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Posters

A METHODOLOGICAL STUDY BASED ON THE ki-67 IMMUNOREACTIVITY IN THE EPIDERMAL CELL. P1 1

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The immunohistochemical determination of the proliferating cells index (PCI) was carried out in normal epidermis, psoriatic lesions and epidermis close to neuroendocrine tumors of the skin. We have used monoclonal antibody MIB-1 raised againts ki-67 nuclear non-histone protein. This antigen is present in all cell cycle phases, but absent in Go. Most studies have shown that the reactive area of the ki-67 antigen in the nucleus increase when the cells enter the cell cycle.

Our findings, which demonstrate an increase of PCI in psoriatic lesions and in the epidermis close to neuroendocrine tumors, are in line with other reports as regards the value of the MIB-1 immunohistochemical evaluation in the study of epidermal cell kinetics.

EXPRESSION OF NCAM IN NERVOUS AND NEUROENDOCRINE TISSUES. P1 2

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The Neural Cell Adhesion Molecule (NCAM) is the best characterized CAM. NCAM exists in the three different isoforms of 120, 140 and 180 kDa.

We have studied the expression of NCAM in nerve and neuroendocrine cells in the skin.

For this immunohistochemical study we have used VC 1.1 monoclonal antibody (Sigma) on formalin fixed, paraffin embedded material. This antibody locates the 140 and 180 kDa transmembrane NCAM isoforms.

NCAM immunoreactivity was observed in those nerve fibers (myelinic and amyelinic) of the dermis and the hair follicles. Striated muscle cells were also immunoreactive to this molecule. The expression of NCAM was also found in the epidermal neuroendocrine cells and in neuroendocrine tumoral cells (Merkel cell carcinomas). We suggest the use of this antibody for the detection of normal and tumoral nerve and neuroendocrine tissue.

P1 3 ACTIVATION OF A_2 RECEPTORS BY ADENOSINE STIMULATES THE L-ARGININE/NITRIC OXIDE SIGNALLING PATHWAY IN HUMAN FETAL ENDOTHELIAL CELLS. L. Sobrevia & G. E. Mann. Vascular Biology Research Centre, Biomedical Sciences Division, King's College, Campden Hill Road, London W8 7AH (U.K.) & Cell. Molec. Physiol. Lab., Dept of Physiology, Faculty of Biological Sci., University of Concepción, (Chile). J. Physiol. Biochem. (Rev. esp. Fisiol.), **53** (1), 96, 1997.

Vasodilatation induced by adenosine is blocked by inhibitors of endothelial nitric oxide (NO) synthase. In this study we have investigated the effect of adenosine on L-arginine transport and NO synthesis in cultures of human endothelial cells. Adenosine and the A_2-agonist CGS 21680, but not the A_1-agonist N^b-cyclopentyladenosine, increased cGMP accumulation in a dose dependent manner. Basal and A_2-agonist-stimulated accumulation of cGMP was blocked by the NO synthase inhibitor N-nitro-L-arginine methylester and by the A_2-antagonists dimethylpropargylxanthine and ZM241385 (Zeneca). Adenosine and A_2-agonists also increased L-arginine transport and influx of the tetraphenylphosphonium and L-arginine transport. In conclusion, adenosine-induced increase in L-arginine transport and NO synthesis are mediated by activation of A_2-receptors in human fetal endothelium.

Supported by British Council, Wellcome Trust (UK), and Dirección de Investigación, University of Concepción (DIUC-Chile). We thank Zeneca Laboratories for supplying ZM241385.

P1 4 CERAMIDE INHIBITS EGF RECEPTOR AUTOPHOSPHORYLATION AND SIGNAL IN A431 AND NIH3T3. L. F. Fanjul, I. Glez-Robayna, I. Hernández, B. López, V. Morales, P. Santana and G. Gallardo. Dpto. Bioquímica y Fisiología, Facultad de Medicina, Universidad de Las Palmas de G.C. (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), **53** (1), 96, 1997.

