate intrathymic T cell differentiation, influencing the generation of the T cell repertoire. Taken together these data clearly illustrate the concept that neuroendocrine circuits exert a pleiotropic control on thymus physiology. Lastly, the intrathymic production of classic hormones such as prolactin and growth hormone suggests that, in addition to endocrine circuits, paracrine and autocrine intreactions mediated by these peptides and their respective receptors may exist in the thymus, thus influencing.

S. 1.
MEMORY CONSOLIDATION: A CENTENARY CONCEPT REVISITED
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Memories are often described in terms of time. Shortly (sec. to hs.) after a learning task, memory is in labile state (short-term memory, STM) that is sensitive to disruption by the learning of a different information shortly after the original one, administration of drugs, brain injuries, etc. Later on (hours to months) memory enters into a stable state (long-term memory, LTM), insensitive to the same disruptive factors. The process of transition from STM to LTM is generally referred to as consolidation. The finding that protein synthesis inhibitors did not prevent the learning of tasks but disrupted memory generated as a consequence of the training experience support the view that there are (at least) two stages of memory and suggests that protein synthesis is required for consolidation. In addition, behavioral, neuropharmacological and neurochemistry studies, provide evidence that STM- and LTM- processes are not sequentially linked, as was proposed early. In spite of being widely accepted in psychology and neuroscience, consolidation theory has been challenged from time to time. One challenge has come from studies showing that memories are not only labile after learning but also after reactivation or retrieval. So, "little is as yet known about system and cellular processes mediating consolidation that continues for several hours or longer after learning to create our lifelong memories. These issue remain to be addressed in this new century of research on memory consolidation" (McGaugh JL, 2000).

S. 2.

REACTIVATION AND RECONSOLIDATION OF LONG-TERM MEMORY IN AN INVERTEBRATE, EVOLUTIONARY PERSISTENCE OF INTRACELLULAR MECHANISMS

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Studies on diverse phases of memory, performed in very disparate animal species, discloses a remarkable constancy both in components and functions at cellular and molecular level. Thus, the memory potential capacity seems to be basically similar for most of the animal species, in contrast with the memory actual capacity that differs widely through evolution and appears to depend on the structure and relations of the neuronal net that subserves a mnesic process in a particular species. The memory model of the crab *Chasmagnathus*, extensively studied at behavioral and mechanistic level, may be taken as a good example of the evolutionary persistence. The crab's associative learning paradigm is based on its escape response elicited by the presentation of a visual danger stimulus, VDS (an opaque rectangle passing overhead). Upon the iterative presentation of VDS, the crab's response declines and a strong freezing is built up. The response decrement lasts for at least a week. This long-term memory is mediated by an association between the environmental features of the training site (the context) and the features of the screen moving overhead (the signal), so that it is termed context-signal memory (CSM). Studies about mechanisms underlying consolidation have shown that CSM consolidation is cycloheximide sensitive; positively modulated by angiotensins; selectively regulated by a muscarinic cholinergic mechanism; mediated by the cAMP signal pathway, by the NFκ-B transcription factor and by NMDA-like glutamatergic receptors. Recent results demonstrated that the robust context-signal memory (CSM) acquired by the crab through spaced-training, again becomes labile after 5 min re-exposure to the learning context, proving vulnerable to cycloheximide- or MK-801-injection. These findings are interpreted according to the view, stemming from findings obtained in vertebrates, that memory retrieved by a reminder passes from a dormant and stable stage to an active and labile one (reactivation), which undergoes a time-dependent consolidation process (reconsolidation).

S. 3.

GENERIC PRODUCTS BIOEQUIVALENCE

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The "generic product" means a pharmaceutical product, usually intended to be interchangeable with the innovator product, which is usually manufactured without a license from the innovator company and marketed after expiry of patent or other exclusivity rights. (WHO. Support Series Nro 5, 1999). The generic products need to conform to the same standards of quality, safety and efficacy required of the originator's product. A clinically interchangeable with nominally equivalent market products must be reasonable assurance.

The ANMAT should ensure that all pharmaceuticals products subject to their control conform to acceptable standards of quality ,efficacy and safety; and that all premises and practices employed to manufacture, store and distribution of this products comply with GMP standards to ensure the continued conformity of the products to these requirements until such time as they are delivered to the end user. There are often economic pressure favouring the use of generic products. However, all pharmaceutical products, including generic products, should be used in country only after approval by the appropriate drug regulatory authority.

The re-formulation of marketed products and the development of generic have lead to situations where the application of medicinal products containing equal amounts of the same active substance in the same dosage form have lead to very different plasma levels in patients. In same instances this resulted in severe side effects due to plasma levels outside the therapeutic window (e.g. digoxina, phenytoin). Regulatory authorities should require that documentation of generic pharmaceutical product addresses three sets of criteria: a- manufacturing (GMP) and quality control, b-product characteristics and labeling and c-therapeutic equivalence.

Pharmaceutically equivalent generic products must be shown to be therapeutically equivalent to one another in order to be considered interchangeable. Several test methods are available to assess equivalence, including: a- Comparative bioavailability (bioequivalence) studies, b-Comparative pharmacodynamic studies, c- Comparative clinical trials and d- In vitro dissolution tests.

S. 4.

PHARMACOECONOMIC CONSECUENCES OF IRRATIONAL PRESCRIBING IN A HEALTH CARE INSTITUTION IN ARGENTINA

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The aim of this presentation is to analyze pharmacoeconomic consecuences of irrational prescribing, considering the results obtained in a Health Care organization belonging to a tertiary Care Hospital in Buenos Aires, Argentina.
We considered irrational prescribing:

1) Prescribing drugs when scientific evidence of effectivity in any pathology is lacking.
2) Prescribing drugs with a different indication that those evidence based indications.
3) Prescribing drugs properly but during the wrong time gap (too short or too long).
4) Prescribing drugs properly but using the wrong dose(too high or too low), without clinical justification.
5) Prescribing more expesive drugs when it is possible to prescribe another with the same efficacy for the indication, in the same chemical group , or in other one.
6) Prescribing drugs when risks overweight clinical benefits.
7) Prescribing drugs when the patient could receive non pharmacological meassures.

It is well known that irrational prescribing shifts economic resources that could be derived to other actions in order to promote the population´s health status. We analized the economic side of some detected irrational prescribing cases and put it in perspective of the actions that could be taken instead.
From the patients point of view, we evaluated the economic impact in therms of basic food supplies that could have acquired with the money spent through irrational prescribing.

S. 5.

MOLECULAR BASIS FOR ANTIMICROBIAL RESISTANCE

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Even if selection of resistant microorganisms occurs independently of the rational basis of therapeutic schemes election, rational and prudent use of antibiotics diminish the speed at which this selection process takes place, improving the use of available therapeutic regimens, diminishing both the costs for antibiotic treatment and antibiotic-associated risks.
Using as an example β-lactam antibiotics (that represent almost 80% of antibacterial prescriptions), resistance mechanisms may be classified as those affecting the molecular target (by recombination leading to mosaic proteins with decreased affinity, mutations in structural genes, the acquisition and/or over expression of low affinity targets), those decreasing the access to these targets (both by a decreased influx through the gram-negative outer membrane and/or active efflux systems, or those destroying the antibiotics by hydrolytic enzymes.
β-lactamases are usually classified (functional or microbiologically) by the substrates to which a bacteria expressing these enzymes show resistance as penicillinases, cephalosporinases, broad spectrum (classical penicillins and cephalosporins), extended spectrum (including third generation cephalosporins and monobactams), and carbapenemases (active on carbapenems that are, in fact, resistant to all the previously mentioned enzymes). Some of these enzymes can be modified in order to be resistant to enzyme inhibitors.
However, this myriad of enzymes is heterogeneous in their structure, even is most of the enzymes share common themes and an active serine at their active site; this serine proteases family can be divided into several structural sub-groups, but these sub-groups do not strictly correlate with the antibacterial resistance acquired by their expression.
Emergence of metalo-β–lactamases of different origins make a good contribution to divergence.
Broadening the substrate spectrum can be followed, at least in some families of enzymes (i.e.TEM, SHV), by single-point mutations of enzymes with reduced spectrum, by flexibilizing access to the active site (even at the loss of some catalytic efficiency). On the other hand, resistance can be acquired after acquisition of better promoters, allowing for better product expression.

S. 6.

MOLECULAR BASIS OF THE RESISTANCE TO ANTIPARASITIC DRUGS

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Drug resistance is defined as a state of not susceptibility or decreased susceptibility to the effect of a given drug concentration that normally causes growth inhibition or cell death. It is heritable and genetic modifications conferring resistance are translated into different biochemical modifications such as: cellular changes affecting the capacity of the drug to accumulate intracellularly, changes on metabolic pathways and/or alteration on drug target cellular receptors. Anthelmintic resistance among strongylid nematode parasites of domestic animals to the main available anthelmintic chemical groups (benzimidazoles, imidazothiazoles /tetrahydropyrimidines, and avermectins/ milbemycins) is a worldwide inconvenient for livestock production. Resistance selection for benzimidazole (BZD) anthelmintics is associated with mutations in β tubulin genes, which reduces or abolishes the high affinity BZD binding sites in nematodes and fungi. Levamisole (LVM) resistance appears to involve genetic modifications at the nicotinic receptors in nematodes, leading to structural changes of the receptor subunits, fewer receptors and/or loss of sensitivity to these cholinergic agonists. The overwhelming evidence suggests that avermectins, such as ivermectin, and milbemycins, as moxidectin, target the same glutamate-gated chloride channels (GluCl), in pharyngeal and somatic musculature of susceptible nematodes. Resistance to both avermectin and milbemycin families is associated with genetic modifications on that glutamate receptor and/or overexpression of a P glycoprotein (P Gp). P Gp is an integral membrane glycoprotein able to pump these glutamaergic agonists out efficiently, avoiding the drugs to reach effective concentrations at GluCl channels. Current research demonstrates that ivermectin-resistant parasites are also side resistance to moxidectin. However, at recommended dose rates, moxidectin is still effective against ivermectin-resistant nematode parasites. Resistance develops slowly at first and then escalates to maximum levels relatively quickly, that is when anthelmintic treatment failures. Enhancement of drug absorption by management of feed intake in treated animals, modulation of drug metabolism and/or excretion are among the most important pharmacokinetic-based strategies under investigation to increase drug availability contributing to optimise antiparasitic therapy. Understanding the relationship between the kinetic behaviour of the drug in the host animal along with and the molecular pharmacodynamic changes occurring in resistant parasites is crucial to define pharmacology-based approaches to delay development of resistance to antiparasitic drugs.

S. 7.

STRATEGIES FOR A PROGRAM OF RATIONAL USE OF DRUGS: RESULTS OF THE APPLICATION OF A THERAPEUTIC FORMULARY IN A SOCIAL SECURITY SERVICE

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In previous pharmacoepidemiologic drug utilization studies, we have demonstrated that drugs are used, at least in part, inappropriately, or in excess, resulting in elevated and no justified expenses in several social security institutions of the Northeast of Argentina. In order to improve the pharmacotherapy in the Instituto de Previsión Social (IPS) of the state of Misiones, which have 131.000 affiliated people, we applied for prescriptions a Rational Therapeutic Formulary (RTF) only composed of drugs with effectiveness and safety previously demonstrated in randomized controlled clinical assays. We also performed comparative drug utilization and pharmacoeconomic studies before and after the obligatory use of the Formulary. For quantitative analyses we used the Anatomical Therapeutic Chemical (ATC) Classification and the Defined Daily Dose (DDD) system. For qualitative assessment we used the Intrinsic Therapeutic Value Classification (Laporte and Tognoni) and for statistical analyses the Epi Info program. **Results**: Before the use of the RTF the more prescribed ATC group (total annual prescriptions, year 2000) was Group N: drugs acting on Central Nervous System with 1.501.619 DDDs, mainly Psycholeptics (1.187.467 DDDs, 79% of the group). Between Psycholeptics the Anxiolytics are in first place (1.034.310 DDDs, 68% of the N group) and Alprazolam is the more prescribed individual drug (734.000 DDDs, 48% of the N group). These results indicate serious irrationalities in prescriptions and consumption of drugs acting on CNS. After the obligatory use of the RTF (total annual prescriptions year 2001) the N Group, is in the thirst place. Number of prescriptions markedly decreased to 573.806 DDDs that mean 38% of prescriptions made during the year before the application of the RTF. Prescriptions of Psycholeptics and particularly Alprazolam also markedly decreased to 367.548 (35%) and 96.490 (13%) DDDs in the year, respectively. Regarding the pharmacoeconomic analyses, our results showed a marked reduction in the expenditure of the N group, from U$S 981.877 in the year before the use of the RTF to U$S 527.911 after. Similar results are seen in the Group A: Drugs for Alimentary Tract and Metabolism (1.252.131 before and 613.011 DDDs after the use of the RTF, total annual prescriptions). In this Group the consumption of Vitamins were markedly reduced from 837.900 to 348.234 DDDs, annual prescriptions, before and after the application of the RTF. Notably, vitamin C reduced the consumption from 709.600 to 194.100 DDDs in the same period. Similar irrationalities were observed in other several Groups of drugs of the ATC Classification. **Conclusion:** On the whole, from the economic point of view, application of a Program of Rational Use of Drugs in the Social Security System of the state of Misiones produced a reduction in pharmacological costs of about U$S 300.000 every month and from the medical point of view, better results are obtained using a rationalized list of essential effective drugs than an open list of drugs, including those of doubtful or null intrinsic therapeutic value, liberally used.

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1.

DUAL EFFECT OF ADENOSINE ON T LYMPHOMA CELL ACTIVITY

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Adenosine is a nucleoside that acts as immunomodulator regulating growth, differentiation and cell death. The aim of this work was to analyze the ability of adenosine to regulate tumor lymphocyte hyperproliferation. Proliferative effects of adenosine have been studied on the murine T lymphoma cell line, BW5147. In this work we show a dual effect of adenosine on lymphocyte proliferation. After a short period of 24 hs of culture, adenosine induced an increase on T BW5147 T cells proliferative activity, as was measured by [^{3}H] thymidine incorporation. Adenosine effect increase in a concentration-dependent manner in a range of 0.1 to 100 μM. After longer periods of culture (48 hs to 96 hs) an inhibitory proliferative effect was obtained. This action was accompanied by the presence of cells with apoptotic nuclear morphology as was observed by Hoechst staining. During apoptotic events adenosine was able to induce an increase in nitric oxide synthase activity with a maximum at 10 μM. We conclude that adenosine is able to modulate the balance between growth vs. cell death in this T lymphoma. These data are important to be considered when proposing adenosine as coadjuvant in leukemic disorders treatment.

2.

SYMPATHETIC DYSFUNCTION IN TYPE-I DIABETES. FUNCTIONAL CONSEQUENCES FOR IMMUNITY

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It has been suggested the existence of a suppression of immune function in diabetic disease. Dysregulation of immune responses in many autoimmune diseases is coupled with sympathetic nervous system dysfunction. We have used multiple low- dose streptozotocin for induce Type I diabetes model in mice. In this model we studied the consequences of sympathetic-immune interactions on disease progression. The results show suppression in T-cell dependent humoral response after one month of diabetic induction. Concerning of the neurotransmitter influence on immune response we analysed the influence of norepinephrine (NE) on T-lymphocyte reactivity. The results show that, at this time, basal mitogen induced T-cell proliferation was similar in normal and diabetic animals, but an alteration in NE lymphocyte sensitivity was observed. NE shows a byphasic effect on normal T cell proliferation, with stimulation at low concentration and inhibition at higher ones. After one month of diabetic induction, only the inhibitory effect was observed. After 6 months of diabetic onset, the lack on NE stimulatory action continued but lower basal lymphocyte proliferation was observed. Experiments performed with specific agonists and antagonist for α_2 and β_2 adrenergic receptors indicate that during early disease stages suppression of NE response, especially for α_2 subtype, occurs. The cAMP response after adrenergic stimulation was according with these findings. These results indicate that an alteration in lymphocyte adrenergic receptors function is observed at early states of diabetes that in turn could be participating in the impairment immune response in this disease.

3.

EFFECT OF ANANDAMIDE ON PLASMA LH IN FEMALE RATS

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Anandamide (AEA) is an endogenous ligand of cannabinoid receptors (CB1) found in brain, including hypothalamus. Previously we have reported that the third ventricular (3V) injection of AEA to adult male rats lowered plasma LH levels. In adition AEA in vitro decrease LHRH stimulated release by NMDA in medial basal hyphotalamus (MBH). In the present work we studied the effect of AEA on plasma LH levels in ovariectomized (OVX) and OVX-primed rats (OVX-E), in vitro release of Gaba and LHRH from MBH. Rats were implanted with 3V cannulae and jugular catheters. AEA (20ng/2ul) or AM251 (CB1 antagonist, 200 ug/2ul)+AEA or vehicle (2ul) were injected into the 3V and blood samples removed every 30 m for 150 m to determine LH and LHRH by RIAand GABA by radioreceptor assay. AEA significantly ($p<0.01$) increased LH plasma levels in OVX-E rats. AM251 antagonized this effect. LH was also decreased ($p<0.01$) in OVX AEA injected rats. AM251 alone lowered plasma LH ($p<0.001$). AEA decreased ($p<0.01$) GABA stimulated release from MBH of OVX-E rats. These results suggest: a) that the increase in LH levels in OVX estrogen-primed rats may be due to the effect of AEA on GABA release. b) that the effect of AEA on LH secretion is dependent of estrogenic status of the animal.

4.

CENTRAL NORADRENERGIC ACTIVITY IN AN ANIMAL MODEL USEFUL FOR SCREENING ANTIPANIC AGENTS

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The activity of Locus coeruleus (LC) noradrenergic neurons and the number of spontaneously active cells/ track were assessed by single unit recording in adult recovered rats undernourished at perinatal age (D) compared to well nourished animals (C). The firing rate of the LC noradrenergic neurons and the number of spontaneously active cells/track were significantly higher in D rats (D: 2.64 ± 0.10 vs. C: 1.44 ± 0.06 spikes/sec; D: 5.19 ± 0.16 vs. C: 3.98 ± 0.12 cells/track). Following a repeated fluoxetine treatment (5 mg/kg/day during 5 days), D rats showed a decrease in the firing rate of noradrenergic neurons and the number of spontaneously active cells/track (1.19 ± 0.07 spikes/sec; 2.93 ± 0.20 cells/track). In addition, D-R curves for the inhibitory effect of clonidine on LC activity showed a shift to the right in D animals (ID_{50} = 5.43 ± 0.50 μg/kg) that was reversed after the treatment with fluoxetine (ID_{50} = 2.97 ± 0.94 μg/kg). These observations suggest that the α_2 autoreceptors subsensitivity showed by D rats was normalized after the fluoxetine treatment. We also evaluated the anxiolytic-like behavior in the Open Field Drink Test (OFDT) after the same treatment. In this paradigm, D rats spent more time drinking (268 ± 7 sec), an effect not observed in C rats (213 ± 9 sec). These data support the hypothesis that neuronal alterations induced by perinatal undernutrition resemble some alterations described in patients with panic disorder. Furthermore, these results support the proposal that D rats may be a useful animal model for screening antipanic agents.

5.

INVOLVEMENT OF NMDA RECEPTOR IN STRESS-INDUCED SENSITIZATION TO STIMULATING EFFECTS OF AMPHETAMINE: MICRODIALYSIS AND *IN SITU* HYBRIDIZATION STUDIES

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Acute restraint stress exposure induces sensitization to stimulating properties of psychostimulants. Dopaminergic areas such as nucleus accumbens and caudate-putamen (CPU) play a critical role in the development of this sensitization. Our main goal was to study the involvement of NMDA glutamatergic receptors from CPU in acute restraint stress-induced sensitization to amphetamine. Wistar male rats (250-350 g) were used. We administered MK-801 (0.1 mg/kg i.p.) and CPP(13.5 ng/0.5µl) intra-CPU previous to stress. Two days after the surgery, animals were immobilized for two hours. The following day we evaluated the effect of amphetamine on: a) locomotor activity (LA) and b) dopamine release from CPU by microdialysis. Amphetamine induced a significant higher increase in dopamine release and LA in animals previously restrained, compared to that observed in the no restraint stress group. CPP abolished the stress-induced sensitization to amphetamine on LA; and MK-801 blocked the sensitization to amphetamine-induced dopamine release. We also studied the mRNA NMDAR1, R2A and R2B content by quantitative in situ hybridization histochemistry in CPU. Amphetamine induced a significantly increase in the mRNA NMDAR1 and NMDAR2A expression in restraint animals compared with control animals. There were no differences among the groups in mRNA NMDAR2B expression. The present findings showed that NMDA receptor stimulation is involved in the development of restraint stress-induced sensitization to amphetamine. Furthermore, a glutamatergic-dopaminergic neurotransmission interaction can be inferred.

7.

UPTAKE OF ANANDAMIDE IN THE RAT SUPERIOR MESENTERIC ARTERY

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In the central nervous system and in cell cultures derived from central and peripheral tissues the endocannabinoid anandamide (AEA) is inactivated by a process of carrier-facilitated diffusion followed by intracellular hydrolysis by fatty acid amide hydrolase (FAAH). We examined whether AEA undergoes carrier-mediated uptake in the superior mesenteric artery of Sprague-Dawley adult male rats. The arteries were cut into 4-6 rings that were maintained in Krebs-HEPES buffer bubbled with 95%O_2 plus 5%CO_2. Incubation with ^{3}H-AEA (0.5 nM; SA: 217 Ci/mmol) was performed at 37ºC. Nonspecific uptake was determined at 0ºC. At the end of the incubation the arterial rings were washed with ice-cold medium containing 1% albumin and digested with 200 µl Soluene. Tissue radioactivity was expressed as % of the initial radioactivity in the incubation medium/mg wet tissue (% initial rad/mg). ^{3}H-AEA was taken up in a time-dependent and temperature-sensitive manner. This process reached a maximum in about 7 min (37ºC: 0.78±0.22% total rad/mg; 0ºC: 0.17±0.03% total rad/mg; n=6). Uptake of ^{3}H-AEA was reduced by the inhibitors of AEA transporter AM404 (100 µM) and VDM11 (10 µM) and by the inhibitor of FAAH, PMSF (200 µM) but not by blockade of either vanilloid-receptors with capsazepine (1 µM) or CB1-receptors with SR141617A (10 µM). These results suggest that in the rat superior mesenteric artery AEA is transported by a carrier-mediated process probably coupled to hydrolysis by FAAH. ***Supported by Grants PICT 99-05-06917, Carrillo-Oñativia 2001 and Fundación Antorchas 14022-112.***

6.

NEURONAL C-FOS ACTIVATION AND DEATH IN THE ACCESSORY OLFACTORY SYSTEM (AOS) AND MEDIAL EXTENDED AMYGDALA (MEXA),AFTER KAINIC ACID ADMINISTRATION

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Glutamic acid is the main excitatory neurotransmiter in the brain. One of its agonists, Kainic acid (KA), binds selectively to a subtype of Glu receptors, producing excitotoxicity in several neuronal systems. Kainic acid has been used as a drug in animal models of epilepsia. In this study of MExA we used KA at the dose of 10 mg/kg ip. to produce seizures in male wistar rats. 24 hours later the brains were fixed, sectioned and stained by immunocytochemistry (ICC) for detection of neuronal activation (c-fos) and parallell sections were processed with the Amino-Cu-Ag method to detect neuronal death. Results: 1) C-fos: in all the nuclei of the MExA and the AOS we observed high level of activity. The fos + nuclei were counted with a Leitz microscope with image processor attached to a PC, and expressed as the X (media) + /- E.S. Significantly less activity was observed in control animals. ($p<0.001$) 2) Neuronal death: the MExA nuclei were severely lesioned by the KA injection. Neuronal bodies and neurites in several degrees of neurodegeneration were seen in all of them. (AMe, PMCo, and BST) as well as the BAOT, second station of the AOS. In the AOB only terminal degeneration in the glomerular layer was detected. This results adds new information about the KA effects in the brain, suggesting that not only the hippocampus and cortex but also the MExA should be included among the areas contributing to affective and conductual simptoms of epilepsy.

8.

STRIATAL µ-OPIOID RECEPTOR CHANGES DURING MORPHINE WITHDRAWAL IN MICE

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Behavioral and neurochemical sex differences have been shown during the morphine (MOR) withdrawal syndrome in mice. In the present study we evaluated striatal µ-opioid receptor binding parameters in male and female mice, during MOR withdrawal and after its prevention with the $GABA_B$ agonist, baclofen (BAC). Swiss-Webster mice (27-33g) 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 and binding of [^{3}H]-DAMGO to µ-opioid receptors was performed. Results (mean ± SEM) were as follow:

	MALE		FEMALE	
	Kd	Bmax	Kd	Bmax
Saline control	1.2 ± 0.1	159.8 ± 4.7	1.1 ± 0.1	170.4 ± 4.3
Withdrawal	1.3 ± 0.2	306.6 ± 13.2*	1.2 ± 0.3	180.2 ± 9.2
Prevention	2.8 ± 0.9*	255.5 ± 31.1*	1.5 ± 0.3	172.6 ± 8.0

Significant changes occur in male striatum during MOR withdrawal and after pretreatment with BAC. On the contrary, no alterations in affinity or density of µ-binding sites occur in females. These results suggest that pharmacological sex differences in the expression of MOR withdrawal would be related to changes in striatal µ-opioid receptor characteristics.

9.

CORRELATION BETWEEN TRANSTEGUMENTAL DIFFUSION AND OCTANOL-WATER PARTITION COEFFICIENTS OF BENZIMIDAZOLE ANTIHELMINTIC DRUGS

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Since benzimidazole (BZD) anthelmintics need to reach their specific β-tubulin binding site in the parasite to exert their action, the intrinsic drug capability to reach the intracellular space is critical to assure clinical efficacy. The current experiments correlate the *ex vivo* diffusion pattern of different BZD drugs with the lipid solubility of each molecule, using the cestode parasite *Moniezia benedeni* as a model. *M. benedeni* were incubated with either albendazole (ABZ), ABZ-sulphoxide (ABZSO), fenbendazole (FBZ), oxfendazole (OFZ), mebendazole (MBZ), thiabendazole (TBZ), flubendazole (FLBZ) or oxibendazole (OBZ), for different periods. Once the incubation elapsed, parasite samples were analysed by HPLC to quantify the amount of drug. Aditionally, octanol-water partition coefficients for each molecule were determined. The *ex vivo* diffusion to the cestode parasite was greater for molecules with higher partition coefficients values (ABZ, FBZ), existing a strong correlation between lipid solubility and transtegumental diffusion. These preliminary results are useful to define further research on different pharmacology-based strategies to enhanced drug diffusion to target helmith parasites.

10.

HYPERHOMOCYSTEINEMIA INDUCES RENAL OXIDATIVE STRESS PROMOTING RENAL HEMODYNAMIC DYSFUNCTION

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Objective: To study whether hyperhomocysteinemia can induce renal oxidative stress promoting renal hemodynamic dysfunction. **Methods:** Control (C, n=7) and treated (T, n=14) male Wistar Kyoto rats received tap water or homocysteine-thiolactone (HTL, 50 mg/kg/day), respectively during 8 weeks. After this period, a half of T group received additionally vitamin C (500 mg/kg/day, ip) for another 3 weeks. Plasma homocysteine was determined by HPLC. Renal superoxide anion radical ($\bullet O_2^-$) production and nitrotyrosine abundance were determined by chemiluminiscence and western blot analysis, respectively. Spectrophotometric assay were used to evaluate the thiobarbituric acid reactive species (TBARS) and conjugated dienes (CD) formation. Inulin and paraaminohippurate clearances were used to evaluate renal hemodynamic function. **Results:** After 11 weeks, T rats showed statistically ($P<0.05$) increased $\bullet O_2^-$ production (+63%) togeher with a greater formation of TBARS (+73%) and CD (+51%), denoting a raise in the oxidative stress and lipoperoxidation. In addition, a higher renal accumulation of nitrotyrosine (+46%), marker of NO inactivation, was also observed. Related to renal function, hypofiltration, hypoperfusion and renal vasoconstriction were observed in the T group ($P<0.05$). Antioxidant treatment normalized the oxidative stress restoring the normal renal hemodynamic function. **Conclusion:** The present work shows that hyperhomocysteinemia induces renal oxidative stress and by this way, promotes renal hemodynamic dysfunction.

11.

[^{3}H]GABA AND [^{3}H]L-SERINE UPTAKE BY BRAIN SYNAPTOSOMES DURING POSTNATAL DEVELOPMENT AND ADULTHOOD

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GABA is the major inhibitory neurotransmitter in brain and participates in the central nervous system development. L-Serine does not seem to act as a neurotransmitter but apart from being a component of proteins and membrane phospholipids, has been proved to be an important neurotrophic factor. GABA uptake is the mechanism of ending the synaptic transmission and L-Serine uptake allow the entry of the amino acid into the neuron.
In this work, we studied these two amino acids transport activity in synaptosomes obtained from neonate brains of 5, 7, 13 and 21 days of age, and from adult brains. The preparations were incubated with 10 nM of [^{3}H]L-Serine or [^{3}H]GABA in either the presence or absence of sodium or potassium chloride, at 2ºC and 30ºC, for different periods up to 30 min.
At all ages [^{3}H]GABA uptake showed a higher activity in the presence of Na+ and at 30ºC. [^{3}H]L-Serine uptake in neonates showed no preference for any ion or temperature condition. However, in adults [^{3}H]L-Serine transport activity was higher in the presence of Na+ and at 30ºC. In these conditions and when incubated for 5 min, [^{3}H]GABA and [^{3}H]L-Serine uptake increased with age reaching values statistically higher in adults.
We conclude that the way in which [^{3}H]L-Serine and [^{3}H]GABA are incorporated into synaptosomes by their specific transporters varies according to the temperature, cation and the age of the rats.
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12.

THE ADMINISTRATION OF HALOPERIDOL PREVENTS NEURONAL NA^+, K^+-ATPASE INHIBITION BY NEUROTENSIN

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We have previously shown that NT inhibits cerebral cortex synaptosomal Na^+, K^+-ATPase activity, an effect fully prevented by SR 48692, antagonist of neurotensin NT_1 receptors. Since diverse treatments with antipsychotic haloperidol produce an increase in neuronal NT levels and changes in NT receptors, this work was extended to analyze potential haloperidol effect on Na^+, K^+-ATPase inhibition by NT. Haloperidol was added *in vitro* at 1×10^{-6} M concentration during synaptosomal membrane incubation (10 min) previous to ATPase assays; it was observed that this drug failed to modify enzyme inhibition produced by 3.5×10^{-6} M NT (-20%). Haloperidol was administered to rats and animals were decapitated 18 hs later; cerebral cortex and striatum were removed and subjected to differential and sucrose gradient centrifugation to obtain synaptosomal membrane fractions. Haloperidol treatment failed to modify basal Na^+, K^+-ATPase activity in both membrane preparations and turned Na^+, K^+-ATPase membrane enzyme insensitive to inhibition by 3.5×10^{-6} M NT. Findings suggest that acute haloperidol treatment modifies NT_1 receptor and therefore Na^+, K^+-ATPase inhibition by NT is impaired.

13.

REACTIVITY TO COCAINE IN ADULT RATS UNDERNOURISHED AT PERINATAL AGE. BEHAVIORAL AND NEUROCHEMICAL CORRELATES

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Adult recovered rats submitted to a protein deprivation schedule at perinatal age, were treated with repeated cocaine administration and subsequently evaluated in the open field test. D-R curve using different doses (5, 10 or 15 mg/kg, i.p. daily injections during 16 days) showed a progressive and higher increase of locomotor activity in deprived rats (D) as compared to controls (C) in response to such drug. Moreover, D animals evidenced behavioral sensitization with the lowest dose of cocaine used, this phenomenon was not observed in C rats. In order to correlate this differential sensitization process with neurochemical parameters, we also assessed dopamine levels in different areas of the mesocoticolimbic system such as nucleus accumbens (core and shell) and prefrontal cortex using a microdialysis technique. Preliminary results showed comparable basal values of the dopaminergic function between sensitized C and D rats. A challenge with cocaine produced an unequal increase in extracellular dopamine levels between both experimental groups. These results indicate that a repeated cocaine treatment produced in D animals a progressive sensitization to the stimulant motor effects, in addition to significant changes in the mesocorticolimbic dopaminergic function as compared with C rats.

14.

CHOLINERGIC MECHANISM AND MEMORY OF AN INHIBITORY AVOIDANCE TASK IN MICE

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Administration of hemicholinium-3 (HC-3), an inhibitory of high-affinity choline uptake (HACU) (1.0 ug/mice, icv) when given either immediately after training or 60 min before the retention test, impaired retention performance of one trial step-through inhibitory avoidance task in mice. In parallel with the behavioral deficit, HC-3 decreased the activity of HACU in hippocampus when injected 60 min before the retention test, but not when was given immediately after training and performance was evaluated 48 hs later. Moreover, the anticholinergic drug atropine (1.0 mg/kg, ip) did not modify retention, however, it was able to reduce the enhancement of retention induced by post-training injections of the anticholinesterase physostigmine (150 ug/kg, ip). These findings suggest that cholinergic mechanisms are involved not only on memory consolidation, but also on memory retrieval of an inhibitory avoidance response in mice.

15.

CHANGES IN CENTRAL MUSCARINIC RECEPTOR BY NEUROTENSIN

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Neurotensin (NT) is a tridecapeptide widely distributed in central and peripheral tissues, where can act as neuromodulator or neurotransmitter. We have previously shown that NT is able to inhibit the activity of synaptosomal membrane Na^+, K^+-ATPase and K^+-*p*-nitrophenyl-phosphatase (under strict conditions), but not of other enzymes such as Mg^{2+}-ATPase and acethylcholinesterase. The purpose of this study was to evaluate the effect of NT on cholinergic muscarinic receptor, by [^{3}H]-QNB binding to CNS membranes. It was found that 10^{-7}-10^{-5} M NT decreased 46-56%, 37-40%, 37-70%, 11-50% ligand binding in striatal, cortical, hippocampal and cerebellar membranes, respectively. With $1x10^{-7}$ M the most sensible area was striatum; however, in hippocampus, though less sensitive than striatum with 10^{-6} M and 10^{-5} M NT binding inhibition attained 70%. Present findings support a relationship between sodium pump and cholinergic system regulations.

16.

COMPARATIVE STUDY OF THE DAILY RHYTHMS OF AA-NAT, HIOMT AND MELATONIN IN RETINA OF VISCACHA

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The aim of this work was to compare the daily rhythms of melatonin and its synthesis enzymes, AA-NAT and HIOMT, in retina of viscacha (*Lagostomus maximus maximus*). Viscachas were sacrificed at 4, 8, 12, 16, 20 and 24 h (4-7 kg, n=5). The retinas were removed. The melatonin content, HIOMT and AA-NAT activities were determinated (Fraser et al., 1983; Axelrod *et al*., 1961; and Champney *et al*., 1984, respectively). Results:

h	Melatonin (pg/retina)	HIOMT (pmol ^{14}C-melatonin /mg protein/30min)	AA-NAT (pmol N-acetilserotonin-^{14}C/ mg otein/10min)
4	381.93 ± 58.16	2.69 ± 0.33	178.5 ± 35.25
8	406.90 ± 55.62	2.83 ± 0.25	158.3 ± 12.31
12	493.63 ± 59.21	3.46 ± 0.15	120.0 ± 8.94
16	191.57 ± 13.95	2.60 ± 0.32	134.0 ± 14.56
20	290.32 ± 18.01	3.04 ± 0.32	129.8 ± 10.84
24	1188.1 ± 207.6	3.78 ± 0.41	157.9 ± 22.52
	Kruskal-Wallis p<0.0001	ANOVA, p<0.03	Kruskal-Wallis, non significant

HIOMT activity and melatonin content exhibit a similar pattern with a nocturnal peak at 24 h and a diurnal increase. The daily fluctuations in the AA-NAT activity are not statistically significant. These results suggest that HIOMT has a relevant role in the indole synthesis in retina of viscacha, thus diffening from what is found in most species

17.

NEURONAL CYTOSKELETAL ALTERATIONS IN AN EXPERIMENTAL MODEL OF DEPRESSION

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Experimental depression is associated with hippocampal morphological changes. Particularly, apical dendrite atrophy of CA3 pyramidal neurons has been described. The aim of the present study was to evaluate the expression of different neuronal cytoskeletal markers in rats exposed to a learned helplessness paradigm, an 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 persisted for 21 days were included in the depressed group. No foot shocks were delivered to control rats. Microtubule-associated protein 2 (MAP-2) and neurofilament (Nf-68, Nf-160 and Nf-200 KDa) immunostaining was analyzed by using morphometric parameters. In the depressed group, Nf-68 immunostaining decreased 55% ($P < 0.01$, Student t test) and 60% ($P < 0.01$, Student t test) in CA3 and dentate gyrus, respectively. In the same areas, MAP-2, Nf-160 and Nf-200 immunostaining did not differ between depressed and control animals. Since Nf-68 is the first step involved in the Nf assembly, it is speculated that depression-associated hippocampal atrophy may be due to a reduced Nf-68 renewal. (This work was supported by Beca Carrillo-Oñativia).

18.

IMMUNOFLUORESCENT CHARACTERIZATION OF GLIAL CELLS IN THE NEUROHYPOPHYSIAL STALK

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Pituicytes are a special class of glial cell, which represents the mayor cellular component in the neural lobe of the neuro-hypophysis. In the neural lobe the pituicytes are closely related to the nerve terminals and show a great morphological diversity, which can be visualized by staining them immunochemically with antibodies to glial fibrilar acidic protein and S-100 protein. In the search for the cytological characteristics of the glia in the pituitary stalk, saggital and frontal cryostat sections were stained by immunofluorescence for S-100 protein. The stained sections showed the presence of a special, morphologically distinct and strongly S-100 positive, class of glial cell in the pituitary stalk. Frontal sections show several cell bodies of about 45 μm in diameter with a dense reticulum of cytoplasmic projections, irradiating from the cell bodies and covering almost the entire suface of the section. Saggital sections show that the bodies of these cells are located in the portion bordered by the *pars tuberalis*, i. e. before reaching the capsular envelope of the hypophysis. Studies are in progress to elucidate the fine structure and the functional features of this particular group of glial cells in the pituitary stalk.

19.

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF *ARTEMISIA COPA*

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Artemisia copa Phil. (Compositae) is a small shrub native of the Northwest of our country, commonly known as "copa-copa" and traditionally used for cold, pneumonia, hypertension, stomach aches and as digestive. Also alcohol macerated leaves are topically applied by rubbing for rheumatism. Analgesic activity of *A. copa* was analyzed by means of writhing, formalin and hot-plate tests in mice. A dose-related antinociceptive response was obtained in the writhing test at doses of 0.5 and 1g/kg p.o. (% inhibition 23.3 and 52.70%) and the second phase of the formalin test was also inhibited (38.81%). This effect was not antagonized by pre-treatment with naloxone 5 mg/kg i.p. Furthermore no significant effect was obtained in the hot-plate test. The antiinflammatory activity was analyzed with the carrageenan-induced paw edema in rats and the ear edema induced by TPA and arachidonic acid in mice. *A. copa* showed antiinflammatory activity in the TPA (88%) and arachidonic acid (37%) tests but no effects were seen at doses between 75-600 mg/kg in the carrageenan test. The results obtained indicate that *Artemisia copa* has analgesic and topical antiinflammatory activities and these would corroborate the traditional use of the infusion in folk medicine.

20.

ABNORMAL INVOLUNTARY MOVEMENTS CAN BETTER EXPLAIN MOTOR RESPONSE COMPLICATIONS IN PARKINSON´S DISEASE

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Repeated treatment with dopamine agonists D_1 and D_2 strongly potentiates contralateral turning behavior in unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rats in the middle forebrain bundle (MFB). This sensitization is believed to be related to motor response complications (dyskinesias, on-off states) that occur during chronic administration of L-DOPA in Parkinson's disease (PD) patients. Most authors conclude that the observed enhancement in rotational response after repeated L-Dopa or Apomorphine (Apo) administration is the experimental equivalent of L-Dopa induced dyskinesia (LID) seen in PD patients. However, there is evidence that rotational behaviour can not represent the complex phenomenon of L-DOPA induced dyskinesia. In this work, we study the presence of c-FOS as a marker of striatal neural activation in 6-OHDA lesioned rats under different regimenes of dopamine agonists and correlate these differences with the behavioral data (Circling Behavior and Abnormal Involuntary Movements, AIM). Briefly, animals were divided into three groups, one was primed with Apo, the second with Qp and the third with vehicle; a month later treatment with Qp or Apo (for three weeks) was started and rotational behavior and an AIM test was observed for two hours in each behavioral session. We found that stereotypies and motor temporal patterns best distinguished between the different treatment regimes and these differences were correlated with the striatal expression of c-FOS. Meanwhile, enhancement in rotational behavior did not differ significantly across groups. We believe this simple evaluation paradigm could be used to differentiate drugs less prone to induce motor complications in Parkinson's Disease and provide an insight into their pathophysiology.

21.