Sphingolipid metabolites have been recently characterized as active components of a novel intracellular signaling pathway initiated in response to ligand-activated sphingomyelinases (SMases) distinguishable by its pH optima and cellular localization. Ceramide, the lipid moiety generated by activated neutral/membrane-bound or acidic/lysosomal-located SMases induce a variety of cellular responses which include cell growth, differentiation and apoptosis. In the present study we evaluated the effect of cell-permeant ceramides (N-acetyl ceramide) in two different cell lines overexpressing the EGF receptor: the human A431 epidermoid carcinoma cell line and a murine NIH3T3 derived clone (EGFR-T17). In both cell types, treatment with C6-cer did not affect the binding capacity of the EGF receptor, but prevented growth factor-induced tyrosine phosphorylation of the EGFR itself. The decreased tyrosine phosphorylation was accompanied by a partial reduction in growth factor-induced and phospholipase Cγ-mediated phosphatidylinositol-4,5 bisphosphate hydrolysis and inositol-1,4,5 trisphosphate generation. Cellular proliferation induced by EGF in EGFR-T17 cells, was also dose-dependently (10^{-7}-10^{-5} M) inhibited with C6-cer and a similar effect was observed on serum-induced proliferation of A431 cells.

REGULATION OF PROSTAGLANDIN BIOSYNTHESIS BY NITRIC OXIDE IN CULTURED OVARIAN GRANULOSA CELLS. P1 5

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It has been recently shown that *in vivo* treatment with L-NAME and other nitric oxide synthase (NOS) inhibitors suppresses ovulation in gonadotropin (hCG)-primed immature rats. In addition, the free NO radical also prevents apoptosis of ovarian granulosa cells (GC) and promotes the survival of cultured rat preovulatory follicles. We recently reported that interleukin-1β (IL1β), induces a time- and dose-dependent NOS synthase activity in cultured GC, an effect that was abrogated by L-NMA or the IL1β–receptor antagonist protein (IL1RA). In the present study we show that NO enhances IL1β-induced PGE_2 biosynthesis in untreated or gonadotropin (FSH)-stimulated granulosa cells, an effect that was specifically abrogated by NOS inhibitors or NO scavengers and reversed by NO generating agents. As demonstrated by RT-PCR analysis, the stimulatory effect of NO on PGE_2 biosynthesis in IL1β- and FSH-treated GC, was exerted by augmented cyclooxygenase-2 (COX-2) mRNA expression.

REGULATION OF ARACHIDONIC ACID RELEASE AND PROSTAGLANDIN FORMATION BY CELL-CELL ADHESIVE INTERACTIONS IN WOUND-REPAIR. P1 6

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Foetal calf serum (FCS) and platelet-derived growth factor (PDGF) induced arachidonate release in subconfluent murine 3T6 fibroblast cultures. However, the magnitude of this effect decreased significantly as cell culture became confluent.

Wound-injury of fibroblast monolayer initiates a repair process that is potentiated by FCS or DPGF and which restores the integrity of cell monolayer. In these experimental conditions, FCS and PDGF induced phospholipase A_2 activity and subsequent arachidonic acid mobilization and eicosanoids production in wounded fibroblast cultures. Finally, it is demonstrated that prostanoids, and specifically prostaglandin E_2, play an important role in cell proliferation induced by FCS and PDGF during wound repair.

This research was supported partially by the DGICYT (PB 94-0934).

P1 7 NUTRIENT-INDUCED DIADENOSINE-POLYPHOSPHATES IN THE PANCREATIC B-CELL. J. M. Rovira, C. Ripoll, J. Pintor, M. T. Miras-Portugal, B. Soria and F. Martín. Depto. Fisiología e Inst. de Neurociencias, Univ. Alicante, 03080 Alicante (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 98, 1997.