A DOUBLE BLIND-PLACEBO CONTROLLED STUDY ON MELATONIN EFFICACY TO REDUCE ANXIOLYTIC BENZODIAZEPINE USE IN THE ELDERLY

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To assess whether melatonin (3 mg p.o.) could be useful to reduce benzodiazepine dosage in old patients with minor sleep disturbance, 45 patients (36 females, 70.5 ± 13.1 years old) regularly taking anxiolytic benzodiazepines in low doses were studied. Overall quality of morning freshness, daily alertness, sleep quality, and sleep onset and offset time were assessed. Patients were randomized to receive either melatonin or placebo for 6 weeks. On day 14 of treatment, benzodiazepine dose was reduced by half and on day 28, it was halted. No significant modifications of sleep or wakefulness were detected after benzodiazepine withdrawal. As compared to basal, there was a general lack of changes in quality of wakefulness or sleep in patients taking melatonin or placebo. Sleep quality of patients taking melatonin during the first two weeks of treatment was significantly lower than that of placebo. Melatonin advanced sleep onset by 27.9 ± 11.9 min and decreased significantly the variability of sleep onset time ($p = 0.03$). The urinary concentration of 6 sulphatoxymelatonin prior to the study did not correlate with any parameter examined. The present study does not support melatonin efficacy to reduce the use of benzodiazepines in low doses. This contrasted with the demonstrable effectiveness of melatonin to reduce benzodiazepine consumption in insomniac patients when used in hypnotic amounts.

22.

INCREASE OF ABSTINENCE SYMPTOMS TO BENZODIACEPINES: A CONSEQUENCE OF THE ECONOMIC CRISIS IN ARGENTINA?

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Argentina is facing an unprecedent economic crisis. The republic´s health system is severely affected as the social security services, one of the most Latin America´s comprehensive and sophisticated health regimens in the past, are now facing a true caos. Public health services have reported 20% increase in heart problems and rises in anxiety, insomnia, gastroenteritis, panic attacks and sexual impotence (Lancet 2001,357:1981; BMJ 2002, 324:192). Under these circunstances, we have observed an increase in Adverse Drug Reactions (ADR) from CNS drugs, mainly abstinence symptoms. During 1996 from 300 reports of ADRs of CNS drugs, 9 were abstinence syndromes. In 1997, from 330 reports we observed 7 abstinence syndromes. In 1998 the relatioship was 538 CNS ADRs/ 3 abstinence syndromes. During 1999 242/23 respectively. In the year 2000 there was 408 ADRs and 8 abstinence syndromes and during 2001 379 ADRs from CNS drugs and 77 abstinence syndromes. All syndromes were moderate or severe and 60% of them were produced by anxyiolitic benzodiacepines, mainly Alprazolam (25%) and Lorazepam (24%). The remainder some allucinogenic mushroom, cocaine and amphetamine-like sympathomimetic drugs.

23.

TROPHIC EFFECTS OF DEPOLARIZATION ON CULTURED CEREBELLAR GRANULE NEURONS

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Our previous results demonstrate that depolarizing KCl concentrations (25 mM, 25K) promote cerebellar granule cell (CGC) survival. The aim of this study was to investigate the molecular mechanism of this trophic effect. CGC were obtained from 6-8-day old rats and grown in free-serum medium in the presence of 5 mM KCl (5K, basal conditions). Treatments with 25K or NMDA were performed two hours after plating and survival was measured 7 days later by the MTT assay. In order to analyze the intracellular pathways activated by elevated KCl concentrations, different channel blockers, protein kinase inhibitors and receptor antagonists were added 1 hour before 25K incubation. Addition of 25K induced a significant increase in CGC survival (55 ± 8%) compared to control cultures, grown under basal conditions during 7 days. This stimulation was completely prevented by pre-incubation with 10 mM $MgCl_2$, 10 μM nifedipine and a CaMKII inhibitor, KN93 (1-30 μM). These results indicate that depolarization evokes a calcium influx through L-type voltage-gated calcium channels (VGCCL) and this leads to CaMKII activation. The partial inhibition of 25K-stimulated CGC survival obtained in the presence of 20-75 μM PD98059, a MEK1 inhibitor, and 1-10 μM SB202190, a p38 kinase cascade blocker, suggests that activation of these kinases is involved. The addition of a non-competitive NMDA receptor antagonist (MK-801, 10 μM) partially blocked 25K actions. Moreover, 100 μM NMDA was as effective as 25K in increasing CGC survival. In conclusion, results presented here demonstrate that depolarization-stimulated CGC survival is mediated by different protein kinase pathways and this effect involves VGCCL and NMDA receptor activation.

24.

MODULATION OF NITRIC OXIDE SYNTASE (NOS) BY PROTEIN KINASE C (PKC) IN NEONATAL GAMMA-IRRADIATED CEREBELLUM

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The exposure of mammals to ionizing radiation during the neonatal period can generate plastic changes, mainly in highly immature organs such as cerebellum (CE). The protein kinase C (PKC) regulates a variety of intracellular and extracellular signals in the developing brain and has been implicated in processes of growth, differentiation and neuronal plasticity suggesting that it might be a target of radiation damage. The possible modulation of nitric oxide synthase (NOS) activity by PKC was investigated in irradiated rat cerebellum. The cephalic ends of neonatal rats were exposed to gamma-rays for up to 72h of postnatal (PN) life with a dose 5 Gy. The NOS and PKC activities (citosolic and membrane fractions) were studied in cerebellum at 7,15,21,30 and 90 PN days. Inmunohisto-chemical analysis for these enzymes was also performed. Data shows that gamma-irradiation promoted translocation of PKC activity to the membrane fraction and a decrease of NOS activity between 7 and 15 days PN. These results indicate that translocation of PKC decreased NOS activity in cerebellar neurons and suggest that phosphorylation of NOS by PKC modulates the catalytic activity of the enzymes in these model. This finding could be related to cognitive and motor deficits induced by neonatal irradiation.

25.

ADAPTIVE CHANGES IN THE RAT HIPPOCAMPAL GLUTAMATERGIC NEUROTRANSMISSION ARE OBSERVED DURING LONG-TERM TREATMENT WITH LORAZEPAM

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Benzodiazepines potentiate GABAergic inhibiton and induce tolerance to most of their pharmacological effects in long-term treatments. The main objective of our work was to investigate whether hippocampal excitatory glutamatergic neurotransmission exerts a compensatory role in such tolerance. In chronically (21 days) and acutely treated rats (lorazepam 1 mg/kg, i.p.) we studied: the *in vitro* glutamate release after a 60 Mm K^+ stimulus, [^{3}H]MK-801 binding, and *in vitro* cGMP efflux in response to the stimulation with 200 nM NMDA.

While no changes were observed in any of the parameters after a single dose of the drug, in chronically treated animals we found an increase of 206% ($p < 0.05$) of the *in vitro* glutamate release, together with an increment of 103%, ($p < 0.0001$) of the NMDA-stimulated cGMP efflux. A decrease (28%, $p < 0.05$) in K_D values for [^{3}H]MK-801 binding to hippocampal membranes was also observed. All these changes strongly suggest that a compensatory increase in the glutamatergic response is developed in hippocampus during the chronic treatment with lorazepam. Our findings could, at least in part, explain the tolerance to hippocampal mediated effects of lorazepam, such as amnesic and anticonvulsant activities. *(This work was supported by FONCYT PICT-397-15 and Beca Carrillo-Oñativia)*

26.

PATTERN OF FOS ACTIVATED AND DAMAGED NEURONS IN MEDIAL EXTENDED AMYGDALA AFTER PENTILENETETRAZOL (PTZ)

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The Lateral amygdaloid complex has been involved as a target for temporal lobe epilepsy (TLE). One animal model is the suppression of the GABA inhibition by the drug Pentilentetrazol (PTZ). The Medial Extended Amygdala (MExA), involved in reproductive, affective and emotional behavior, received less attention from the researchers. Conductual simptoms elicited from this structures, as aggression, fear, or sexual attacks could be originated after epileptic activation of MExA neurons. Here we show results by immuno-cytochemistry (ICC) for the early gene c-fos, in the nuclei of the MExA, after administration to adult male wistar rats with a schedule of Ip Injections of PTZ a 40 to 70 mg/kg to produce epileptic seizures (SE). Two to 72 hours after, the brains were fixed, sectioned and stained for c-fos (ICC). Nuclei were counted with a Leitz microscope with image processor attached to a PC . Results are expressed as the X (media) +/- ES. The Nuclei of the MExA, Medial Nucleus (AMe), PosteroCortical (PMCo), Bed nucleus of stria Terminalis (BST) and the AOS (Acces. Olfactory System) Accessory Olfactory Bulb (AOB) and Bed Nuc. Of Acc. Olfactory Tract (BAOT) were also strongly labeled after PTZ. No activation was observed in control animals. The Cupro Argentic technique was used to search if neuronal death occurs after PTZ. Only CA3 area of hippocampus shows terminal degeneration. We conclude that at the doses and timing used, PTZ hiperactivates neurons of MExA/AOS not producing neural death.

27.

***In vivo* AND *in vitro* STUDIES OF DIFFERENT FRACTIONS OF DERMATAN SULFATE WITH ANTITHROMBOTIC PROPERTIES**

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Dermatan sulfate (DS) selectively inhibits thrombin (T) through heparin cofactor II or indirectly by Protein C activation.We have described previously the experimental conditions for interaction between DS and the first protein complex of the complement system (C1). The aim of the present report was to study the biological activity of different DS subpopulations obtained by interaction with C1.These fractions are studied in a thrombosis rat model induced by stasis. C1 was obtained by affinity chromatography after a selective precipitation of human plasm. DS was 100% pure (Syntex). The interaction between both macromolecules requiered very strict experimental conditions. After centrifugation, DS biological activity was studied in the supernatant and in the precipitate, by anti T activity using chromogenic sustrate S2338 (Cromogenics). The DS subpopulation isolated in the precipitate represents 6% of the total and has a biological activity of 1.1 which increased significantly in comparison with DS of the supernatant (0.22). The interaction between DS and C1 allows a concentration of the biological activity.

28.

EFFECT OF KETAMINE INCUBATION ON VASCULAR REACTIVITY IN RATS

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When ketamine (Ket) is administered in bolus i.v injection it may be observed an hypertensive response. Furthermore hypotension may occur if sympathetic stimulation is prevented by other drugs. This last response seems to be due to an inhibitory action on vascular smooth muscle and endothelium-dependent relaxation. Rats were killed by decapitation and the thoracic aorta was removed. Rings were mounted on stainless steel hooks and suspended in tissue baths. Tension development was measured by isometric force transducers. Two rings were used as controls and two were incubated in the presence of Ket (10^{-4} and 10^{-5}M), and then cumulative dose-response curves to phenylephrine (Phe) and acetylcholine (Ach) were performed. Ket 10^{-4} M significantly attenuated the contraction elicited by KCl (83% vs controls, $p < 0.001$). and Phe (Ket : 46,8 ± 5,1% vs Control: 75,3 ± 2,9% respectively, $p < 0.01$). When Ket was added to the incubation chamber at 10^{-5} M, Phe (10^{-5} M) contraction was also reduced (Ket: 65.72 ± 2.78% vs Controls: 75.3 ± 2.9% $p < 0.05$). On Ach-induced relaxation it was observed a dual effect: Ket 10^{-4} M inhibited (Controls: 60.5± 2.96% vs Ket 11,22±2.21%, $p < 0.001$) and Ket 10^{-5} M increased the relaxation (Controls: 78.27 ± 2.99% vs Ket : 88,02 ± 1.16%, $p < 0.05$). These results are compatible with the view that in addition to the cardiovascular activation of ket, due to the central stimulation of the sympathetic nervous system, this drug exerts direct actions on arterial vessels.

29.

CARDIOVASCULAR IN VIVO EFFECTS OF THE ARGENTINIAN PLANT AMBAY *(Cecropia adenopus)*

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Cecropia adenopus Mart. (Moraceae) is a plant commonly known as "ambay" that grows from Mexico to the North of Argentina. Its leaves are used in herbal therapeutics as expectorant and antitussive. Nevertheless, there are reports about its cardiac toxicity. To study this point we evaluate and compare the cardiovascular in vivo effects of Ambay growed in Misiones rainforest (Ami) vs. Ambay cultivated in Cordoba province (Aco). Blood pressure (BP) of rats was measured by a cannula in the internal carotyde connected to a pressure transducer and A/D digitized to estimate also heart rate (HR) and differential pressure (systolic minus diastolic, DP). Rats had an initial BP of 86.3 ± 6.3 mmHg (n=9) and HR of 249 ± 20 beats/min. Methanolic extract of Ami produced hypotension with a DE50 of about 100 mg/kg until reaching a BP of 52.4 ± 5.7% of initial (n=5), without significant changes in HR. Aqueous crude extract of Aco produced a biphasic hypotension with a DE50 of about 46 mg liofilized/Kg (extract yield: 9%w/w). The transient fall in BP reached 69 ± 6% of initial, while the steady BP was 85.2 ± 6.8% of initial (n=4). HR decreased until 87 ± 7% of initial during the transient phase of hypotension, while DP increased to 149 ± 30% of initial. Rats resisted doses 4 to 6 times higher than DE50 without letality. The results suggested that both types of Cecropia adenopus produced hypotension but they differ in cardiac effects. The cultivated Aco was less effective as hypotensive than that from rainforest, Ami. With grant from Colegio Farmaceuticos Pcia. Bs.As

30.

CANNABINOID AND VANILLOID SPINAL RECEPTORS COULD HAVE A ROLE IN THE CONTROL OF THE BLOOD PRESSURE

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It has been reported that the cardiovascular effects of cannabinoids are related to their actions in blood vessels and heart, but little is known about the effects of cannabinoids on cardiovascular nuclei in the central nervous system. In this study we examined whether intrathecally (i.t.) injected cannabinoids elicit cardiovascular effects in male Sprague-Dawley urethane-anesthetized rats. A femoral artery was cannulated for the recording of the blood pressure (BP). A PE_{10} catheter was placed in the spinal subarachnoid space (T_{12}-L_1) for i.t. injection of drugs. The endocannabinoid anandamide (AEA: 25 to 100 nmol) induced a dose-related decrease in the BP that was abolished by nicotinic ganglionic blockade with hexamethonium. The decrease in BP caused 100 nmol AEA (-17.6±2.1 mmHg; n=4) was prevented by either the CB1-cannabinoid receptor antagonist SR141716A or the VR1-vanilloid receptor antagonist capsazepine. Pretreatment with the inhibitor of NO synthesis L-NAME (70 mg/day during 4 weeks) produced a 100% increase in the hypotensive effect of AEA. The mean BP in L-NAME-treated rats was higher than in the controls (118.4±3.4 mmHg; n=10 vs. 77.7±2.1 mmHg; n=10; $p<0.01$). It is suggested that AEA participates in the regulation of the BP through the activation of cannabinoid and vanilloid spinal receptors. The spinal action of AEA appears to be enhanced in hypertensive animals. *Supported by Grants PICT 99-05-**06917, Carrillo-Oñativia 2001 and Fundación Antorchas 14022-112.***

31.

MECHANISMS UNDERLYING ANANDAMIDE POTENTIATION IN MESENTERIC BEDS ISOLATED FROM ENDOTOXEMIC RATS

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It has been reported that the relaxant effects of peripheral endogenous cannabinoid system can contribute to the vascular disorders related to the septic shock. The aim of the present work was to study the mechanisms underlying the potentiation of anandamide (AEA) effects in mesenteric beds isolated from endotoxemic rats. Adult male Sprague-Dawley rats (300-350 g) were injected i.p. with 5 mg/kg lipopolysaccaride (LPS) and killed 6 hs after LPS administration. Vascular responses to bolus injections of NA were measured in the mesenteric bed as changes in perfusion pressure. AEA (0.01-10 μM) induced a concentration-dependent reduction of NA contractions that was significantly potentiated in mesenteric beds isolated from LPS treated rats. The inhibitor of AEA metabolism 200 μM PMSF significantly reduced anandamide effects in LPS treated mesenteric beds but not in control preparations. AEA relaxations were significantly attenuated by the vanilloid receptor antagonist 1 μM capsazepine. The vanilloid receptor agonist capsaicin (0.01-100nM) induced concentration-dependent reductions of the contractile responses to NA that were also potentiated after LPS treatment. Endothelium removal did not modify the relaxations induced by AEA in LPS treated mesenteric beds. It is concluded that the potentiation of AEA effects after LPS treatment might be mediated at least in part by vanilloid receptors and could involve the participation of AEA metabolites.
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32.

POTENTIATION OF BRADYKININ (BK) B_1 RECEPTOR CONTRACTILE RESPONSE BY INHIBITION OF METALLOPEPTIDASES IN HUMAN UMBILICAL VEIN (HUV)

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Introduction and goals: Kinins are hydrolysed by metallopeptidases which are localized in the plasmatic membrane of cells. The aim of this study was to evaluate the effect of the inhibition of the given enzymes on BK B_1 induced response in HUV. **Methods and results:** HUV rings were mounted under isometric tension in Krebs solution at 37ºC. After 5h, concentration-response curves (CRCs) were obtained to the selective BK B_1 receptor agonist Lys-des-Arg^9-BK (LDABK). When 1μM of Captopril (**C**, Angiotensin converting enzyme inhibitor) or 10μM of Amastatin (**A**, Aminopeptidase M inhibitor) were applied, CRCs to LDABK were shifted to the left (pCE_{50} control: 9.15±0.03; **C**: 9.39±0.07; **A**: 9.47±0.07, $p<.05$). However, no significant difference was evident when 10μM of Phosphoramidon (**P**, Neutral endopeptidase inhibitor) was applied (pCE_{50} control: 9.15±0.03; **P**: 9.30±0.06). Concomitant exposure to **C** (1μM) and **A** (10μM) or **P** (10 μM) and **A** (10μM) produced a leftward shift of the CRCs that was significantly different from the CRCs in which the inhibitors were applied individually (pCE_{50} control: 9.15±0.03; **C**+**A**: 9.81±0.05, **P**+**A**: 9.89±0.05, $p<.05$, ANOVA). **Conclusion**: These results indicate that metallopeptidases inactivate BK B_1 receptor endogenous agonist LDABK in biophase.

33.

BRADYKININ (BK) B_1 RECEPTOR MEDIATED RESPONSES IN HUMAN UMBILICAL ARTERY (HUA): POTENTIATION BY INHIBITION OF METALLOPEPTIDASES

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HUA rings were placed under isometric tension in Krebs´ solution at 37ºC. Concentration response curves (CRCs) to des-Arg^9-kallidin (DAKD), selective endogenous BKB_1 receptor agonist, were performed after 5 h of incubation. Peptidase inhibitors and the BKB_1 receptor antagonist, des-Arg^9-Leu^8-kallidin (DALKD), were added to the bath 30 min before and during the CRC to DAKD. Results are expressed as mean ± SEM. Maximum responses to DAKD, expressed as percentage of maximum responses to serotonin 10^{-5} M, were increased by pretreatment with captopril 10^{-6} M or amastatin 10^{-5} M (control: 36.31 ± 5.26%; treated: 68.75 ± 15.83%, $P<0.05$ and 65.24 ± 7.67%, $P<0.05$, n=9, respectively). Phosphoramidon 10^{-5} M also increased maximum responses to DAKD (control: 46.86 ± 6.84%; treated: 84.52 ± 11.73%, $P<0.05$, n=6). In agreement with this, maximum responses to DAKD were markedly increased by simultaneous exposure to captopril, amastatin and phosphoramidon (control: 38.62 ± 4.25%; treated: 89.38 ± 4.25, $P<0.01$, n=16). Under the latter conditions, contractile responses were competitively and potently antagonized by DALKD (pA_2 8.6). These results support the view that angiotensin converting enzyme, neutral endopeptidase and aminopeptidase M are involved in the biological inactivation of DAKD in HUA.

34.

HUMAN UMBILICAL VEIN (HUV): PHARMACOLOGICAL CHARACTERIZATION OF ACETYLCHOLINE (ACH) INDUCED CONTRACTILE RESPONSES

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The aim of the present study was to evaluate the involvement of both cholinesterases and cholinergic receptor/s on ACh contractile-response in HUV. **Methods and results:** HUV rings were mounted under isometric tension in Krebs solution at 37ºC. After 2.5h, concentration response curves (CRCs) to ACh were obtained. Data are expressed as mean ± SEM. Exposure to neostigmine (acetylcholinesterase inhibitor, 10 μM) and tetraisopropylpyro-phosphoramide (iso-OMPA, butyrilcholinesterase inhibitor, 100 μM) did not modify CRCs to ACh (pEC_{50} control: 6.04±0.09; neostigmine: 6.21±0.09, $p>.05$; pEC_{50} control: 6.28±0.04; iso-OMPA: 6.25±0.06; $p>.05$). Atropine (1nM, 3nM, 10nM) produced a competitive rightward shift of CRCs to ACh (pA_2: 9.75; slope: 0.94±0.31). Pirenzepine (M_1 receptor antagonist, 0.17 μM) caused a rightward shift of CRC (pEC_{50} control: 5,96±0,07; pirenzepine: 4,99±0,07, $p<.05$) without affecting maximal responses and its apparent pA_2 was 7.58. Each drug was applied 1h before the CRCs. **Conclusion:** In HUV, aparently the cholinesterases activity does not modified the ACh effective concentration in biophase. Moreover, the estimated affinity of atropine is consistent with a functional muscarinic receptor population in this tissue but the pirenzepine apparent pA_2 value does not discriminate between the muscarinic receptors subtypes.

35.

5-HYDROXYTRYPTAMINE (5-HT) OR ENDOTHELIN-1 (ET-1) SUBTHRESHOLD CONCENTRATIONS POTENTIATE ADRENALINE MEDIATED CONTRACTION IN HUMAN UMBILICAL VEIN (HUV)

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Objetive: We tested the hypothesis that subthreshold concentrations of 5-HT or ET-1potentiate adrenaline-induced contraction in this tissue. **Methods:** HUV rings were mounted in isolated organ baths suspended in Krebs solution. After 2 hs equilibration period rings were pretreated with 5-HT (1 nM and 1.7 nM) or ET-1 (0.1 nM) during 10 min. Then, concentration responses curves (CRC) to adrenaline were constructed (0.01 to 100 μM). Data are expressed as mean ± SEM. **Results and conclusions:** 5-HT 1.7 nM produced a leftward shift of adrenaline CRC ($p<0.05$) while 5-HT 1nM did not show any potentiating effect. pEC_{50} values were 6.99 for control, 7.09 and 7.60 for rings pretreated with 5-HT 1.0 and 1.7 nM respectively. ET-1 10nM also shifted adrenaline CRC to the left in a significant manner ($p<0.05$). pEC_{50} values were 6.71 for control, and 7.12 for treated rings. Neither 5-HT nor ET-1 modified adrenaline maximum response. We conclude that adrenaline response potentiation observed with subthreshold concentrations of 5-HT or ET-1 could be important in acute fetal hypoxemia and fetal distress where catecholamine levels are significantly increased in umbilical venous blood.

36.

CARDIOVASCULAR ACTIVITY OF SODIUM KAURENATE, A NATURAL DITERPENE. COMPARATIVE STUDY WITH ATENOLOL

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The present study was performed to evaluate the effects of sodium kaurenate, a natural diterpene with biological activity, on the medium arterial pressure and cardiac frequency of SHR/N rats. Results were compared with atenolol used as a reference. A non-invasive method was used on fifteen SHR/N male rats (240–300 g). Study parameters were evaluated at 0 (basal), 1, 6, 12, 48, and 72 hours after the administration of a DE_{50} hypotensive dose of sodium kaurenate (20 mg/Kg) (n=8) intraperitoneally (IP) or atenolol (0.31 mg/Kg), IP (n=7). The two compounds restored the parameters analyzed in hypertensive animals to normotension, with a significant decrease of blood pressure ($p<.01$) and heart rate ($p<.01$) in contrast with the basals. Additionally, the rats treated with the kaurene presented effects with lower latency period and action duration in relation to atenolol ($p<.01$). The results suggest that sodium kaurenate decreases cardiovascular parameters with an effect equivalent to that atenolol, a β_1-selective adrenergic antagonist. Grant Number 010-ME-2000 / CDCHT- UCLA.

37.

CYTOPROTECTION BY MELATONIN AND GROWTH HORMONE IN EARLY RAT MYOCARDIAL INFARCTION AS REVEALED BY FEULGEN DNA STAINING

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OBJECTIVE. To examine the cytoprotective effect of melatonin or recombinant human growth hormone (hGH) on the early phase of a running myocardial infarction in rats by using the Feulgen staining. METHODS. Rats were subjected to surgical ligature of the left coronary artery or its sham-operation and were studied 1.5 - 3 h later. Melatonin was administered in the drinking water (100 µg/ml water) for 7 days before surgery. Recombinant hGH (2 IU/kg) was given ip at the time of surgery. Feulgen-stained histological cardiac sections were examined by light microscopy and image analysis. RESULTS. Infarcted rats receiving vehicle exhibited large, diffuse cardiac lesions with a marked positivity for Feulgen reaction. About 18 - 20% of the total area recorded became injured 1.5 or 3 h after infarction, respectively. Infarcted rats treated with melatonin or hGH, or the combination of both, and killed 3 h after surgery, showed cardiac sections with scattered lesions and only a few isolated injured muscle fibers. A similar effectiveness of melatonin and hGH, alone or in combination, to decrease injured area by 86-87% and the number of cardiac lesions by 75-80% was observed. CONCLUSION. A significant cytoprotective effect of melatonin or hGH is demonstrable in an early phase of myocardial infarction in rats.

38.

ASPIRIN IMPROVES NITRIC OXIDE BIOAVAILABILITY AND REVERTS HOMOCYSTEINE-INDUCED HYPERTENSION

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Objective: To study if aspirin reduces the oxidative stress and hypertension induced by hyperhomocysteinemia. **Methods**: Control (C, n=7) and treated (T, n=14) male Wistar Kyoto rats received tap water or homocysteine-thiolactone (50 mg/kg/day), respectively during 8 weeks. After this period, a half of T group received additionally Aspirin (10 mg/kg/day, ip) for another 3 weeks. Arterial superoxide anion production ($\bullet O_2^-$) and nitrotyrosine abundance were determined by chemiluminiscence and western blot analysis, respectively. Aorta glutathion (GSH) content was measured by spectrophotometric assay. Femoral arterial blood pressure was measured using a catether connected to a Statham pressure transducer. **Results**: After 11 weeks, T rats showed increased $\bullet O_2^-$ production together with an 1.5 fold greater formation of arterial nitrotyrosine, a footprint of NO inactivation by reactive oxygen species ($P<0.05$). In addition, arterial GSH content was depleted in T group. On the other hand, T rats also develop diastolic (C: 81± 13; T: 103±9, $P<0.05$). and systolic (C: 127±12; T:151±12mmHg, $P<0.05$) hypertension. The treatment with Aspirin reverts the oxidative status, restoring the arterial GSH content and normalizing the nitrotyrosine production. Finally,arterial hypertension was reverted. **Conclusion**: The present work supports that Aspirin counteracts the oxidative stress and hypertension induced by hyperhomocysteinemia, improving NO bioavailability.

39.

CASTRATION DECREASE AMYLASE RELEASE ASSOCIATED WITH MUSCARINIC ACETYLCHOLINE RECEPTOR DOWN REGULATION, IN RAT PAROTID GLAND

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The mechanism and receptor subtypes involved in carbachol-stimulated amylase release (Ar) and its changes after castration, were studied in parotid slices from males rats. Amylase activity determination was carried out using the method described by Bernfeld in 1951 and Inositol phosphates (IP) levels were measured using a method modified from Berridge *et al.* (1982). Carbachol (Cch) induced both, Ar and IP accumulation in parotid slices from control (C) and castrated (Ca) rats, but castration induced a decrease of Cch maximal effect. The effect of castration was reverted by testosterone replacement. The selective M_1 and M_3 muscarinic receptor (mR) antagonists inhibited Cch-stimulated Ar and IP accumulation in a dose-dependent manner in slices from C and Ca rats. A diminution of binding sites of mR in membranes from Ca rats was observed. Competition binding assays showed that both, M_1 and M_3 mR subtypes are expressed in membranes of parotid glands from C and Ca rats, being M_3 the greater population. These results suggest that Ar induced by Cch in parotid slices is mediated by IP accumulation. This mechanism appear to be triggered by the activation of M_1 and M_3 mR subtypes. Castration induced a decrease of the maximal effect of Cch evoked Ar and IP accumulation accompanied with a diminution in the number of parotid gland mAch receptors.

40.

DETERMINATION OF REPRODUCTIVE PARAMETERS IN RATS OF BOTH SEXS EXPOSED TO 2,4-DICHLORO-FENOXYACETIC ACID (2,4.D)

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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. We studied: **body growth curves**, **days of fur appearance** (FA) and **eye-opening** (EO) in all groups. Females: **estrous cycle** (EC); **first proestrus** (FP); **vaginal opening** (VO); **progesterone** (P), **estradiol** (E2), **prolactin** (PRL) and **growth hormone** (GH) serum levels. Males: **day of testes descent** (TD) and **testosterone** (T) level.

There was a decrease in pup weight gain of treated groups. FA, EO and TD showed no differences between groups. VO occurred within the expected period in all females. The median day of FP was delayed and the dispersion was much greater for the treated group, that also showed a greater number of rats with abnormal cycles. Hormones: T and PRL showed no differences in any groups; E2 were significantly diminished in 60 days treated and P in 45 days treated; GH was significantly diminished in 45 days treated and normal in 60 days treated. 2,4 D induced important changes in growth and reproductive hormones in both sexes, suggesting alterations in gonad histology and function, that need to be confirmed by histology.

41.

SEASONAL CHANGES IN THE PITUITARY FSH OF THE MALE VIZCACHA (*L.m.m.*)

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Seasonal variations in sexual activity in mammals are suppose to involve endocrine factors and photoperiod. Levels on sex hormones are known to change during different seasons in several mammalian species. The adult males vizcacha, a nocturnally active photoperiodic rodent, exhibit in their natural habitat an annual reproductive cycle characterized by a gonadal regression period during the short days of July-August.The involution ranges from a substancial reduction in the number of the spermatids and mature spermatozoa in some animals to an almost complete loss of spermatogenesis in others.The objective of the present study was to investigate the pituitary FSH levels in the different month of the year. The pituitaries were removed monthly (n:6) from April to December, FSH concentration was determined by Inmunoradiometric Assay (Magnetic Solid Phase). Results: FSH (mUI/ml, means±SEM) April: 27.42 ± 2.86, May: 18.70 ± 2.10, June: 10.80 ± 0.86, July: 14.56 ± 1.36, August: 21.20 ± 1.57, Setember: 16.38 ± 1.50, October: 15.11 ± 2.43, November: 14.26 ± 3.12, December: 16.65 ± 2.78 (One-way ANOVA, $P<0.0001$). Our findings indicate that the male adult vizcacha exhibits characteristic of an annual reproductive cycle. There is a close relationship between changesin the pituitary FSH levels and the efficiency of spermatogenesis (Muñoz *et al*., 1997).

42.

SEXUAL BEHAVIOR ALTERED BY 2,4-DICHLORO PHENOXYACETIC ACID (2,4-D) EXPOSURES

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Pregnant rats were individually housed in plastic breeding cages in a controlled temperature (22-24°C) and on a 12-h light/dark cycle room. Twenty-four hours following parturition (PND1), dams were divided into two groups. **2,4-D-treated** dams, that were orally exposed to 50, 25 or 15 mg/kg/ day of 2,4-D in their diet until PND 23 and their pups were maintained on the 2,4-D diet until PND 90. **Control** dams were fed with untreated pellets. On PND1, litters were examined and culled to eight pups. Pups were weaned on PND 23 and separately housed by sex per cage. Three subgroups of rats were observed. **a)** Control males and females; **b)** control males and 2,4-D exposed females and **c)** 2,4-D exposed males and control females. An n=12 rats per group were assessed. Male sexual behaviors were assessed according to the following scores: number of mounts, penis intromissions, ejaculation over a period of 40 min; and post-ejaculatory refractory periods following presentation of a receptive female in a mating arena. Lordosis was the score for females undergoing oestrus. All tests were carried out under red light during the dark period, and consisted of placing each female in an observation cage housing a sexually active male. An inhibition of lordosis behavior was registered in 2,4-D exposed female rats. Only 15% of them became pregnant. 2,4-D exposed male rats were sexually inactive (quiescent) when they were exposed to females. These results indicate that perinatal exposure to 2,4-D has long-term effects on the reproductive behavior of rats.

43.

GENE TRANSFER IN THE HYPOTHALAMUS AND HYPOPHYSIS OF RATS BY MEANS OF HERPETIC AND ADENOVIRAL VECTORS

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In previous studies we demonstrated that herpetic and adenoviral vectors constitute suitable tools for gene transfer into anterior pituitary (AP) cells *in vitro*. In order to extend these studies to *in vivo* systems we used the same type of vectors to transfer reporter genes at hypothalamic and pituitary level in rats. Here, we used three vectors: a) *ts*K/β-gal, an HSV-1 derived mutant harboring the E *coli* β-galactosidase gene. b) Ad.RSV.nls/β-gal, an adenoviral vector harboring the β-gal gene targeted to the nucleus. c) RAd $(eGFP\text{-}TK)_{fus}$, an adenoviral vector carrying a DNA sequence coding for the green fluorescent protein (eGFP) fused to the gene for HSV1 thymidine kinase (TK). These vectors were sterotaxically injected in the hypothalamus and AP of female rats. Two days later the animals were sacrificed, the glands and hypothalami dissected and processed by the X-gal method to detect the presence of the β-gal in the tissues exposed to the appropriate vectors. In the animals injected with $RAd(eGFP\text{-}TK)_{fus}$, the transgene product was identified by fluorescence microscopy. Our results showed that the herpetic vector was the most efficient in the AP, whereas the two adenoviral vectors showed a lower, although significant, transduction efficiency in the AP. At hypothalamic level, both types of vector displayed comparable transduction efficiencies. We conclude that adenoviral and herpetic vectors are potentially useful tools to implement gene therapy in the neuroendocrine system. (We acknowledge Sirex SRL for the generous loan of an Olympus BX-51 fluorescence microscope used in this study).

44.

EFFECT OF MELATONIN ON VASCULAR REACTIVITY "IN VITRO" AFTER SUBTOTAL PANCREATECTOMY IN WISTAR RATS

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Pancreatectomy(Ppx) is one of the standard procedures in diabetes research. Based on the existence of an alteration in vascular reactivity(VR) in diabetes, the aim of this study was to evaluate the effect of melatonin(Mel) on VR of rats turned intolerant to carbohydrates. Intolerance was obtained by subtotal Ppx. Rings of thoracic aorta were placed in organ chambers, and isometric tension was recorded. Dose response curves to Phenylephrine (PhE) and Acetylcholine (Ach) were performed with and without Mel (10^{-5} M). The effect of incubating the aortic rings in a Krebs solution with glucose (G) 44mM was also evaluated. PhE response did not differ between groups. Rings pre-treated with G presented a diminished relaxation to Ach as compared to rings incubated with G+Mel: Ach 10^{-4} (G 24,85±1,70vsG+Mel 7,43± 1,50%$p<0.01$), 10^{-5} (G 26,79±1,32vsG+Mel 8,32±1,34% $p<0.01$), 10^{-6} (G 36,06±1,92vsG+Mel 17,03±2,59% $p<0.001$), 10^{-7} (G 65,75±3,63vsG+Mel 41,48±6,91%$p<0.001$), 10^{-8} (G 86,58±2,31 vs G+Mel 67,15±6,05 $p<0.001$). These results shows that: 1) Ppx rats develops an alteration in vascular response; 2) Mel significantly increased the Ach evoked relaxation of rings incubated with G. Since exposure of vascular tissue to a high glucose medium impairs endothelium-dependent vasodilatation through superoxide anion production, the mechanism of Mel action can be related to the antioxidant properties of Mel.

45.

DIFFERENTIAL EFFECTS OF PROPYLTHIOURACIL (PTU) INDUCED HYPTHYROIDISM ON T AND B LYMPHOCYTE MEDIATED RESPONSES

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Previously the modulation of the immune response by thyroid axis was demonstrated. Here we show the effect of PTU treatment on lymphocyte proliferation and antibody (Ab) production. Hypothyroid [PTU, diluted 0.05% w/v in drinking water for 15 (P15), 20 (P20) and 30 (P30) days], and euthyroid (E) BALB/c mice were used. Triiodotyronine (T3) and thyroxine (T4) serum levels were measured by radioimmunoassay and PTU mice displayed lower levels of both hormones than E animals from day 15 of anti-thyroid treatment onwards. Spleen and lymph node cells were cultured with T and B selective mitogens. T and B cell proliferation, assessed by [3H]-Thymidine incorporation, was risen by T4. T cell proliferation was lower in P15 and higher in P20 than E. B cell proliferation was lower in P15 and P20 but higher in P30 than E. PTU (at day 15 of treatment) and E mice were immunized with the T-dependent antigen (Ag), sheep red blood cells (SRB), or the T-independent Ag, lipopolisacharide (LPS). One week after antigenic challenge, specific Abs were evaluated, by haemagglutination. Anti-LPS titers were lower in PTU than in E mice, while no significant differences were found between anti-SRB titers. In conclusion, reduction in thyroid hormones levels is able to regulate lymphocyte proliferation with different kinetics on T and B cells, but with a paralell effect on humoral responses depending on antigenic challenge.

46.

REGULATION OF TRANSCRIPTIONAL ACTIVITY OF THE P450scc & StAR GENE PROMOTERS IN GENETICALLY MODIFIED STABLE GRANULOSA CELLS

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Immortalized porcine granulosa cells, the JC-410, were stably transfected with genomic constructs containing the cytochrome P450 side-chain cleavage (P450scc) or steroidogenic acute regulatory (StAR) protein genes linked to the luciferase (LUC) reporter gene. The expression of LUC and responsiveness to cholera toxin (CT), were determined by a luminometric assay. CT (100 ng/ml) and estradiol-17β (E_2, 30 μM) increased the transcription of P450scc after 24 h of incubation (1.3- and 1.7-fold, respectively). Insulin (5 μg/ml) increased transcription by 2.3-fold and potentiated the effects of E_2 (1.9- vs. 4.2-fold) and CT, after 48 h. The 24-h treatment with E_2 (10 μM) or CT (100 ng/ml) resulted in 1.5-fold increases in the transcription of pStAR. Insulin had no effect. In summary, both CT and E_2,stimulated transcriptional activity of P450scc and pStAR. Insulin increased the transcription of P450scc and had the additive effect on CT- and E_2-induced transcription of P450scc and pStAR. The JC-410 cells stably transfected with P450scc and pStAR provide a useful tool for studying the function of steroidogenic genes.

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47.

TOXIC EFFECTS OF CADMIUN ON THE RAT PLACENTA

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The development of the fetus in the uterus of mammals is strongly related (anatomically, physiologically and metabolically) to the placenta, a temporary organ with a high metabolic activity, that protects and maintains the fetal life. The placenta acts as a selective filter in the transference of nutrients, and, also, of endotoxins and exotoxins. In this study, the effect of the administration of one dose of $CdCl_2$ to pregnant rats on placenta histoarchitecture was evaluated. Four groups of pregnant Wistar rats were used. They were injected subcutaneously with 10 μg Cd/g BW at the following days of pregnancy: group I, day 7; group II, day 9 and group III, day 11. The group IV (control) received saline solution. At the day 20 of pregnancy, all the rats were sacrificed; their placentas were removed and weighed. One portion of placenta was used to determining Cd concentration (ppm DM). The rest of the organ was fixed with buffered formol, dehydrated with alcohol and embedded in paraffin. Sections of 5μ were stained with H/E. No significant differences (P>0.05) in placenta weights among experimental and the control group were observed. The highest Cd concentrations were found in placentas of rats treated on day 11 of pregnancy. (non significant (P>0.05)). The ratio ***placenta Cd concentration / fetus Cd concentration*** on days 7, 9 and 11 of pregnancy was constant. The histological studies showed, only in Cd treated groups, the presence of numerous infarcts, hemorrhages, fibrinoid deposits and granulocyte infiltrations.

48.

INVOLVEMENT OF MICROTUBULES IN THE RELEASE OF SECRETORY PRODUCTS FROM THE *PARS TUBERALIS* IN THE RAT.EFFECTS OF ALBENDAZOLE

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The p*ars tuberalis* (PT) of the pituitary gland has cells that release secretory products of unknown function up to date. Albendazole (ABZ) is an anthelmintic benzimidazole whose mechanism of action is to despolimerize microtubules (MT); these participate in the secretion of cellular compounds, requiring their integrity and the preservation of the equilibrium between the polimerized (MT) and soluble (dimers of α and β-tubulin) forms. The present work evaluated the modifications generated by ABZ on the release of secretory products from PT of rats. Two groups of adult Wistar rats were used (control and experimental, n=3). The experimental group was administered 2 g/k body weight of ABZ, orally, and sacrificed 48 h after administration. The secretory product location and the MT organization of the PT were determined by immuno-histochemical techniques using monoclonal antibodies against the secretory product and α-tubulin, respectively. In control brain slices the secretory product distribution was paranuclear and, in ABZ treated rats this product was dispersed in all the cytoplasm, as was the distribution of tubulin. These results show the involvement of MT in the secretion from the PT and help in the understanding of the mechanism of action of ABZ.

49.