We have recently described that diadenosine polyphosphates are effective blockers of K_{ATP} channels in pancreatic B-cells. We have also demonstrated that diadenosine polyphosphates concentration in B-cell cytosol increases in the presence of stimulating levels of glucose. Both results suggest an active participation of diadenosine polyphosphates in insulin secretion. To further investigate the involvement of diadenosine polyphosphates in the secretory process, we have used HPLC techniques to quantify the concentration changes of diadenosine polyphosphates in diverse conditions that stimulate insulin secretion in pancreatic islets. Our results demonstrate that: a) diadenosine polyphosphates concentration increase is glucose dose-dependent; b) other nutrients, like 2-ketoisocaproate, enhance as well diadenosine polyphosphates concentration; c) diadenosine polyphosphates levels are not modified by cell depolarization, either with tolbutamide or potassium. These results suggest that the rise in diadenosine polyphosphates content in B-cell requires the metabolization of fuel secretagogues and that metabolic pathways independent from glycolysis can account for these changes in diadenosine polyphosphates concentrations.

P1 8 REGULATION OF PANCREATIC B-CELL ELECTRICAL ACTIVITY AND INSULIN RELEASE BY PHYSIOLOGICAL AMINO ACID CONCENTRATIONS. S. Bolea, J. A. G. Pertusa, F. Martín, J. V. Sánchez-Andrés and B. Soria. Depto. Fisiología e Inst. de Neurociencias. U. de Alicante, 03080 Alicante. (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 98, 1997.

The effects in the electrical activity and insulin release of a mixture of amino acids and glucose at concentrations found in fed (aa_{FD}) and fasted (aa_{FT}) animals were determined in freshly isolated mouse islets. Islets perifused with aa_{FD} mixture showed an oscillatory pattern of electrical activity in response to lower glucose concentrations (5 mmol/l) than islets perifused with the aa_{FT} mixture and with glucose (G) alone (10 mmol/l). The concentration response curve of the fraction of time of membrane potential in the active phase in aa_{FD} stimulated islets was found to be significantly left-shifted and had a smaller slope than that for G. Insulin release followed the same pattern. We have also found that leucine, isoleucine, alanine and arginine are mostly responsible for the effects observed. This effect was more pronounced in the second phase of insulin release and depended on intracellular calcium. These findings indicate that amino acids account for most of the left-shift in the concentration-response curve to glucose and that a reduction in the threshold for glucose-induced oscillatory electrical activity response and in the generation of calcium spikes accounts for triggering of insulin release at lower glucose concentrations.

CHOLESTEROL INCREASES NCEH ACTIVITY IN GH_4C_1 AND NIH3T3 CELLS. P1 9

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Neutral cholesterol ester hydrolase (NCEH) is the enzyme responsible for the degradation of cholesteryl esters (CE). The enzyme is activated by cAMP through PKA phosphorylation. The present experiments were carried out to determine the effect of cholesterol (Ch) cell content on enzyme activity in GH_4C_1 and NIH3T3 cells. We measured NCEH and PKA activities in cells cultivated in the presence of 10 % lipoprotein deficient serum (LPDS). Ch and CE levels decreased markedly by 24 h incubation. NCEH was decreased by 40 %, and was not stimulated by the addition of cAMP. However, the PKA activity measured with the addition of specific substrates was normal. The addition of Ch, or the HMGCoA inhibitor 25-HC to LPDS recovered NCEH activity and the stimulation by cAMP. These data indicate that, contrary to macrophages, in which Ch loading inhibits CEH activity and produces CE accumulation, Ch addition to Ch depleted GH_4C_1 and NIH3T3 cells up-regulates NCEH activity, probably to avoid CE accumulation.

IN VITRO CAPTURE, AMPLIFICATION AND INDUCTION OF OSTEOPROGENITOR CELLS FROM RAT BONE MARROW. P1 10

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In order to capture and expand a population of mesenchymal stem cells (MSC) with osteogenic potential, we have devised an *in vitro* culture system with a mesenchymal cell compatible collagen framework, containing a genetically engineered recombinant TGF-β1, fusion protein (rTGF-β1-F2).

Bone marrow cells were cultured inside the collagen I matrix during 16 days with 0.5 % and 10 % FBS. During the last 2 days, the cells were exposed to dexamethasone and β–glycerophosphate. At the end of the experimental period, samples were analyzed for DNA content, alkaline phosphatase activity and osteocalcin expression.