ALBENDAZOLE EFFECTS ON WISTAR RAT TESTICULAR AND EPIDIDIMAL MORPHOLOGY

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A number of drugs have toxic effects on reproductive organs. This work is included in a more broad research plan aimed to determine albendazole (ABZ) effects on testicular and epididimal morphology. For this purpose, we worked with two sexually mature Wistar rat groups (control and experimental); to the experimental group a single dose of ABZ, 2 g/k body weight, was administered by gastric intubation; to the control group an equivalent amount of water was given as a placebo. Organs were excised and fixed in Bouin medium, and processed until inclusion in paraffin. Five micrometers-transversal serial cuts were performed, placed on a slide and stained with Mayer hematoxylin and eosin. Taking into account average diameter of seminiferous tubules and the histological appearance of the seminiferous epithelium, they were classified into normal or altered ones. Also, the epididimal histoarchitectural was evaluated. In the experimental group of animals, there was a significant ($P<0.001$) decrease of tubular diameter and 19.5% of seminiferous tubules were altered; in addition, no sperm were observed. There was a partial loss of the germ line and membranous vacuoles between Sertoli cells showed up. In the epididimus, significant differences in the thickness of epithelium were found between experimental and control groups, but without tissular alterations.

50.

BIDIRECTIONAL EFFECT OF STRESS. ROLE OF LYMPHOCYTE-STRESS HORMONE RECEPTORS

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Many studies have suggested that stress has profound effects on immune system depending on the intensity, duration and type of stressor. The present work was carried out to study the effects of acute and chronic stress on T-cell dependent humoral response. Moreover, we analyze catecholamines and corticosterone influence on immune response. For this purpose acute stress was administered by placing animals in well-ventilated restrainers for 2 h. For chronic stress animals were exposed to mild stressors for six weeks. Normal and stressed animals were inoculated with sheep red blood cell. We observed that acute stress induced an enhancement of antibody formation and a significant increase of catecholamine and corticosterone levels. On contrary, chronic stress have a suppressive effect on humoral response without modification on stress hormones levels. On the other hand both, catecholamine and corticosterone, have a dual effect on mitogen-induced normal T cell proliferation. A stimulatory effect was observed for lower concentrations while an inhibitory effect was obtained with higher ones. Similar effects were observed on proliferative response of lymphocyte from animals subjected to acute stress. However, stress hormones induced only an inhibitory effect on chronic stress lymphocyte reactivity. These results indicate that lymphocyte sensibility to stress hormones could be playing an important role in bi-directional effects of stress.

51.

THYROID STATUS MODULATES LYMPHOCYTE ACTIVITY AGOUTI AND NON-AGOUTI MICE

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The obesity syndrome caused by the dominant agouti alleles includes alterations in the regulation of feeding behavior and metabolism. Thyroid hormones are able to modulate immune responses. The aim of this work was to analyze the immune status and its relation to thyroid status in yellow agouti obese mice (B6.Cg-A^y strain) in comparison with the non-agouti (control) brothers. T and B lymphocytes were purified from lymph nodes and spleens and proliferative responses to selective mitogens were evaluated by [^{3}H]thymidine incorporation. Serum levels of T3 and T4 hormones, as well as hypothalamic TRH were determined. Three months old agouti mice show a non-significant increase in T and B cell proliferative responses with no changes in thyroid hormones levels respect to controls. However older animals display a significant increase both in T and B cell proliferation. This was accompanied by an increase in hypothalamic TRH, with non significant decrease in T3 and T4 serum levels, probably suggesting a subclinic hypothyroid state in agouti mice with aging.

These results show that agouti mice develop an alteration in both immune system and thyroid axis with aging. The interrelationship between immunity and thyroid axis and the participation of TSH hormone in these effects are now under study**.**

52.

EFFECTS OF COMBINED TREATMENT WITH FILGRASTIM AND ERYTHROPOIETIN ON GRANULOPOIESIS RECOVERY OF CYCLOPHOSPHAMIDE PRETREATED MICE

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Erythropoiesis and granulopoiesis are fed by common ancestors cells. Accelerated differentiation into one of these lineages may be accompanied in vivo by diminished differentiation into the other pathway. Here we report the effects of treatment with filgrastim (G-CSF) plus eritrhopoietin versus filgrastim alone, on granulopoiesis recovery of cyclophosphamide pretreated mice. After the cyclophosphamide administration (100mg/kg on day 0) femoral and splenic granuloid precursors cells level decreased on day 4, and then returned to normal values. G-CSF treatment (30ug/kg/día) increased pheripheral neutrophils values on day 8 and later increased these cells, though no significantly. Femoral granulopoietic precursors contents rose significantly in mice treated with G-CSF on days 16 - 20 cells. In contrast, the combined treatment with G-CSF and Epo was unable to stimulates the bone marrow granulopoiesis. In the spleen, G-CSF plus Epo administration caused a dramatic, although transient increase in the numbers of granuloid cells on day 12, but the splenic contribution to granuloid recovery (measured as granuloid precursors cells number) was small. This results suggests that a competition between granulopoiesis and erythropoiesis may be present and are necessary careful analyses before combination of this factors for treatment in humans.

53.

PHYSIOLOGICAL RESPONSE OF HEMOPOIESIS BY PENTOXYFILLINE

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Pentoxyfilline (PTX) shows high homology to Lysofilline (LSF), which it has cytostimulant properties on hematopoietic system. Moreover both drugs interfere in cytokines production. The aim of this study is to elucidate if Ptx has cytostimulant effects as LSF. PTX was injected daily for fourteen days (100 mg/kg, i.p.). Bone marrow (BM), spleen (Sp) and peripheral blood (PB) samples were taken each day to perform experiments, comparing to controls. Transient reticulocytosis was found in BM, Sp and PB at different times. Colonies growth of early BM progenitors decreased strikingly since day 1 till day 6, then CFU-GEM and CFU-GM returned to control values. BFU-E appeared to be stimulated on day 14. In Sp, all mentioned progenitors were affected also, but kinetics of recovery was different. BFU-E returned to control since day 8. CFU-GM didn't show differences to controls and CFU-GEM remain decreased till the end of experience. CFU-E were enhanced on the first half of the experimental schedule but decayed under controls on day 14 in both, BM and Sp. Hematocrits didn't show significant differences through experience. PTX does not seem to exert similar effects as LSF on normal hematopoiesis. It causes cellular damage affecting hematopoietic lineages. It appears to enhance maturation of previously committed progenitors helping in the homeostasis of peripheral parameters. This feature could mask effects of PTX on normal hematopoiesis

54.

HEMOPOIETIC RECOVERY POST-PACLITAXEL: ERYHTROID PROTEIN EXPRESSIONS AND APOPTOTIC PROFILES

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Paclitaxel (Px) became an important agent against human cancers. Its properties may be explained not only to microtubules stabilization but also by its effects on gene expression. Cytotoxic injury causes deeply alterations on hemopoietic progenitors. Erythropoietic restitution involves changes of critical proteins, such as c-myb and GATA-1. c-Myb activates immature genes and suppresses terminal differentiation of several lineages.GATA-1 is crucial for erythroid differentiation. It inhibits proliferation, suppresses *gata*-2 and c-*myb* expressions and promotes survival of red blood cells. The aim of this study is to correlate changes in c-Myb and GATA-1 expressions with apoptotic variations post-Px single injection (29 mg/Kg, i.p.). Samples were taken at 0, 24, 48, 72, 96 and 120 hs, determining apoptotic indexes by TUNEL assays, transcription factors expressions by inmunoblottings and total bone marrow and differential cellularities by standard methods. We observed that erythroid restoration involves increased expressions of c-Myb and GATA-1 at 24 hs while myeloid recovery requires maintained expression of both till 4 days post Px. Apoptotic indexes and erythroid transcription factors expressions were maximal at 24 hs. Experimental data suggest that drug injury causes lineage differential recovery profiles and that survival cells increased transcription factors expressions despite of cellular depletion.

55.

MOBILIZATION KINETIC OF HEMATOPOIETIC STEM CELLS BY PACLITAXEL TREATMENT

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Paclitaxel (Px) is a promising drug for cancer treatments. It also may play a role in hematopoietic stem cells (HSC) mobilization, as an alternative to cyclophosphamide. We investigate the HSC-mobilizing potential of Px in a murine model. CF1 mice were primed with ip injection of Px (29 mg/kg) and were sacrificed 2, 5, 7 or 10 days later. Spleens were collected and processed to obtain mononuclear cell (MNC) pools for different assays. Total and differential cellularities and apoptotic indexes were determined at each day of the experimental schedule. Total MNC decreased between day 5 to 7 vs. control ($p<0.01$), in a close relation with high apoptotic index ($p<0.001$). At day 5, the number of hematopoietic progenitors (CFU-C) were significantly higher compared to control ($p<0.001$). From day 7 to 10, CFU-C decreased under control. In contrast to other cytotoxic drugs, mobilization with Px was associated with less perturbation on mature lymphocytes. These results indicate that Px is an efficient early mobilizer of HSC (from day 2, maximum at day 5). It also has the advantage to preserve lymphoid lineage. Lastly, hematopoietic stem cells mobilized by Px comprise cells with high self-renewal potential, and they may be used for hematopoietic reconstitution after myeloablative therapy.

56.

STIMULATED SECRETION OF ERYTHROPOIETIN IN NON-POLYCYTHEMIC-HYPOXIC MICE

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Plasma erythropoietin (pEPO) levels markedly increase in response to hypoxia (HX) to stimulate erythropoiesis. During sustained hypoxia, pEPO declines before an increase in the blood oxygen carrying capacity is evident. To define the role of the size of the circulating red cell mass on the pattern of pEPO changes during exposure to HX, we have serially measured pEPO in untreated control mice and in mice whose hematocrits were kept constant by either phenylhydrazine (PHZ) administration or repeated phlebotomies. Animals were placed for 9 d in a hypobaric chamber in which the air pressure was kept at 556 mbar. Hypoxia-stimulated EPO production was derived from pEPO (ELIZA, Medac Diagnostika, FRG). Untreated control mice exhibited a steady rise in hematocrit, which remained practically unchanged in the hypoxic groups that were subjected to regular phlebotomies or PHZ injections. At day 8 of continuous exposure, reticulocytes were 6.72 $\pm$ 0.63% in the former and 43.0 $\pm$ 1.34 in the latter. Hypoxic-control mice exhibited a rise in pEPO with two peaks of around 100 mU/ml during days 1-2 and 5-6 of exposure; pEPO was 3-4 times higher in experimental mice. A negative correlation between pEPO and hematocrit was established (Pearson r -0.7285, r^2 0.5307, $p<0.0001$). It is concluded that EPO production under hypoxic conditions is highly influenced by the circulating red cell mass, thus providing evidence for the adaptive role of the hemoglobin mass in non-genetically adapted animals.

57.

STIMULATED PRODUCTION OF ERYTHROPOIETIN IN POST-HYPEROXIC MICE

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Current evidence suggests that a modulatory action on O2-dependent EPO secretion is exerted by the erythroid-precursor cell population in the erythropoietic organs through a negative feedback system. The aim of the present investigation was to estimate hypoxia-stimulated EPO secretion in mice with hyperoxia-depressed red cell production. Female CF#1 mice aged 70 d were divided in control (C) and experimental (E) groups. The former was maintained in plastic cages in a normal environment, while the latter was placed in a environment of 60%O2/40%N2 in a 85 dm^3 atmospheric chamber with a air flow of 1 L/min. CO2 was removed from the chamber by washout and absorption with calcium hydroxide. Erythropoiesis was evaluated in 10 C and 10 E mice by iron kinetics performed 3 h after i.v. injection of a tracer dose of ^{59}Fe. The fraction of iron going to erythroid tissue was 65.7% ($p<0.0001$) lower in E than in C mice, showing the erythropoiesis-lowering effect of hyperoxia after 72-hour exposure. Hematocrit values were similar in both groups. Oxygen-dependent EPO production was derived from pEPO (ELISA, Medac Diagnostika, FRG) changes after 4-hour exposure to 506 mbar in a decompression chamber. Plasma EPO was 173.2% higher ($p<0.05$) in E than in C mice. Data support the concept that the rate of erythropoiesis, perhaps through the number of the erythroid progenitor/precursor cell population, modulates O2-dependent EPO production.

58.

LEPTIN VALUES IN TWO MODELS OF LIPID DIET SUPPLEMENTATION IN BOVINES

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In two separate experiments, the effects of additioning lipids to the diet of bovines on plasma leptin levels were analyzed. In the first experiment, 37 multiparous Holstein lactating cows in grazing conditions received 5.4 kg/d of a basal concentrate to which 0, 0.5, or 1 kg of partially hydrogenated oil (melting point 58 to 60°C) was added. Jugular blood samples were taken on days 30 and 60 of lactation. In the second experiment, 38 Aberdeen Angus and Aberdeen Angus X Hereford feedlot steers (124.5 ±12.3 kg mean body weight (BW)), were paired by strain and BW. Animals within each pair were randomly assigned to one of two diet treatments: with or without calcium salts of unsaturated fatty acids in a 0.13% of mean BW. Jugular blood samples were taken twice, at 177 and 225 Kg of mean BW. In plasma samples of both experiments leptin concentration was measured by RIA with an ovine specific antiserum. Leptin levels were higher in cows than in steers and augmented between 30 and 60 days of lactation in cows. No differences were observed in leptin values during development in steers. Additioning oil to the diet didn't change leptin levels even though fat dorsal deposition was enhanced in males.

59.

SOME PHARMACOKINETICS PARAMETERS OF R (-) AND S (+) KETOPROFEN IN THE DAIRY CATTLE

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Ketoprofen (3-benzoilphenylpropionic acid) is a non-steroidal anti-inflammatory drug (NSAID). The aim of this work was to study the possible modifications of the pharmacokinetics of ketoprofen enantiomers that may result from age, lactation or gestation in dairy cattle. Twenty-four Holando Argentino bovines were divided into three groups: 8 cows in early lactation, 8 pregnant cows and 8 newborn calves. Four animals from each group received the enantiomer R-(-) ketoprofen, the other four animals received the S-(+)enantiomer, all at a dose of 0.5mg/kg. Blood samples were withdrawn at predetermined times after administration. Plasma enantiomer concentrations were measured by HPLC. Significant differences between the three categories of animals were obtained in elimination half-life (t1/2) (1.52, 0.87, 0.31 and 1.71, 0.69 and 0.26 h), retention half-time (MRT) (0.45, 1.25, 2.20 and 0.38, 0.99, 2.47 h) and area under the plasma concentration-time curve (AUC) (0.87, 2.93, 3.24 and 0.67, 2.78, 5.13 µg-h/ml) for R (-) and S(+) ketoprofen, respectively. R(-) and S(+) ketoprofen showed significant differencess in AUC (3.24 v 5.13µg-h/ml) and ClB (0.16 v 0.10 ml-h/kg) in calves. In this study, a different enantioselective pharmacokinetic behaviour of one compound in one species under different physiological situations has been clearly demonstrated.

60.

***IN VIVO* AND *IN VITRO* PHARMACODYNAMIC PROPERTIES OF METOPROLOL IN AORTIC COARCTATED RATS**

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It was studied the chronotropic effect of β-adrenoceptor antagonist metoprolol *in vivo* after intravenous administration and *in vitro* in isolated atria of aortic coarctated (ACo) rats and in sham operated (SO) control animals.

Metoprolol levels in arterial dialysates and changes in arterial pressure and heart rate were determined after iv administration of metoprolol. Affinity of metoprolol by means of shifting of noradrenaline concentration-response curve and the proposed inverse agonism activity of metoprolol were studied in isolated atria.

In vivo, after iv injection, IC_{50} of metoprolol cardiac effect was smaller in ACo rats (13.0±5.4 $ng.ml^{-1}$, n=5, $P<0.05$ vs. SO rats) than in SO rats (36.2±4.6 $ng.ml^{-1}$, n=5).

In isolated atria, no difference was found in metoprolol pK_b between both groups (SO: 7.2±0.2, n=5; ACo: 7.5±0.2, n=5). The inverse agonist activity of metoprolol in ACo rats (pEC_{50}: 6.4±0.2; E_{max}: -27.8±4.2%, n=5) was not different than in SO rats (pEC_{50}: 6.2±0.1; E_{max}: -27.3±2.0%, n=5).

In conclussion, a biggest sensibility to the metoprolol chronotropic effect was found in aortic coarctated rats. The affinity and inverse agonism activity of metoprolol would not be altered in isolated atria of aortic coarctated rats.

61.

PHARMACOKINETIC STUDY OF LEVOFLOXACIN AFTER REPEATED ORAL DOSING IN CATS

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Introduction: Levofloxacin (LVX) is a fluoroquinolone broadly used in human medicine, but poorly studied in domestic animals. Up to date there are not studies on plasma disposition of LVX after repeated oral dosing in cats.

Materials and Methods: 5 adult cats weighed 4.66 ± 1.21 kg received LVX orally at a dose rate of 10 mg/kg once daily for 4 days. Blood samples were withdrawn at pre-determined times. LVX plasma concentration was determined by microbiological assay using *Klebsiella pneumoniae* (ATCC 10031) as test micro-organism. Plasma disposition curves were analyzed by non linear methods using PcNonlin software.

Results: The analysis of plasma curves disposition of LVX showed a limited accumulation (R: 1.47±0.60) after 4 days administration. LVX was quickly absorbed ($Tmax_{(ss)}$ 1.62±0.84) with a half life for this process of 0.18±0.12 h. Moreover, the drug achieved high plasma concentration levels ($Cmax_{(ss)}$ 4.70±0.91 mcg/ml). LVX elimination was slow with a ClB/F of 0.18±0.07 l/kg.h. A long T˚ß (8.39±2.14 h) is reflecting the low ClB mentioned as well as a wide volume of distribution (1.42±0.41 l/kg). It can be concluded that LVX has interesting pharmacokinetic features, such as high plasma levels with a slow elimination, pointing it as a useful tool for feline practice.

62.

PHARMACOVIGILANCE IN PUBLIC HOSPITALS OF CÓRDOBA CITY. ARGENTINA

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There was designed Pharmacovigilance's study (FVG), centred on three Public Hospitals of Cordoba, seeking to detect RAM and / or Lack of Efficacy and Technical Faults in Biomedical Material. They have added to this study other Hospitals and Private Centers.

Methods: There were realized forming courses of Human Resources in FVG. There were distributed FVG's cards of own design, recovered then.

For statiscal calculus was applied INFO-4.

Results: 42 informed cards recovered. The Pharmacological involved Groups were analyzed: Anaesthetics: 25%, Ansiolitics: 15%, Cardiovascular: 10%, Anticoagulants: 10%, Immunosuppressants: 10%, Hipolipemiantes: 5%, Analgesics: 5%, Anticonvulsivantes: 5%, Oncológicos: 5%, Cicatrizantes: 5%.

The principal organs affected by RAM were: CNS: 40%, Skin: 30%, Blood: 10%, Cardiovascular System: 10%, Immunological System: 5%, Digestive System: 5%.

The RAM meant 13, 63% of the sample, the lack of efficacy 31, 82%, the faults in biomedical material: 54, 55%. The analysis of imputability indicated: definite RAM: 10%, probable RAM: 90%. The distribution of responsibility for Generating Agents was: Physicians: 61, 36%, Nurses: 34%, Pharmacists: 2, 32%, Others: 7%. The principal information centers were: Hospital Cordoba: 70 %, National Hospital of Clinics: 18%, Private Hospital: 5%, Others: 7%.

One concludes that the major percentage of difficulties takes.

63.

KINETIC ANALISIS OF CYNARIN IN RATS

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Artichoke (*Cynara scolymus* L.) is native to the Mediterranean region in Europe, and is also cultivated for medicinal and food purposes. Cynarin (1,5-dicaffeoylquinic acid, $C_{25}H_{24}O_{11}$) is a compound found in artichoke leaves which is included in various pharmaceutical preparations for its renown therapeutic properties such as hepatoprotective, antioxidant and hypocholestorolemic.

In order to study cynarin biliar elimination, a reverse phase, isocratic HPLC method with UV detection was developed. This system was run for 30 minutes. Two clean-up methods were used in order to separate cynarin from other components present in the sample. In the first method, alumina was used. In the second, bilis was precipitated with perchloric-acid. In both cased, linearity (r = 0.99) and recovery were calculated, and the detection limit was also determined. The detection limit for alumina was higher due to the fact that recovery in this case was 48.7%+/- 11.06 , in comparison to that obtained for perchloric-acid, equal to 102.6%. +/- 10.13 However, we decided to use the former method since using alumina avoids interference during further runs.

64.