During the capture period, cells decreased in numbers. These cells were able to produce AP, but not osteocalcin. The rTGF-β1-F2 group showed higher cell number than commercial hTGF-β1, during the amplification and induction periods. At this time cells treated with rTGF-β1-F2 were able to form colonies, and after addition of inducers visible calcium deposition around cell nodules could be detected.

Here we report the first demonstration that a population of cells present in rat bone marrow, with many characteristics of osteoprogenitor MSCs, can be selected, expanded within a collagen matrix by modified GFs, and induced *in vitro*. This bioactive osteoinductive-conductive matrix, combined with the targeted delivery of fusion proteins, should be able to stimulate osteogenesis at sites of bone repair or remodelling.

This work was supported by grants PB95/1134 (DGICYT, Spain) and AG02577 (NIH, USA).

P1 11 FUNCTIONAL AND METABOLIC STATUS OF ERYTRHOCYTES FROM SHED AND BANKED BLOOD. Y. Sánchez-Arrieta, J. J. García-Vallejo, S. Gómez-Ramírez, F. Mérida, M. Morell and M. Muñoz. Dept. of Biochemistry and Molecular Biology, School of Medicine, University of Málaga, 29071-Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 100, 1997.

Postoperative reinfusion of shed blood after major surgeries is becoming a common practice. However, the controversy about its effectiveness still exists regarding the functionality and viability of shed erythrocytes in comparison with those of banked blood. In order to address the former question, in the present study we have examined: 1) the haematological and biochemical characteristics of shed blood, collected up to six hours in the postoperative period in cardiac and orthopaedic surgeries, and blood banked in CPD-A up to four weeks; 2) the functional integrity of erythrocyte membranes: median corpuscular fragility (MCF), glucose and amino acid uptakes, and morphology; and 3) the erythrocyte metabolism by measuring ATP and 2,3-BPG contents. The comparative analysis revealed that postoperative shed blood, even with lower haematological values and higher levels of plasma free hemoglobin than banked blood, presented less serum ionic alterations and its erythrocytes showed a better functionality since membrane integrity and energy metabolism were totally preserved.

Shed blood samples were kindly provided by Dr. J. M. Salas (Cardiovascular surgery), Dr. C. Sebastián and Dr. C. Ferrer (Orthopaedic surgery).

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P1 12 FUNCTIONAL ROLE OF A CONSERVED GLUTAMATE IN K^+ CHANNELS. P. Ortega-Sáenz, R. Pardal and J. López-Barneo. Departamento de Fisiología Médica y Biofísica. Fac. Medicina. Univ. Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 100, 1997.

Voltage-dependent K^+ channels are tetrameric proteins. Each monomer is composed of six transmembrane segments connected by extra- and intracellular loops. The loops contain clusters of dicarboxylic amino acids that at physiological pH act as fixed negative charges. We have investigated the role of the asp and glu located in the external loops. Our basic construct was the *Shaker* B Δ6-46 lacking N-type inactivation, which was mutated using mismatched oligonucleotides. Mutant channels were expressed in CHO cells transfected with cDNA and studied using the whole-cell patch clamp technique. Neutralization of a dicarboxylic cluster (EEDE) in positions 433 to 436 of loop S3-S4 (mutation 433-436QQQQ) did not apparently alter activation or inactivation time course of the channels. A similar lack of apparent effect was observed in a mutant (433-436QQQQ, E422Q) where we also replaced glu 422, located in the prepore region, for gln. However, neutralization of glu in position 418, resulted in a channel (43-436QQQQ, E422Q, E418Q) that exhibited an almost 100-fold acceleration of C-type inactivation time course. The time constant changed from 1.3 ± 0.16 s (n=6) in the wild type to 10.8 ± 2.4 ms (n = 10, +20 mV) in the mutant channel. C-type inactivation rate was decreased by high extracellular K^+ and TEA^+. These data demonstrate that, among negatively charged amino acids, residue 418, highly conserved in K^+ channels, has a critical role in channel function. Interestingly, residue 418 regulates C-type inactivation, a process that was thought to depend on amino acids in the pore.

DIFFERENTIAL EFFECT OF HYPOXIA ON Ca^{2+} HOMEOSTASIS IN CONDUIT AND RESISTANCE PULMONARY MYOCYTES. P1 13

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The effect of hypoxia on spontaneous cytosolic Ca^{2+} spikes were studied in fura-2 loaded myocytes isolated from conduit and resistance branches of the rabbit pulmonary artery. Ca^{2+} spikes were mainly due to release of Ca^{2+} from ryanodine and IP3-sensitive stores but they were modulated by basal Ca^{2+} which was influenced by the influx of Ca^{2+} through L-type Ca^{2+} channels of the plasmalemma. Internal Ca^{2+} regulated membrane potential through activation of Cl^- and K^+ conductances. The frequency and the amplitude of the Ca^{2+} spikes were sensitive to changes in the O_2 tension of the external solution. In conduit, main trunk, myocytes, the most typical effect was a decrease of basal Ca^{2+} and the frequency of the oscillations, but an increase in the amplitude of the spikes. In contrast, in some resistance, distal, myocytes the hypoxic response consisted in an elevation of basal Ca^{2+} and the decrease in the amplitude of the Ca^{2+} oscillations. In a parallel study performed in voltage-clamped smooth muscle cells, we have observed that hypoxia reversibly reduces the macroscopic Ca^{2+} current of conduit myocytes, whereas near the resting potential it potentiates the Ca^{2+} current in a population of resistance myocytes. These findings, demonstrating that hypoxia can differentially modulate cytosolic Ca^{2+} in smooth muscle cells in the pulmonary artery, may help to explain the segmental differences in the response of the pulmonary vasculature to low PO_2.

EFFECT OF ACUTE AMPHETAMINE ADMINISTRATION ON SOLUBLE AND MEMBRANE-BOUND PGLU-AMINOPEPTIDASE ACTIVITIES IN SEVERAL AREAS OF THE RAT BRAIN. P1 14

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Amphetamine has been widely used because of its central stimulant action and its anorectic effect. It has been reported that in the central nervous system amphetamine administration releases dopamine. Pharmacological studies indicate that TRH increases dopamine release and metabolism and evokes a number of behavioral changes which resemble those induced by psychomotor stimulants. The aim of this study is to ascertain the possible role of pGlu-aminopeptidases in the mechanism of action of amphetamine. pGlu-aminopeptidase activities were measured using pGlu-β–naphtylamide as substrate. The brain areas, taken by dissection, were frontal, parietal and occipital cortices, thalamus, amygdala, medulla, hypothalamus, hippocampus, striatum and the pituitary gland. Acute treatment with amphetamine generates a statistically significant reduction on soluble pGlu-aminopeptidase in hypothalamus and amygdala. However, the soluble activity in the other brain areas and the membrane-bound form did not show significant changes. pGlu-aminopeptidase has been proposed as the enzyme which metabolizes TRH, so these results suggest that amphetamine effects can be modulated by the action of this enzyme on TRH's metabolism.

P1 15 ANTIDOPAMINERGIC-LIKE ACTIVITY OF GAMMAHYDROXYBUTYRATE IN AGONISTIC ENCOUNTERS BETWEEN MALE MICE. J. M. Manzaneque, C. Pedraza, M. Martín and J. F. Navarro. Area de Psicobiologia, Facultad de Psicología, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 102, 1997.

This study was designed to assess the effects of a single administration of gammahydroxybutyrate (GHB) (20-120 mg/kg, ip), a catabolite of GABA which has been proposed as a possible central neuromodulator, on agonistic behaviour elicited by isolation in male mice. Individually housed mice were exposed to anosmic "standard opponents" 30 minutes after the drug administration and the encounters were videotaped and evaluated using an ethologically based analysis. GHB significantly decreased time spent in offensive behaviours (threat and attack) without an impairment of motor activity (100 and 120 mg/kg). This selective antiaggressive activity is very similar to that described with some atypical neuroleptics that exhibit an anti-D2 pharmacological profile, suggesting that GHB shows an antidopaminergic and neuroleptic-like activity in agonistic encounters between mice.