MULTIPLE-DOSE PHARMACOKINETICS OF CIPROFLOXACIN IN DOGS

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Fluoroquinolones are currently used in antimicrobial veterinary therapeutics, however not many pharmacokinetic multiple dose studies have been conducted in dogs. Ciprofloxacin (CIP) is a very potent fluoroquinolone with good antibacterial activity on *Enterobacteriaceae* and *Pseudomonas aeruginosa.* In this study the pharmacokinetics of ciprofloxacin in healthy dogs was investigated after multiple dosing. Eight adult male dogs (12.3 ± 4.6 kg) received 7 twice daily doses of CIP (15 mg/kg) orally. Serial blood samples were taken after first and last administration, predose and 1.5 h after administration blood samples were taken on days 2 and 3. CIP plasma concentrations were determined by microbiological assay with *Klebsiella pneumoniae* ATCC 10031 as the test organism. Plasma CIP concentrations vs time curve were analysed by nonlinear methods (PCNonlin, 4.0). Disposition curves were best described by a one-compartment model. The main pharmacokinetic parameters were as follows, as mean (SD): AUC (µg.h/ml) 11.35 ± 2.12; Cl_t/f (ml/min.kg) 1.13 ± 0.15; V_d/f (l/kg) 10.82 ± 5.26; $t_{1/2abs}$ (h) 0.17 ± 0.11; $t_{1/2el}$ (h) 6.87 ± 3.83; C_{max} (µg/ml) 1.28 ± 0.56; t_{max} (h) 1.27 ± 0.60; t_{lag} (h) 0.40 ± 0.42. The long elimination half-life and peak plasma concentrations obtained in this experience point this drug as a possible tool in antibacterial treatment in dogs.

65.

PHARMACOKINETICS OF GENTAMICIN IN NEONATES: ONCE-DAILY VS STANDARD DOSING REGIMEN

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Knowledge of antibacterial mechanisms and pharmacodynamics properties of aminoglycosides has led to a reconsideration of dosing regimens. In adults, once-daily dosing compares favorably with standard regimens. On the basis of the large volumes of distribution and slow renal clearance of aminoglycosides in neonates, we calculated that larger doses per body weight and longer dosing intervals would be suitable for neonates. The aim of this work was to study the pharmacokinetics of gentamicin in neonates in order to compare standard vs once-daily dosage regimen. Therapeutic drug monitoring of gentamicin was carried out in 18 neonates and bayesian non-linear analysis was performed in order to estimate pharmacokinetic parameters (PP), elimination rate constant (Ke) and distribution volume (VD). Laboratory data (serum creatinine and urea, Na excreted in urine, etc) together with other covariates such as weight and post-natal age were also obtained. The mean PP estimated were: Ke: 0.14 h^{-1}, t1/2: 5.7 hs, VD: 0.47 L. In 11 neonates the dosing regimens were changed to once-daily dosing (6.5-17.0 mg/24 h), showing no changes in renal function in comparison to traditional dosing.

In conclusion the results suggest that once-daily dosing regimen could be more safety and clinical effective, than traditional regimen in neonate population.

67.

PHARMACOLOGICAL CHARACTERIZATION OF PROSTANOID RECEPTORS MEDIATING CONTRACTION IN HUMAN UMBILICAL VEIN (HUV)

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Prostanoids have been implied in the contraction of HUV. The aim of this study was to asses which is/are the prostanoid receptors involved in this effect. **Methods and results:** HUV rings were mounted under isometric tension in Krebs´ solution at 37ºC. After 2h, concentration -response curves (CRCs) to several prostanoids were obtained and pEC_{50} estimated (indomethacine 30µM was added 30 min before CRCs): U46619 (8.08), $PgF_{2\alpha}$ (6.04), fluprostenol (5.47),17-Phenyl-PgE_2 (5.40), misoprostol (5.15) and PgE_2 (5.13).TP receptor antagonist: ICI192605 and SQ29548, produced a rightward shift of the CRCs to U46619 without modifying the maximal response and led us to estimate a pK_b value of 9.10 and 7.90 respectively. ICI (0.1µM) completely abolished PgE_2 responses while $PgF_{2\alpha}$ mediated contraction were not blocked neither by ICI (0.1µM) nor by AH6809 (1µM). Data are presented as mean ± S.E.M. **Conclusion**: The potency of U46619 and the antagonist pK_b values suggest that TP receptor are present in HUV. The low potencies of PgE_2, misoprostol and 17-Phenyl-PgE_2 and the blockade of this response by ICI suggest that this contraction is mediated by TP receptors. The lack of blockade of $PgF_{2\alpha}$ with ICI and AH indicates that this contraction is not mediated by EP_1 or TP receptors while the low potencies of $PgF_{2\alpha}$ and fluprostenol are not consitent with FP receptors involvement.

66.

EFFECT OF DEXAMETHASONE ON THE PHARMACOKINETIC PROFILES OF OXFENDAZOLE AND ALBENDAZOLE SULPHOXIDE IN SHEEP

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The co-administration of albendazole and dexamethasone (DXM) showed to be useful in the treatment of neurocysticercosis in man. The current study examined the effect of DXM co-administration on the dispositon kinetics of oxfendazole (OFZ) and albendazole sulphoxide (ABZSO) and its metabolites, respectively, in sheep. **Experiment 1.** In PHASE 1, eight adult sheep (30-38 kg) were allocated in 2 different groups (n=4). Animals in Group 1 were intravenously treated with OFZ (5mg/kg). Group 2 received identical treatment that Group 1 plus an intramuscular injection of DXM phosphate (0.2mg/kg). After 21 days, the treatments were reversed and the experiment repeated as PHASE II. **Experiment 2:** it was identical to that described for Experiment 1 using ABZSO as anthelmintic drug. Jugular blood samples were taken at 0 h (blank), 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 32, 48 and 72h post-treatment. Samples were analised by HPLC. Area under the concentration vs.time curve values (AUCs) obtained in Experiment 1 after DXM treatment, increased 55% for OFZ and 60% for fenbendazole sulphone metabolite. However, the AUC values obtained for ABZSO and its sulphone metabolite (Experiment 2) were not affected by the presence of DXM. The results reported here could have important implications on the search of kinetic interactions resulting on enhanced anthelmintic efficacy.

68.

STUDY OF RANITIDINE PERMEATION ACROSS INTESTINAL SACS OF RATS

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The H_2 antagonist ranitidine is a small and relatively hydrophilic drug believed to cross the intestinal epithelium passively via a paracellular route and so considered not to be a typical substrates of P-glycoprotein. This protein is implicated in the outward transport of different drugs such as the antitumor, leading to the phenomenon of multidrug resistance.

We aimed to study the permeation of ranitidine 0.1 mM across the intestinal sacs of rats (ileum), and determine the influence of P-gp on the process.

The accumulated amount of drug that permeates across the everted intestine after 50 min of incubation is statistically superior to the amount quantified in the non-everted sacs ($p<0.05$). The slopes of the lines amount of drug accumulated vs time are also different ($p<0.05$).

Considering ranitidine to permeate via a transcellular route, we tried to block its permeation across the everted sac with quinidine 0.2mM. We found no statistical difference in the accumulated amounts of drug ($p>0.05$)nor in the slopes of the lines. To verify that the concentration of quinidine was sufficient to block Pgp, we assessed the permeation of quinidine in presence or absence of verapamil 0.1 mM finding a significant difference in the amount of drug accumulated in the medium when comparing between groups. From the results, we proposed that there are two components of ranitidine transport across the intestinal ileum of rats but probably the most important in the absorption of the drug in our biological system is the passive paracelullar transport.

69.

NEUROHISTOLOGICAL, PHARMACOKINETIC AND BEHAVIORAL ANALYSIS OF RATS TREATED WITH MK-801 AND ETHANOL

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The combination of PCP and alcohol (EtOH) has been reported to be heavily psychotoxic in humans. Therefore it seems important to verify weather EtOH administered at a low dose (1,5 g/kg i.p.) to female rats previously treated acutely with a single high dose of MK-801 (a PCP analog, 10mg/kg i.p.), has or not an additive effect on the neurodegeneration (ND) induced by this latter drug. For that purpose, we analyzed the EtOH blood levels (BAL) at different post administration times (t=5, 15, 30, 75, 120, 180 min) both on control rats (EtOH alone), and on EtOH/MK-801treated rats. For the demonstration of ND the amino-cupric-silver stain was used. Finally, we evaluated the duration of the MK-801 induced recumbency, as well as the weight loss in rats exposed to different treatments. The main results were that the rats treated with both EtOH and MK-801 presented, at naked view, a greater amount of degenerated granular cells (GR) in the dorsal dentate gyrus than in those treated only with MK-801, whereas no ND was present in control groups (ClNa or EtOH alone). On the other hand, the BAL were significantly greater in the EtOH group, and both recumbency and weight loss showed significant differences between controls and experimental groups. In conclusion the present work shows that altought the i.p. administration of a low (subneurotoxic) dose of ethanol potentiated the ND induced by high dose of MK-801 in the dentate GR cells, this added action did not affected the duration of the MK-801 induced recumbency.

70.

ESTIMATION OF PHARMACOKINETIC PARAMETERS OF IRBESARTAN FROM MICRODIALYSIS DATA

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The aim of our study was to compare pharmacokinetic parameters of irbesartan obtained from microdialysis data (MD) and conventional blood samples (BS). A vascular shunt microdialysis probe was inserted into the carotid artery and one femoral vein was cannulated for i.v. administration of irbesartan. Microdialysis samples were collected every 15 min. Blood samples were taken every 15min. Levels of drug were measured by HPLC. Pharmacokinetic parameters were estimated using TOPFIT program. Corrected MD were compared with BS taken at same time to determine protein binding. The irbesartan protein binding did not change during the experiment. The estimated Ke from MD and BS were similar (MD: 1.8±0.3 hs^{-1}, n=5; BS: 1.7±0.2 hs^{-1}, n=5). After protein binding correction for the MD, the estimated values of Vd (MD: 1.2±0.4 l, n=5; BS: 1.1±0.4 l, n=5), Cl (MD: 32.3±7.3 $ml.min^{-1}$, n=5; BS: 30.7±8.2 $ml.min^{-1}$, n=5) and AUC (MD: 7.7±3.2 ?$g.ml^{-1}$.hs, n=5; BS: 8.8±3.4 ?$g.ml^{-1}$.hs, n=5) were similar between MD and BS.
In conclussion, the microdialysis technique is an alternative method for the estimation of pharmacokinetic data. On the other hand, the microdialysis technique is also useful to study protein binding of drugs and saturation in protein binding.

71.

ACE INHIBITOR´S TREATMENT DURING PREGNANCY AFFECTS KIDNEY DEVELOPMENT OF THE FETUSES RAT. MOLECULAR AND HISTOLOGICAL EVIDENCES

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Angiotensin II (Ang II) plays an important physiological role in the regulation of blood pressure and thirst response. All components of the RAS has been demonstrated on the fetal tissues. The aim of the present study was to assay the effect on development of Enalapril and Captopril (ACE inhibitors), administered during late gestation.. Adult Wistar rats with 13 days of pregnancy were treated with Enalapril (E), Captopril (C) or saline (Control) by mini-osmotic pump (Alzet 2001). Treatment with E –a compound that does not cross the placental barrier- in PND0 animals leads to a thickened nephrogenic zone. In PND8 this phenotype reversed to that of treated animals. In contrast, addition of C –a compound that crosses the placental barrier- in PND0 pups caused an underdeveloped nephrogenic zone. This effect was maintained in PND8 rats. AT_2R expression was determined by semiquantitative RT-PCR using β-actine as standard and quantified by using Scion Image program. A marked reduction was observed on the level of AT_2R expression in treated newborn rats. In contrast, at eight days of age, there were no significant differences in AT_2R levels between treated and control animals. This is probably due to the decreased AT_2R expression in eight days old animals. Our observations indicate that the IACE has a remarkable effect on the kidney development of the embryo.

72.

DIURETIC ACTIVITY OF AN AQUEOUS EXTRACT OF *Cuscuta xanthochortos* MART

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Cuscuta xanthochortos Mart. ex Engelm (Cuscutaceae) is a climbing parasitic plant native of the Northeastern regions of our country, Brazil and Paraguay, commonly known as “cabello de ángel” or “cabello de la virgen” and traditionally used in infusions as diuretic. The preliminary phytochemical screening revealed the presence of tannins, steroids and/or triterpenoids. Investigations performed in other species (*Cuscuta chinensis, Cuscuta reflexa*) revealed the presence of different compounds (neutral heteropolysaccharides, flavonoid glycosides) with antioxidant activities. The aim of this preliminary study was to validate the diuretic effect of an aqueous extract of *C. xanthochortos* (10% P/V) in Wistar rats. Three groups of orally hydrated rats (5 ml/l00g) received a single p.o. dose (1g/kg) of *C. xanthochortos,* hydrochlorothiazide (25 mg/kg) and vehicle (water) respectively and then were placed individually in metabolic cages. Urine was collected over a period of 8hr every 2hr intervals for volume and electrolyte determinations. At 6 and 8 hr after administration, the aqueous extract produced a significant increase in urine volume (ml/100g b.w.) and electrolytes (Na^+, K^+ and Cl^-)($p<0.01$ ANOVA-Tukey´s test) compared with control values. Similar effects were seen with the thiazide but starting earlier (4hs). These results would corroborate the traditional use of the infusion of *C. xanthochortos* in folk medicine.

73.

THE USE OF DIETARY N-3 FATTY ACIDS AS AN EXPERIMENTAL TOOL FOR CHANGING THE LIPID CONTENT AND COMPOSITION OF PERIPORTAL AND PERIVENOUS RAT LIVER CELLS

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The liver plays a central role in the maintenance of lipid homeostasis. Previous work showed that cholesterol metabolism differs between periportal (PP) and perivenous (PV) liver cells. We also showed that, in mice fed fish oil rich diets, the content and fatty acid (FA) composition of liver lipids is modified, the new composition remaining constant from day 4 onwards, as long as the new diet is administered. The aim of this work was to determine how diet-induced changes affect subpopulations of PP and PV cells. These were obtained by centrifugal elutriation from the liver of rats fed a chow diet and an n-3 FA-enriched diet (9% of fish oil). In control rats, the lipid content and FA composition were very similar between PP and PV cells. The administration of fish oil resulted in lipid changes quite similar to those found previously in whole liver (decrease in the amount of triacylglycerols and increase in the percentages of n-3 FA in lipids). Some differences between PP and PV (e.g. higher percentages of long-chain n-3 FA in PP lipids) were observed only at day 4, both supopulations reaching a similar composition at day 7 and onwards. Dietary n-3 FA can thus be used as an experimental tool to obtain liver PP and PV cells enriched in different types of FA in relatively short times (not more than 10 days). These cells can be useful models in which to study how dietary pressure affects biotransformation processes.

74.

INTERACTION BETWEEN PROSTAGLANDIN E_2 (PGE_2) AND NITRIC OXIDE (NO) IN RAT ADRENAL GLAND

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In a previous study we found that five days of alcohol (EtOH) (3g/kg) administration twice a day decreased cyclooxygenase (COX) and nitric oxide synthase (NOS) activities in adrenal gland (AG) of the rat. Presently we studied the effect of NO using sodium nitroprusside (NP) as donor of NO on PGE_2 and corticosterone (B) release from AG of adult male rats in a static incubation system in vitro. NP (600uM) increased highly significantly PGE_2 ($p<0.001$) and B ($p<0.01$) release into the media. Addition of EtOH (100mM) inhibited PGE_2 release ($p<0.01$) but did not block NP stimulated PGE_2 release. On the other hand, incubation of AG with PGE_2 (10^{-7}M) did not modify NO production (measured as NOS activity by ^{14}C-citrulline method) and inhibited B secretion ($p<0.001$). These results indicate that: 1) NO has a stimulatory action on COX, 2) PGE_2 does not affect NOS activity. Furthermore, NO and PGE_2 play a role in the intra-adrenal regulation of steroid release probably by a paracrine pathway. (Pict 99 #5-6117 & Beca Carrillo-Oñativia).

75.

REACTIONS OF NIFURTIMOX WITH CRITICAL THIOL-CONTAINING BIOMOLECULES. POTENTIAL TOXICOLOGICAL AND PHARMACOLOGICAL RELEVANCE

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Nifurtimox (Nfx), a chemotherapeutic agent in the treatment of Chagas' disease, has shown toxic side effects in clinical use. Reaction of the drug with thiol-containing biomolecules was analyzed *in vitro.* Glutathione (GSH), cysteine (RSH), lipoic acid (LA) and coenzyme A (CoA) produced nitrite formation from Nfx that was followed spectrophotometrically. Nfx concentration and metabolites formed in the reactions were determined by HPLC using a DAD detector. Reaction rates were CoA>LA≥GSH>RSH. Nitrite formation increased and Nfx concentration decreased simultaneously in the presence of GSH and RSH. GSH transferase activity measured by nitrite formation was not observed in the hepatic cytosol of SD rats. On the contrary, nitrite was determined in urine of SD rats as well as Nfx and two more polar metabolites. Nfx reactivity with thiol-containing molecules could be related not only to the undesirable side effects of the drug but also to the therapeutic action against *Trypanosama cruzi.* Besides that, GSH could exert a crucial Nfx detoxifying role in patients.

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76.

GENOTOXIC EFFECTS OF LONG - TERM EXPOSURE TO CADMIUM ON MURINE HEMOPOIESIS

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Cadmium is a toxic metal for all organisms. The aim of this study is to evaluate genotoxic effect of cadmium chloride (Cl_2 Cd) on hemopoiesis along 8 weeks. CF-1 mice were injected ip (28 mg/kg/day). Clonogenic assays on bone marrow (BM) and spleen (SP) progenitors were performed at 0, 6, 12, 21, 35, 42, and 62 days. Total and differential cellularities (erythroid-myeloid-lymphoid) were determined in both compartments. Erythroid lineage was mainly affected. A drastic anemia was noticed by the end of the experience. General chronic toxicity was evident in the loss of body weight along the experience. Total and differential BM and SP cellularites were affected. BM total cell counts decreased with a concomitant increment of apoptosis on day 62. Mean while spleen failed to show significative changes by the end of the experience. Early erythroid burst (BFU-E), granulocyte-erythroid-macrophage (GEM) and granulocyte-macrophage progenitor (GM) showed differential sensitivity. GEM decreased on the 3 and 5 weeks in BM, while SP mixed progenitors enhanced in the first week of Cd administration. This fact reveals progenitors mobilization from BM to SP in response to injury. GM progenitors showed low sensitivity to Cd in both tissues. These results suggest that Cd induces deep perturbations in hemopoiesis probably due by induction of microenvironmental inhibitory cytokines production

77.

COMPARISON OF THE CHROMATOGRAPHIC PROFILES OF *PHILODRYAS BARONII* AND *PHILODRYAS PATAGONIENSIS* VENOMS

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The salival secretion of colubrid snakes belonging to the genus Philodryas, considered as not poisonous, has caused serious injuries. Fibrin(ogen)olytic enzymes, which are responsible of tissue injuries and hemostatic alterations in bitten individuals, have been isolated from *P. olfersii* venom. This work presents a preliminary study about *P. baroni* and *patagoniensis* venoms, which inhabit Argentina and whose secretions have not been studied yet. The profiles of elution are compared by ion exchange chromatography (DEAE-celulose equilibrated and eluted with 10 mM buffer Tris-HCl, pH=7,5). The eluted proteins were followed by the measurement of A_{280}, and the proteolytic activity was measured on casein. Crude venoms of both epecies showed a strong proteolytic activity (*P. patagoniensis*=253 U/mg; *P. baroni*=88 U/mg). The elution profile of *P. patagoniensis* venom showed a peak corresponding to unbound proteins (basic proteins) with proteolytic activity; and three peaks corresponding to fractions that eluted after raising the ionic strength of the elution buffer, of wich only the most acidic peak did not show proteolytic activity. The elution profile of *P. baronii* venom showed a peak corresponding to unbound proteins (basic proteins) with proteolytic activity; and two peaks corresponding to fractions that eluted after raising the ionic strength of the elution buffer, wich did not show proteolytic activity. From this comparative study, we can make evident that both venoms differ in their compositions; detecting proteolytic enzymes, which award toxicity to these venoms.

78.

EFFECT OF ACACIA VISCO METHANOLIC EXTRACTS ON CHRONIC INFLAMMATION AND LYMPHOID ORGANS. PHITOCHEMICAL STUDY

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The effect on the chronic inflammation, the corporal, spleen and thymus weights and the spleen cell number, in both *Acasia visco (A.v.)* leaft and bark methanolic extracts were studied. Bioactive compounds were investigated. Wistar rats were divided into groups: normal, inflammation control, reference (dexametasone) and experimental (200 mg/kg *A.v*. methanolic extracts). Granulome with cotton pellet was induced (Meier *et al*.) and the treatment was given (s.c.) for 6 days. Granulome, spleen, thymus and corporal weights and spleen cell number were assessed. Active compounds were identified using liquid gas chromatography and mass spectrometry. Both *A. v.* leaf and bark methanolic extracts reduced granuloma weight ($p<1.8 \times 10^{-7}$ and $p<0.02$, respectively) without modify the corporal, spleen and thymus weights. *A.v*. bark methanolic extract decreased the spleen cell number. Triterpens: lupeol, alpha-amyrin and beta-amyrin were indentified. Conclusions: both *A.v.* leaft and bark methanolic extracts showed anti-inflammatory activity without influence on corporal and lymphoid organ weights. This anti-inflammatory effect would be attributed in part to lupeol, alpha-amyrin and beta -amyrin.