P1 16 SEROTONIN UPTAKE INHIBITOR GIVEN LOCALLY OR SYSTEMICALLY INCREASES EXTRACELLULAR SEROTONIN IN MICRODIALYSIS FLUID FROM RAT HIPPOCAMPUS. I. Míguez*, J. M. Míguez, F. J. Martín and M. A. Aldegunde. Dpto. Fisiología, Facultades de Biología y Farmacia*, Universidad de Santiago de Compostela (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 102, 1997.

The selective serotonin reuptake inhibitors (SSRIs) constitute a relatively new group of antidepressant drugs that appear to exert their action by enhancing central serotonin (5-HT) function. In order to characterize more fully the acute effect of uptake inhibitors, we have studied the dose-dependent effects of locally (0.5 and 3 μM) or systemically (2 and 10 mg/kg) administered paroxetine (SSRI) on extracellular 5-HT in the rat hippocampus. Male rats were anaesthetized and implanted with a dialysis probe into the ventral hippocampus. Dialysate 5-HT and 5-HIAA were determined by HPLC with electrochemical detection. The local administration of paroxetine resulted in a significant dose-dependent increase in the extracellular level of 5-HT (800 %), with no change in dialysate 5-HIAA. In another experiment, systemic paroxetine significantly increased extracellular 5HT to 200 % and decreased 5-HIAA (30 %). These results show that paroxetine causes a rapid and sustained increase in extracellular concentration of 5-HT in a serotonergic terminal area, probably by inhibiting the serotonin uptake carrier selectively.

ANTIDEPRESSANT ACTIVITY OF S-ADENOSYL-L-METHIONINE VS IMIPRAMINE AND BUSPIRONE IN THE FORCED SWIMMING TEST IN THE RAT. I. Bellido, A. Gómez and F. Sánchez de la Cuesta. Department of Pharmacology and Clinical Therapeutics, School of Medicine, Malaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 103, 1997. P1 17

Antidepressant effect of SAM is very rapid in contrast to the typical delay of up to several weeks with standard antidepressants. Our aims were: 1) to compare SAM antidepressant effects in acute vs chronic treatment; 2) to determine possible potentiation of imipramine and buspirone antidepressive effects by SAM in both acute and chronic treatment.

Wistar rats were treated with saline SAM, (50 mg/kg ip.) imipramine (10.9 mg/kg i.p.), SAM+imipramine, buspirone (1.25 mg/kg i.p) SAM+buspirone, methionine (18.72 mg/kg ip.), and SAM+methionine in acute treatment (x 24 h) and in chronic treatment (x 20 days). The forced swimming test (method of Porsolt) was used to evaluate the antidepressant activity. The Opend-Field test was used to evaluate exploratory and locomotor activity.

The rank order of potency obtained of Porsolt test immobility response were (in bracket seconds of immobility time): i) In acute treatment: SAM+imipramine (133.2 ± 7 s) > SAM+methionine (142.3 ± 13 s) > SAM (152.5 ± 10 s) > SAM+buspirone (173.8 ± 15 s) > buspirone (193.9 ± 13 s) > methionine (207.7 ± 9 s) > imipramine (224.3 ± 9 s) > control (232.1 ± 4 s); ii) In chronic treatment: SAM+imipramine (73.5 ± 6 s) > SAM+buspirone (83.8 ± 8 s) > imipramine (114.8 ± 11 s) > buspirone (126.5 ± 17 s) > SAM (131.4 ± 10 s) > control (224.7 ± 7 s).

Conclusions: SAM showed similar antidepressant activity in both acute and chronic treatment. Methionine did not change SAM antidepressant effect. Neither imipramine nor buspirone acute treatment did not show antidepressant activity. SAM potentiated imipramine and buspirone antidepressant activity in both acute and chronic treatment.