79.

EFFECT OF CHRONIC INHALED SALBUTAMOL ON PULMONARY SURFACTANT AND LUNG ALVEOLAR MACROPHAGES OF MALE RATS

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Introduction. Previous studies in our laboratory have shown that inhaled salbutamol in high doses modifies the lipid and the phospholipid content of the pulmonary surfactant (PS) and alveolar macrophages (AM). **Objective**. The purpose of this study was to evaluate the possible existence of a dose-response of inhaled salbutamol on these biochemical effects. **Methods**. Adult Wistar male rats were used that were arranged into four groups: (I) inhalated with 1.3 mM salbutamol (low dose); (II) inhaled with 13 mM salbutamol (therapeutic dose); (III) inhaled with 130 mM salbutamol (high dose) and (IV) inhalated with saline (Control), for 5 min., twice/day during 15 days. After treatment, all rats were killed and protein and lipid content was biochemically evaluated. **Results.** A significant increase was observed in PS total lipids at all three doses used compared to control: (I) 28,4 ±2,1mg; (II) 30,4 ± 0,5mg; (III) 33,2 ±1,5 mg Vs 13,23± 2,4.mg ($p <0,001$). PS protein content was also modified. (I) 3.07± 0,4 mg/ml; (II) 2,52 ± 0,2 mg/ml; (III) 2,75 ± 0,5 mg/ml Vs 1,45 ± 0,1 mg/ml ($p <0,05$). However, protein AM was not affected. (I) 12,0 ± 0,5 mg/ml; (II) 12,95 ± 1,1 mg/ml; (III) 14,54 ± 1,7 mg/ml Vs 13,0 ± 1,3 mg/ml (n.s.). **Conclusions.** Present data suggest a net effect of inhaled salbutamol on lipid and protein content of the pulmonary surfactant; this effect did not follow a clear dose-response relationship.

80.

APPLICATION OF THE DYNAMIC SPECKLE TECHNOLOGY TO EVALUATE DRUG EFFECTS ON PARASITE MOTILITY UNDER *EX VIVO* CONDITIONS

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Testing drug effects on target parasites under *ex vivo* conditions is a useful tool to assess the pharmacological activity of anthelmintic drugs. The speckle technique is referred to an interference image that arises when a rough surface is illuminated by a coherent light source, such as a laser. Dynamic speckle is a related phenomenon occurring when laser light is scattered by objects showing some type of activity or movement (i.e. parasites). The change in the image is analyzed through an algorithm that quantifies the velocity of change of the speckle diagram image. The research goal of this work was to characterize the *ex vivo* anthelmintic activity of different antiparasitic molecules using dynamic speckle. Infective third-stage larvae (L_3) of the nematode *Haemonchus contortus* were incubated with the following anthelmintic drugs: albendazole, levamisole and ivermectin at different concentrations for different time periods. The application of this technology allowed the detection of differences in L_3 motility according to the drug treatment and time of parasite exposure to different drugs. Although further work is required to adjust different technical procedures, the results obtained are highly encouraging.

81

TRICLABENDAZOLE INTERACTION WITH *Fasciola hepatica CYTOSOLIC PROTEINS*

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The anthelmintic activity of the benzimidazole compounds (BZD) depends on the prolonged presence of the drug at its site of action, the microtubules. Despite the significant amount of available knowledge in its pharmacokinetic behaviour and tissue distribution, the mode of action of triclabendazole (TCBZ) has still not been fully elucidated. TCBZ has greater flukicidal activity than albendazole (ABZ), one of the most used BZD for treating other helminth parasites although with less efficacy against this trematode. The present work was aimed to developunderstanding in certain molecular aspects related with the mechanism of action of TCBZ in adult stages of *F. hepatica*. Cytosolic proteins of *F. hepatica* were incubated with TCBZ in the presence or absence of different concentrations of ABZ. By using gel filtration columns (Sephadex G25), free fractions of TCBZ and ABZ were separated from those bound to proteins and quantified by HPLC. Although ABZ concentrations in the incubation media were raised more than 3-fold, results showed that TCBZ was not significantly displaced from its binding site, indicating that tubulin would not be the only target protein for TCBZ in the trematode. These results are a further step to understand the differential pharmacological activity of these benzimidazole drugs against helminth parasites. The search for alternative TCBZ target proteins in liver flukes may be important to fully understand its mechanism of action.

83.

EFFECT OF AN AISLATED TOXIN FROM *CROTALUS DURISSUS TERRIFICUS* SNAKE VENOM, CALLED GIROXIN, ON CENTRAL NERVOUS SYSTEM OF NEONATE RATS

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The *Crotalus durissus terrificus* snake venom contents several toxins, among them giroxin. Previous results of our laboratory showed that this toxin causes modifications on the profiles and contens of gangliosides on differents exposed neonates rat brain areas. The present work consisted in analizing the behaviour of neonates rats that were exposed to subacute intoxication with this toxin. After that, the same animals were sacrificed in order to carry out histopathological study of the brain. Results showed neurobehavioral alterations in the evaluations of reflex responses and motor activity test, however, it did not happen in muscular force test. The histopathological study showed degeneratives injuries belonging to the vacuolar type in cerebellum, prefrontalcortex and striatum tissue. Our results demonstrated evident injuries on different brain areas, compatible with behavioral alterations caused by the intoxication. How there is not any bibliographical reference about the giroxin effect on Central Nervous System, these results could to be useful as support to subsequent studies in neurotoxicology area.

82.

OXIDATIVE STRESS GENERATED BY DIESEL ON THE DIGESTIVE GLAND OF THE ANTARCTIC LIMPET *Nacella concina*

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The limpet *Nacella concina* is a gastropod mollusc widespread along Antarctic coasts. Due to its intertidal-subtidal habitat, this species is particularly accessible to floating organic pollutants. Hydrocarbons can penetrate into the organism by diffusion across the body surface and through the digestive system, since limpets feed on algae that are easily reached by floating pollutants. We investigated the possible effects of a fuel commonly used in Antarctica (diesel) on the activity of antioxidant enzymes and oxidative damage on the digestive gland of the limpet *Nacella concina,* as possible biomarkers for hydrocarbon pollution in Antarctic coasts. Three groups of 45 individuals each were kept in seawater containing 0, 0.05 or 0.1% diesel. Superoxide dismutase, catalase, glutathion S transferase and glutathion peroxidase (GPx) activities as well as lipid peroxidation and protein oxidation were studied in 15 animals of each group after 24, 48 and 168h of exposure. There was a general trend to increased enzyme activity with increasing doses of diesel. GPx showed the clearest effect, with significantly higher activity in both treated groups than in controls, it was also significantly higher in the 0.1% than in the 0.05% group. Both lipid peroxidation and protein oxidation were significantly increased by diesel after 168h, being both higher in the group exposed to the lowest dose.

84.

STUDY I*N VITRO* OF THE METALLOPROTEINASE ACTION AND PHOSPHHOLIPASE-A2 OF SNAKE VENOM IN THE EQUINE LAMINITIS

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Utilizing explants of equine foot as an experimental model, metaloproteinase and phospholipase A2 actions on tissue Laminitis of equine foot were studied. Venom of *Bothrops alternatus* snake was used to isolate basic metaloproteinasas for Ion-Interchange Chromatography was carried out (DEAE-cellulose column equilibrated and eluted with Tris-HCl 0.01M, pH=7.5 buffer). PLA2 was isolated by filtration trhough Sephadex-G75 at pH 4.5, filtration through Sephadex G-25 at pH 7.6 and cromatography on DEAE-cellulose at pH 7.6.Different concentrations of whole venom, metaloproteinase and PLA2 were inoculated in the explants of normal equine foot using broth cultures of Dulbecco·s Modified Eagle·s Media (D-MEM) and incubated anaerobically at 37°C, 5% CO2 and 95% relative humidity for 48 h. Explants were pos-fixed in 10% paraformaldehyde, processed for histopathology and stained histochemically with haematoxylin-eosin and periodic acid-Schiff (PAS). The optic microscopy revealed an extensive separation of the basement membrane from the epidermal cells with metaloproteinase and whole venom. In contrast, the lesions were slight with PLA2. We conclude that the equine laminitis induced by venom from *Bothrops alternatus* snake is caused by basic metaloproteinases present in this venom.

85.

EFFECT OF DEHYDROEPIANDROSTERONE (DHEA) ON CA^{2+} MOBILIZATION AND HORMONAL SECRETION OF NORMAL PITUITARY CELLS *IN VITRO*

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In a previous work we showed that DHEA *in vivo* was able to partially reverse DES-induced pituitary hyperplasia, hyperprolactinemia and loss of body weight. The aim of this study was to determine the effects of DHEA on normal pituitary cells *in vitro.* Pituitary cells of 60-day old female rats were cultured and stimulated with DHEA, OH-DHEA, androstenediol, dopamine (DA) and GHRH. After 48h prolactin (PRL), growth hormone (GH) and luteinizing hormone (LH) secretion was measured by RIA. The intracellular Ca^{2+} mobilization in a suspension of cells stimulated with DA or GHRH in presence or absence of DHEA was also determined by the Fura-2 fluorometric method. DHEA was able to significanlty reduce the basal PRL secretion (31 $\pm$8% for DHEA 10^{-7} M), but also to reverse in a dose-dependent manner the PRL inhibition induced by DA. Accordingly, DHEA blocked the decrease in intracellular Ca^{2+} mobilization achieved by DA. DHEA 10^{-7} M increased GH secretion induced by GHRH (in a 34 $\pm$13%), although this effect was not related with the intracellular Ca^{2+} release induced by GHRH. We conclude that DHEA has direct *in vitro* effects that correspond with some of its endocrine actions *in vivo.*

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86.

EFFECT OF MELATONIN INCUBATION ON LIVER OXYGEN CONSUMPTION DURING RAPID RESPIRATION

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In previous studies we showed that, 1), pretreatment of rats with melatonin (Mel) during 45 days, reduced oxygen consumption (VO_2) in liver mitochondria, 2), this effect was abolished when the administration of Mel was suspended 15 days before sacrifice, and 3), the incubation of liver specimens in the presence of Mel (10^{-3} to 10^{-9} M) during a dose-response curve using succinate (SU) or beta-hydroxybutyrate (HB) as substrates also disminished VO_2. In the present work we studied the effects of incubating liver specimens (obtained from 5 months old untreated rats) in the presence of Mel (10^{-3} to 10^{-9} M) during a cumulative dose-response curve. Liver mitochondrial VO_2 was measured polarigraphically (Gilson Medical Electronics oxygraph) at 30 °C using a Clark electrode. SU, malate (MA) and HB were used as substrates. VO_2 was measured in state 4 (without ADP) and state 3 (with ADP 0.82 mM). Results were expressed as ng atoms $O_2/min^{-1}/mg$ mitochondrial $protein^{-1}$. Mel (10^{-3} to 10^{-7} M) reduced state 3 liver O_2. Percentage inhibition using SU, MA, HB were: 10^{-3} M Mel (41,3$\pm$ 2,69; 48,5 $\pm$ 2,8; 27 $\pm$ 3,4), 10^{-4} M Mel (52,3 $\pm$ 2,02; 55,5 $\pm$ 1,94; 34,83 $\pm$ 1,99), 10^{-5} M Mel (66,5 $\pm$ 3,67; 74,26 $\pm$ 2,5; 44,67 $\pm$ 2,87) and 10^{-6} M Mel (82,25 $\pm$ 1,38; 88,25 $\pm$ 2,14; 55,6 $\pm$ 5,35) ($p < 0.01$). At 10^{-7} M Mel (99,75 $\pm$ 3,57; 100,5 $\pm$ 2,9; 67,1 $\pm$ 1,02), VO_2 was also lower ($p < 0.05$). Respiratory control ratio (State 3/State 4) was significantly reduced in the presence of 10^{-3} M - 10^{-6} M Mel. These results demonstrate that Mel effectively reduces state 3 VO_2 of liver mitochondria and suggests a direct inhibitory effect of pro-oxidative phenomena by the pineal methoxyindole.

87.

STUDY OF DEHYDROLEUCODINE ON THEIR ANTI-INFLAMMATORY ACTIVITY USING THE TRANSCRIPTION FACTOR NF-κB AS TARGET

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Dehydroleucodine (DhL), a sesquiterpene lactone (SQL) of the guaianolide type isolated from *Artemisia douglasiana* Besser, prevents damage in ulcerative colitis in rats. Moreover, DhL have an important anti-inflammatory effect in adjuvant-carragenin and cotton pellet tests. Extracts from Mexican medicinal plants used in traditional indigenous medicine for the treatment of inflammations contain SQL, specifically inhibit the transcription factor NF-κB. To obtain information regarding the molecular targets which might be affected by DhL, an *in vitro* bioassay was performed: DNA binding activity of the transcription factor NF-κB was evaluated by electrophoretic mobility shift assay (EMSA). HeLa cells were pre-incubated with DhL at a concentration of 5 μg/ml. The following oligonucleotides were used: NF-κB, 5′-TTAGTTGAGGGGACTTTCCCAGGA-3′ and 5′-TTGCCTGGG AAAGTCCCCTCAACT-3′. Band-shift experiments with total cell proteins revealed that DhL prevent NF-κB induction. Several SQL have the structure which can form covalent bond with biological functional group of proteins (Schmidt *et al.*, 1997) and Lyss *et al.* (1997, 1998) reported that a SQL alkylates cystein residue of NF-κB p65 unit. We may postulate that DhL has also alkylating activity with α-methylen-γ-lactone group essential for this activity. The exact mechanism of DhL inhibition of NF-κB activation is needed to be further studied.

88.

INHIBITORY EFFECT OF DEHYDROLEUCODINE ON INTESTINE *IN VIVO* AND *IN VITRO*

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Dehydroleucodine (DhL), isolated from *Artemisia douglasiana* Besser, reduced the damage in experimental colitis, accompanied by significant decreases in diarrhea. Based on decreases in diarrhea and its use in traditional folk medicine, we decided to study the inhibitory activity of DhL on intestine. Small intestinal transit: DhL (60-80 mg/kg) inhibits small intestinal motility in mice. The α_2-adrenergic receptor antagonist yohimbine and phentolamine effectively antagonized the effect of DhL ($p<0.01$). Prazosin, atropine and naloxone failed to modify the effect of DhL on intestinal transit. Stimulated intestinal transit: the inhibitory action of DhL was produced in rats. DhL reduces the intestinal transit in castrol oil treated rats, with respect to the control group treated with castrol oil only ($p<0.001$). Inhibitory effect on intestine *in vitro*: when DhL was added to the organ bath containing a segment of rabbit jejunum, it inhibited the spontaneous contractions. This inhibition was dose-dependent in the range 0.025-0.6 mg/ml. It is probably due to the action of α_2 adrenergic receptor. The present results suggest therefore that DhL produces an inhibitory action on intestinal function, and may have some antispasmodic property. DhL could represent an useful tool in relieving gastrointestinal colic, diarrhea and/or other gastrointestinal disorders.

89.

ANTI-ULCEROUS ACTIVITY AND EFECT ON GASTROINTESTINAL TRACT OF *ACACIA VISCO* EXTRACTS

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The gastric anti-ulcerous activity and effect on normal defecation of *Acacia visco* (*A.v.)* extracts were studied. Wistar rats were given (p.o.): saline (normal and ulcer control groups) and 25, 50, 100 or 200 mg/Kg of *A.v*. leaf or bark methanolic extracts (experimental groups). After 1 hour, absolute ethanol was administrated to experimental and ulcer control groups. The ulcer grade was evaluated after 1 hour according to Marazzi Uberti and Turba scale and expressed as ulcer index (UI). Normal defecation was study in mice by administration p.o. of: saline (control), Loperamida (reference), 400 mg/kg *A.v*. leaf, bark or seed aqueous extracts or 200 mg/Kg of *A.v*. leaf or bark methanolic extracts (experimental groups). The number, weight and humidity of faeces were scored. The *A.v*. leaf methanolic extract UI compared with ulcer control (UI=4.6) were: 3.5 ($p<0.02$), 2.0 ($p<0.003$), 0.7 ($p<0.0001$) and 0.025 ($p<5x10^{-6}$). The *A.v.* bark methanolic extract UI were: 4.0 (n.s.) , 4.1 (n.s.), 2.9 ($p<0.04$) and 1.6 ($p<0.01$). *A.v.* leaft and bark methanolic extracts reduced the number and weight of faeces. Conclusions**:** *A.v.* methanolic extracts exhibited a dose-depend anti-ulcerous activity with higher effect in leaf extract. Both extracts decreased the gastrointestinal transit.

90.

HEMOPOIETIC RECOVERY POST-PACLITAXEL: ERYHTROID PROTEIN EXPRESSIONS AND APOPTOTIC PROFILES

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Paclitaxel (Px) became an important agent against human cancers. Its properties may be explained not only to microtubules stabilization but also by its effects on gene expression. Cytotoxic injury causes deeply alterations on hemopoietic progenitors. Erythropoietic restitution involves changes of critical proteins, such as c-myb and GATA-1. c-Myb activates immature genes and suppresses terminal differentiation of several lineages.GATA-1 is crucial for erythroid differentiation. It inhibits proliferation, suppresses *gata*-2 and c-*myb* expressions and promotes survival of red blood cells. The aim of this study is to correlate changes in c-Myb and GATA-1 expressions with apoptotic variations post-Px single injection (29 mg/Kg, i.p.). Samples were taken at 0, 24, 48, 72, 96 and 120 hs, determining apoptotic indexes by TUNEL assays, transcription factors expressions by inmunoblottings and total bone marrow and differential cellularities by standard methods. We observed that erythroid restoration involves increased expressions of c-Myb and GATA-1 at 24 hs while myeloid recovery requires maintained expression of both till 4 days post Px. Apoptotic indexes and erythroid transcription factors expressions were maximal at 24 hs. Experimental data suggest that drug injury causes lineage differential recovery profiles and that survival cells increased transcription factors expressions despite of cellular depletion.

91.

THYROXINE STIMULATES NORMAL AND TUMOR T LYMPHOCYTE PROLIFERATION VIA DIFFERENT PATHWAYS INVOLVING PROTEIN QUINASE C ISOENZYMES AND NITRIC OXIDE SYNTHASE

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Previously, we have shown a different protein kinase C (PKC) isoform pattern and a high activity of nitric oxide synthase (NOS) in a T lymphoma cell line BW5147 (BW) respect to normal T cells. Regulation of immune responses by thyroid hormones was demonstrated. In this work, the effects of thyroxine (T_4) upon normal and tumor lymphocyte activities were studied. T_4 stimulated both, mitogen-activated normal and tumor T cells proliferation in a doses-dependent manner, measured by [^{3}H]-thymidine incorporation. After 24-72 hs of culture in the presence of T_4 a rise in total PKC content accompanied by an increased membrane associated PKC activity was observed on both cell types. This was accompanied by an increase in the atypical, Ca^{2+}- independent PKCζ or in the classical PKC isoforms on BW and mitogen-stimulated normal T cells, respectively, as assessed by Western blot. T_4 augmented NOS activity in BW, but not in mitogen-stimulated normal T cells, as determined by [^{14}C]-citrulline production from [^{14}C]-arginine. We conclude that T_4 modulates proliferation of both normal and tumor T lymphocyte through PKC activity, but involving different PKC isoenzymes and stimulating NOS activity only on BW cells. This points to a differential effect of T4 on normal and tumor T cell activation.

92.

EFFECT OF *TILIA CORDATA* MILL. AQUEOUS EXTRACT ON TUMORAL AND NORMAL LYMPHOCYTES PROLIFERATION

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Aqueous extract (AE) obtained from flowers of Tilia species have been used for years as antiaxient and psicological depressor and are used for colds, chills and bronchitis. Cientifics studies dealed with flavonoids which are able to interact with benzodiacepine receptors (BZR). In a previous work, low concentrations of Tilia extract (20 μg/ml) exerted stimulatory action on normal lymphocyte (NL) proliferation interacting with peripheral type BZR. In this work, we propose 1) to analyze the effect of an AE of Tilia on proliferation of: a lymphoma cell line BW 5147 and on BALB/c male mice normal Con A stimulated lymphocytes (CSL) 2) to study the viability on CSL and NL 3) To analyze the participation of peripheral BZR on the action of AE. The action on proliferation was determined by tritiated thymidine uptake and trypan blue exclusion methods (X ± SEM of three experiments) in presence or ausence of a BZR antagonist PK1111 10^{-6} M. Proliferation: tumoral cells: EC_{50} : 5000 ± 150 μg/ml; +PK1111 EC_{50} : 4800 ± 120 μg/ml; CSL: EC_{50}: 600 ± 20 μg/ml; + PK1111: EC_{50}: 580 ± 10 μg/ml. Cell viability: control: 80% ± 6; NL: 78% ± 2; CSL: 75% ± 5. We can conclude that, the AE of Tilia cordata had antiproliferative action on tumoral and on Con A stimulated lymphocytes without affecting normal cell viability. These effects were not mediated by BZR

93.