RECOMBINANT HUMAN GROWTH HORMONE INDUCES A BIPHASIC RESPONSE ON GROWTH PERFORMANCE IN FEMALE AND MALE BALB/c MICE FROM 21 TO 50 DAYS OF AGE. A. Agis-Torres, M. E. López-Oliva, M. T. Unzaga, E. Muñoz-Martínez. Secc. Depto. Fisiología Animal, Facultad de Farmacia, U. C. M., Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 103, 1997. P1 18

Female and male BALB/c mice were used to investigate the effects of exogenous recombinant human growth hormone (rhGH) administration on the growth performance between 21 and 50 days of age, allowed to consume 20 % dietary protein feed *ad libitum*. Sixty mice were assigned within each sex (female [F] and male [M]) to treatment of either 20 μL g^{-1} saline (Fs and Ms groups) or 74 ng rhGH g^{-1} body weight (BW) in 20 μL saline (FGH and MGH groups). Body weight and feed intake were recorded daily. Body growth in the experimental period showed a biphasic behaviour. During the first stage (25-35 days of age) rhGH treatment induced a caloric deficit by means of decrease of both the feed intake and the feed efficiency, leading to the growth impairment, manifested in a negative growth. In the second stage (35-50 days of age), through the self-regulated increase in the feed intake and improved feed efficiency the body weight of treated mice was enhanced, attaining body weight of control mice in a similar way of catch up growth mechanism. In conclusion, rhGH treatment induced a differential growth performance in BALB/c mice of both sexes between weaning and puberty: early body growth fails and later growth is self-recovered to normal values.

P1 19 BEHAVIORAL AND NEUROANATOMICAL CORRELATES OF LONG-TERM DETELENCEPHALATION IN PIGEONS. S. M. Cerutti*, L. Cintra, S. Díaz-Cintra and E. A. M. Ferrari*. *Lab. de Sistemas Neurais e Comportamento, Dep. de Fisiologia e Biofisica, IB, UNICAMP, 13083-970 Campinas, SP (Brasil), and Centro de Neurobiologia, UNAM (Mexico). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 104, 1997.

Operant discriminative behavior provides a useful baseline for analyzing the effects of lesions both in mammal and avian brain. The neural substrates of the discriminative behavior include integrative functions by telencephalic and subtelencephalic systems. The present work was conducted to quantify behavior under discrimination contingencies and morphological characteristics of visual pathways components in detelencephalated pigeons. Morphological evaluation was done using the Klüver-Barrera staining and the Histo 2000 (BIOCAM, France) image processing program. It was analyzed the Optic Tectum (OpT) and the Nucleus Rotundus (NR) in telencephalon lesioned pigeons (LL), sham-lesioned (SL) and non-lesioned (NL) previously submitted to extensive discrimination and reversal discrimination training. LL and control group comparisons showed that lesioned birds had more sessions for key peck acquisition and steady-state behavior ($p < 0.05$), higher response rates ($p < 0.05$) and did not show reversal discrimination learning. In the NR of LL birds it was observed increases in perimeter, neuronal soma and vascularity ($p < 0.05$) contrasting with a decreased number of neurons ($p < 0.05$). The OpT analysis showed a major disorganization in cell layers, associated to increased thickness of layers 1, 2 and 3. These evidences of plastic changes of subtelencephalic systems after long-term detelencephalation suggest functional mechanisms correlated with learning and neural plasticity in pigeons.

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P1 20 INFLUENCE OF MANIPULATION AND δ OPIOID RECEPTOR BLOCKADE ON NOCICEPTIVE AND BEHAVIOURAL RESPONSES IN THE NEONATAL RAT. M. P. Viveros, B. Fernández, M. T. Antelo, M. J. Alfaro* and M. I. Martín*. Dept. Biol. Anim. II, Fac. Biol., UCM, and *Dept. Farmacol., Fac. Med., UCM. 28040 Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 104, 1997.