INHIBITION OF *TRYPANOSOMA CRUZI* GROWTH IN VITRO BY TWO *BOSWELIA* SPECIES

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Due to the absence of an efficient and safe chemotherapy for Chagas disease, new compounds are urgently needed. This work assessed the activity of gum resins obtained from *B. serrata* and *B.carterii* on two stages of *T.cruzi*: A) Epimastigotes (clon Bra C15C2, $5x10^5$ cells) were cultured in F-29 medium at 27°C in the presence of the two extracts (5-50 µg/ml). After 72 hours the parasites were isolated and counted in a Neubauer chamber. Allopurinol was used as reference drug. Both, *B. carterii* and *B. serrata* inhibited the growth in a dose-dependent manner. The IC50 values were 21.4 µg/ml and 9.9 µg/ml respectively. B) Trypomastigotes (clon H510C8C3) obtained from blood of infected CF1 mice were cultured with blood from healthy mice to a final concentration of $1x10^7$ parasites/ml and they were incubated 24 hours at 4°C in the presence of 250 µg/ml of both resins. At the end of the incubation period, the presence of the parasite was determined and the treated blood was injected to healthy mice. After 19 days postinfection, analysis of blood samples obtained from these mice revealed the presence of trypomastigotes. Thus these extracts, in the concentrations used, inhibit the proliferative stage of *T. cruzi* whereas it has no effect on the invasive stage of the parasite.

94.

SPECIFIC ANTIBODIES ANTI-GYROXIN PRODUCTION IN RABBITS-PRELIMINARIES STUDIES

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Gyroxin, a toxin of *Crotalus durissus terrificus* venom, was isolated and used for the production of antibodies in rabbits. Antigens diluted in buffer PBS with Tween 20, in which was added the adyuvante (INTA), was used for the vaccine preparation. The immunization was carried out in rabbits of the genus *Oryctolagus cuniculus*, which received 2 ml of vaccine distributed in different body points (i.m. in both paw and 4 applications s.c. in different loin points). Three booster separated for a period of 2 weeks and 2 months respectively, were carried out (after the first and second booster antibodies were not obtained, however, after two weeks of third booster they could be obtained). Blood samples were extracted of outer ear marginal vein. Serums of all inoculated rabbits showed an increase of immunoglobulin, it was detected through the bands of similar quality and intensity in γ region obtained by electrophoresis in cellulose acetate. Immunoprecipitation in agar gel tests are being used to establish the antibodies titer.

95.

GASTROINTESTINAL ACTIVITY OF *BACCHARIS POLIFOLIA* GRISEB. IN RATS AND MICE

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Baccharis polifolia Griseb. ("Quincha mali"), placed in the Cuyo region, has been used in folk medicine for gastrointestinal disorders. This work aimed at evaluating the gastrointestinal activity of several extracts obtained from *B. polifolia* in rats and mice. Plant material was deffated using n-hexane twice. The solvent was evaporated and the material was extracted twice with boiling EtOH. After several liquid-liquid fractionation three main extracts were recovered, namely "Residue I", "Residue S", and "Residue EtOH". Gastric lesions were produced according to the method of Robert et. al. (1979). The results were expresed in terms of an Ulcer Index (UI) from 0 to 5 (maximal damage). Only Residue EtOH prevents the formation of gastric lesions induced by absolute ethanol (UI: 1.30 ± 0.20, $p<0.001$ *vs.* damage control). Intestinal transit was measured by method of Ueda et. al. (1969). Residues I and S don't modify the transit of charcoal. Residue EtOH decreased small intestinal transit in mice.
Conclusion: The Residue EtOH of *Baccharis polifolia* reduces the intestinal transit in mice and prevents ethanol induced gastric damage in rats. These facts support the use in traditional medicine of *Baccharis polifolia* to treat digestive disorders.

96.

EVALUATION OF THE USE OF CIDR FOR THE "RESCUE" OF LATE CALVING COWS: EFFECT ON PREGNANCY RATE AND LEPTIN LEVELS

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In the sub-humid areas of Argentina the length of calving season is usually 3 months. The cows that calf on the last 30 days are expected not to reach acceptable pregnancy rates on the new breeding season (December 1 to March 1). The objective of the present experiment was to evaluate if progesterone-estradiol priming with the application of a CIDR device plus an estradiol-benzoate injection improve these results. Sixty-seven Aberdeen Angus x Hereford multiparous cows that calved from November 6 to December 6 were identified by rectal palpation as "non-cyclic" cows and body corporal scores (BCS) was determined. On day 40 from the last deliver the cows were bled and randomly assigned to one of two groups. Cows in group 1 received a CIDR device plus 2 mg of Estradiol benzoate, and cows in group 2 remained as untreated controls. After ten days CIDR were removed and all the cows were bled. Pregnancy was evaluated by rectal palpation on day 50 after the end of the breeding season.
Pregnancy rates were 50% for the control cows and 64% for the treated ones. When cows were classified by BCS the response to treatment was significant in BCS's 3 and 4 (scale 1 to 9) and not in BCS 2. Leptin values directly correlated with BCS in both groups but did not differ between treatments.
The use of CIDR plus Estradiol benzoate can be a useful tool for the rescue of late calving cows if they have a moderate BCS, but not in very thin animals.

97.

SOME PHARMACOKINETICS PARAMETERS OF FLURBIPROFEN IN DAIRY CATTLE

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Flurbiprofen (FBP) (racemic-2-[2-fluoro-4-biphenyl] propionic acid) is an asymmetric NSAID. The aim of this work was to study the pharmacokinetics of FBP in different age and physiological status in dairy cattle. Ten Holando Argentino bovines, were divided into three different groups: 3 cows in early lactation, 3 cows in gestacion and 4 newborn calves. All the animals received racemic FBP (50:50) at a dose of 0.5 mg/kg by intravenous administration. Blood samples were withdrawn at predetermined times after administration. Plasma enantiomer concentrations were measured by HPLC. ClB of S-(+)-FBP was higher in calves than in cows (74.95v30,95 - 29,16 ml-h/kg). MRT for R (-) enantiomer was higher for cows in early lactation than for cows in gestation (3 v 4.16 h). MRT for S-(+)-FBP was lower for calves than for cows in early lactation. T1/2 for R-(-)-FBP was higher for cows in early lactation than for other categories (2,76 v 2,05-2,10 h). In calves, the disposition kinetics was stereoselective. AUC (6.1 v 3.38µg-h/ml) and MRT (3.35 v 1.98 h) were higher for R-(-)-FBP than for S-(+)-FBP. ClB was lower for R- (-)-FBP than for S-(+)-FBP (46.25v74.95ml-h/kg). In this study, a different enantioselective metabolic behavior of FBP under the physiological situations studied has been clearly demonstrated.

98.

DEVELOPMENT OF AN HPLC ASSAY TO DETERMINE MOXIDECTIN RESIDUES IN MILK AND DAIRY PRODUCTS

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Moxidectin (MXD) is a potent antiparasitic compound active against a wide variety of nematode and arthropod parasites. Parasitism causes important losses in sheep and cattle dairy farms and the extra-label use of moxidectin, ivermectin and similar compounds is widespread. However, the presence of drug residues in dairy products derived to human consumption is an unresolved inconvenient and the availability of analytical techniques to valorate residual concentrations is a key issue. This work describes an analytical development addressed to validate a simple and rapid high performance liquid chromatographic (HPLC) method to quantify MXD residue concentrations in milk and dairy products (cheese). Drug-free milk and cheese samples were fortified with MXD in the concentration range between 0.1 and 100 $ng.g^{-1}$. Abamectin was used as internal standard. Liquid and solid-phase extraction procedures were used for sample preparation and clean-up. Calibration lines for MXD in the range of 0.1-10 and 10-100 $ng.g^{1}$ were plotted using the peak area ratios between MXD and the internal standard vs. analyte concentrations. The developed HPLC method allowed the quantification of MXD up to 0.1 $ng.g^{-1}$ in sheep milk and cheese with international accepted coefficient of variations (lower than 15%). Recovery values were higher than 70%. This HPLC method is reliable to determine the presence of residual concentrations of MXD in milk and cheese, which contributes to assure consumer´s safety.

99.

CHRONOBIOLOGICAL STUDY OF A KETAMINE-MIDAZOLAM COMBINATION PHARMACOLOGICAL RESPONSE IN DOGS

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Administration of ketamine in combination with midazolam is currently recommended for induction of general anaesthesia in small animal surgery. The aim of this study was to analyse the pharmacological response of a ketamine-midazolam combination injected at 11.00 and 23.00 h. in dogs. The experience was conducted in June 2002. Six Beagle adult dogs (2 females and 4 males) weighing 8.4 to 12.6 kg (mean ± SD 10.7 ± 1.7 kg) from the kennel of Facultad de Ciencias Veterinarias, UBA were administered intramuscularly ketamine ClH (10 mg/kg) and midazolam (0.5 mg/kg) at 11.00 and 23.00 h (local hour), with a washout period between administrations of 3 weeks. Duration of pharmacological response (minutes) was recorded by visual assessment starting from the administration of the combination (t0) as follows: duration of latency period (t0/beginning of ataxia), pre-recumbency ataxia (t0/recumbency), begining, duration and end of recumbency, post-recumbency ataxia (recovery of recumbency/recovery of normal gait), duration of total pharmacological response (t0/recovery of normal gait). No testing for analgesia was made. Duration of post-recumbency ataxia (minutes) was significantly lower ($p<0.05$) for the 11.00 h group (median 9.78, range 4.1-18.38) when compared with the 23.00 h group (median 19.7, range 10.93-27.7), as determined by means of the Wilcoxon matched pairs test. Results of this study indicate that time of administration may partially affect ketamine-midazolam pharmacological response in dogs.

100.

AGE-DEPENDENT CHANGES IN 24-HOUR RHYTHMS OF CATECHOLAMINE CONTENT AND TURNOVER IN HYPOTHALAMUS, CORPUS STRIATUM AND PITUITARY GLAND OF RATS INJECTED WITH FREUND'S ADJUVANT

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Little information is available on the circadian sequela of an immune challenge in the brain of aged rats. To assess them, we studied 24-h rhythms in hypothalamic and striatal norepinephrine (NE) content, hypothalamic and striatal dopamine (DA) turnover and hypophysial NE and DA content, in young (2 months) and aged (18-20 months) rats killed at 6 different time intervals, on day 18th after Freund's adjuvant or adjuvant's vehicle administration. Aging decreased anterior and medial hypothalamic NE content, medial and posterior hypothalamic DA turnover, and striatal NE concentration and DA turnover. Aging also decreased NE and DA content in pituitary neurointermediate lobe and augmented DA content in the anterior pituitary lobe. Immunization by Freund's adjuvant injection caused: (i) reduction of DA turnover in anterior hypothalamus and corpus striatum; (ii) acrophase delay of medial hypothalamic DA turnover in old rats, and of striatal NE content in young rats; (iii) abolition of 24-h rhythm in NE and DA content of neurointermediate pituitary lobe, and in DA content of anterior lobe, of old rats. The decline in catecholamine neurotransmission with aging could contribute to the decrease of gonadotropin and increase of prolactin release reported in similar groups of rats. Some circadian responses to immunization, e.g. suppression of 24-h rhythms of neurointermediate lobe NE and DA and of anterior lobe DA were seen only in aged rats.

101.

COMPARATIVE STUDY OF THE DAILY RHYTHMS OF AA-NAT, HIOMT AND MELATONIN IN PINEAL OF VISCACHA

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The aim of this work was to compare the daily rhythms of melatonin and its synthesis enzymes, AA-NAT and HIOMT, in pineal of viscacha (*Lagostomus maximus maximus*). Viscachas were sacrificed at 4, 8, 12, 16, 20 and 24 h (4-7 kg, n=5). The pineals were removed. The melatonin content, HIOMT and AA-NAT activities were determinated (Fraser *et al.*, 1983; Axelrod and Weissbach, 1961; and Champney *et al*., 1984, respectively). Results:

h	Melatonin (pg/mg tissue)	HIOMT (pmol ^{14}C-melatonin /mg tissue/30min)	AA-NAT (pmol N-acetilserotonin-^{4}C/mg tis/10min)
4	299.00 ± 70.37	1.63 ± 0.27	33.74 ± 4.27
8	449.36 ± 56.72	1.62 ± 0.26	39.54 ± 6.13
12	458.65 ± 55.65	1.70 ± 0.16	41.28 ± 7.50
16	157.39 ± 33.11	1.64 ± 0.40	21.49 ± 2.78
20	357.26 ± 36.77	2.00 ± 0.19	31.64 ± 5.56
24	720.45 ± 133.9	3.24 ± 0.29	111.5 ± 25.86
	ANOVA, p<0.0003	ANOVA, p<0.002	Kruskal-Wallis, p<0.006

AA-NAT activity and melatonin content exhibit a similar pattern with a nocturnal peak at 24 h and a diurnal increase, while HIOMT activity only shows the nocturnal peak at 24 h. Does AA-NAT have a more relevant role in the indol synthesis in pineal of viscacha as such in the most species?

102.

A MULTIFACTORIAL APPROACH EMPLOYING MELATONIN TO ACCELERATE RESYNCHRONIZATION OF SLEEP-WAKE CYCLE AFTER A 12 TIME-ZONE TRANSMERIDIAN FLIGHT IN ELITE SOCCER ATHLETES

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The aim of the present study was to test the timely use of melatonin treatment, exposure to light and physical exercise to hasten the resynchronization of a group of elite sports competitors and their coaches to a westerly transmeridian flight comprising 12 time zones. Twenty-two male subjects were included in the study. They were professional soccer players and their coaches who traveled to Tokyo to play the final game of the Intercontinental Coup. The day prior to departure, urine was collected from each subject from 18:00 h to 06:00 h to measure the melatonin metabolite 6-sulphatoxymelatonin. All subjects received 3 mg of melatonin p.o. daily at expected bedtime at Tokyo immediately after leaving Buenos Aires. Upon arrival at Tokyo the subjects performed a daily physical exercise routine outdoors at two restricted times of the day (08:00-11:00 h; 13:00-16:00 h). Exposure to sunlight or physical exercise at other times of the day was avoided. There was a general absence of significant changes in subjective sleep parameters as compared to pre-flight assessment. Sleep quality and morning alertness at Tokyo correlated significantly with pre-flight 6-sulphatoxymelatonin excretion. Mean resynchronization rate of sleep-wake cycle to the 12 h-time shift was 2.13 ± 0.88 days. The results underlines the efficacy of this treatment to help to overcome the consequences of jet-lag.

103.

MATERNAL BEHAVIOR DISRUPTED BY 2,4-DICHLORO PHENOXYACETIC ACID

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Pregnant rats were individually housed in plastic breeding cages in a controlled temperature (22-24°C) and on a 12-h light/dark cycle room. Three treated groups of rats were observed A) 2,4-D-treated dams exposed by diet to 50, 25 or 15 mg/kg/day of 2,4-D and B) Control dams fed with untreated rodents chow. Twenty-four hours following parturition, litters were examined and culled to eight pups. A special camera and a time-lapse video were used to record dams and pups behavior in the housing cage and in an arena. All tests were carried out under red light, starting at 5 p.m. and ending at 6 p.m. on PND 1, 3, 5 and 7. An analysis of maternal care - during 1/2 h - such as nest building, licking, mouthing, retrieval of the pups from the opposite end of the cage, grouping, crouching and time of in/out nest period was made. Dams exploring, self-grooming and frequency of resting position were also registered. Retrieval, sniffing and licking of pups were reduced and suspended after 2,4-D treatment. Dams did not cover their offsprings. 2,4-D treated mothers showed less frequency in resting position than control mothers; they have high movement along cage peripheries, showed high self-grooming and rearing, and their gait was on their tiptoes. 2,4-D exposure produced changes in parental behavior that would result in offspring maturation delay

104.

ATTENUATED *SALMONELLA ENTERICA* SEROVAR TYPHI (*S.* TYPHI) INVADES AND INDUCES CELL DEATH IN A T-CELL LYMPHOMA

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Salmonella is a facultative intracellular pathogen that offers several advantages to be considered a potential anticancer vaccine. They can grow under either aerobic or anaerobic conditions, such as those that occur within solid tumors; they express specialized systems for invasion and survival within both epithelial cells and macrophages. The uptake of *Salmonella* into non-professional phagocytes is an active process induced by the bacteria.
In this work we investigated the ability of attenuated *S.* Typhi to invade T-lymphoma cells in order to use it as an antitumor vector. Tumor cells were implanted subcutaneously into naive mice and ten days later 2 x 10^9 CFU of attenuated *S.* Thypi were inoculated intratumor. Animals were euthanized at different days and the tumors were removed. Several portions of the tumor were prepared for microscopic evaluation. Apoptosis assays were performed. The remainder of the tumor was homogenized and lysed to quantify intracellular bacteria. Our results showed that attenuated *S.* Typhi can invade and survive within tumor cells *in vivo*. Furthermore, this bacterial strain was able to induce tumor cell death by mechanisms of apoptosis and necrosis. These findings suggest that this attenuated *Salmonella* strain could be useful to induce inherent antitumor activity as well as to deliver *in vivo* therapeutic DNA or proteins to cancer cells.

105.

LONG-TERM EFFECTS OF NEONATAL TREATMENT WITH A GNRH AGONIST WITH 30 DAYS OF ACTION

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In rats, sexual maturation is critically dependant on adequate sexual steroids actions during the first 30 days of life. The aim of this study was to characterize the long-term effects of the treatment with a controlled release GnRH agonist upon sexual development. To this end, 1-day-old Sprague-Dawley female and male rats were injected sc with a single dose of a controlled-release formulation containing 0.085 mg of Leuprolide acetate (Takeda Chemical Japan) Animals were killed on day 70. In all animals, trunk blood and gonads were taken. Sera were store at -20°C until hormone assay by RIA. Female rats were observed daily after weaning for vaginal opening. Data are expressed as mean ± SEM. Statistically significant differences were determined by ANOVA and Tukey's test. Results: GnRH administration induced: i. a significant increase in serum LH in male rats (59.02±14.72 vs 25.01±1.42) and in female rats (41.92±7.3 vs 21.5±2.1) ii.a significant reduction in testicular wt (2.008±0.12 vs 3.022±0.12) and in ovarian wt (57.66±3.08 vs 75.58±2.38) iii. Testosterone concentrations significantly lower (334.6±26.34 vs 571.1±29.74) iiii. Delay in vaginal opening. Conclusions: The mechanisms of sexual maturation were disturbed during the first 30 days of life by the blockade of pituitary GnRH receptors in such a way that it was possible to observe the effects of the drug at 70 days old rats. It confirms that the disruption of the organizational action of sexual steroids is critical in normal sexual develpment.

106.

LEUPROLIDE DEPOT: PHARMACOLOGICAL EVALUATION BY GNRH RECEPTOR BLOCKADE OF PITUITARY GONADOTROPES IN MALE RATS

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GnRH is a hypothalamic decapeptide that plays a predominant role in gonadotropin regulation, particularly LH and hence gonadal function. In the present study we have analyzed the effects of a single dose administration of a GnRH agonist with long lasting action (30 days) in normal male rats on their hypothalamic - pituitary-gonadal axis, in order to investigate the best response to be applied in pharmacological comparative evaluation.
With this purpose, animals were injected sc with a single dose of a sustained-release formulation containing 0.085 mg of Leuprolide acetate (Takeda Chemical, Japan) and were sacrificed at 1,2,3 and 4 weeks after injection. Hypothalamic GnRH, pituitary response to natural GnRH, testicular weight and serum testosterone were evaluated. Results: GnRH agonist administration induced: 1) a significant decrease in hypothalamic GnRH at 2, 3 and 4 weeks 2) no pituitary response to natural GnRH at all the times considered 3) decrease in testicular weight during the 4 weeks and 4) decrease in serum testosterone levels at all the times considered. Conclusions: We were able to verify a direct blockade of pituitary GnRH receptors and a positive correlation with serum testosterone. Therefore, in order to evaluate the biological action of the drug we consider the first task to test the pituitary receptors blockade and secondarily to include the levels of serum testosterone.