The aim of this work was to assess if neonatal δ receptor blockade by the selective antagonist naltrindole (NT) (1 mg/kg, i.p., days 0-19) affected nociceptive and behavioural responses to the δ agonist DPDPE, at 20 days, an age at which δ-receptor is not fully developed. The effects of manipulation were studied by including a non-manipulated group. We used 94 Wistar male rats which were subjected to tail immersion test and open field (O.F.). Acute treatments consisted of a single injection of s.s. or DPDPE (4 mg/kg, i.p.) and reversion was assessed by administering a previous injection of NT (1 mg/kg, i.p.). DPDPE did not produce analgesia at this age and only reduced external ambulation in the O.F. ($p < 0.05$), an effect that was not reversed by NT. The acute NT+DPDPE treatment decreased O.F. activity ($p < 0.05$) and the quotient of nociceptive latencies (post/pre-treatment). The effects on activity were more marked in the non-manipulated group, and neonatal NT treatment attenuated or prevented them. Non-manipulated animals showed lesser O.F. activity ($p < 0.05$) and latency quotients than manipulated groups.

The present results suggest an effect of NT *per se* and point out the δ receptor plasticity.

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HIGH AFFINITY GLUTAMATE TRANSPORTER INHIBITORS BLOCKED THE RELEASE OF GLUTAMATE BUT NOT DOPAMINE, INDUCED BY AMPHETAMINE IN STRIATUM OF THE FREELY MOVING RAT. P1 21

A. del Arco and F. Mora. Dept. Physiology, Fac. Medicine, Complutense University, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 105, 1997.

Previous studies have shown that amphetamine (AMPH) produces a calcium independent release of glutamate (GLU) in striatum of the rat. The present study was designed to investigate the role of the high affinity GLU uptake carrier in the release of GLU induced by AMPH in this area of the brain. For that, two different GLU transporter blockers were used: *Dihydrokainic acid* (DK) (4, 8 mM) (non-competitive) and *L-trans-pyrrolidine,2-4,dicarboxylic acid* (PDC) (1, 4 mM) (competitive). Intrastriatal microdialysis were performed with CFS at a flow rate of 2.5 µl/min. Samples were collected every 15 min. GLU and DA content of samples were analyzed by fluorometric and electrochemical detection. AMPH (20 µg/µl), infused through the microdialysis probe for 10 min, produced 3.72 ± 0.30 µM increase on [GLU] and 204 ± 11.68 nM increase on [DA]. DK (8 mM) and PDC (4 mM) reduced increases on [GLU] induced by AMPH from 3.72 ± 0.30 to 0.69 ± 0.25 (DK) and 0.50 ± 0.47 (PDC) (µM). Neither DK nor PDC modified the increases on [DA] produced by AMPH. In contrast, GLU and DA basal levels were increased by both GLU uptake inhibitors. DK (8 mM) produced 4.5-fold increase on [GLU] and 5-fold increase on [DA]. PDC (4 mM) produced 30-fold increase on [GLU] and 7-fold increase on [DA]. These results show that high affinity GLU uptake carrier is strongly involved in AMPH-induced increases in extracellular concentrations of GLU.

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AMPHETAMINE INCREASES THE EXTRACELLULAR CONCENTRATIONS OF GABA THROUGH THE HIGH AFFINITY UPTAKE CARRIER: A MICRODIALYSIS STUDY. P1 22

T. Rodríguez, A. del Arco and F. Mora. Dept. Physiology, Fac. Medicine, Complutense University, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 105, 1997.

Amphetamine (AMPH) produces a calcium independent increase in extracellular concentrations of dopamine and glutamate (GLU) in striatum of the rat. The aim of the present study was to investigate the effects of AMPH in extracellular concentrations of GABA and the role of the high affinity GABA transporter in striatum of the freely moving rat. Intrastriatal microdialysis were performed with CSF at a flow rate of 2.5 µl/min. After baseline concentrations of amino acids were established, samples were collected every 15 min. GABA and GLU content of samples were analyzed by HPLC-fluorometric detection. AMPH (10 and 20 µg/µl), infused into the striatum for 10 min, increased extracellular concentrations of GABA, and GLU. At the highest dose (20 µg/µl), AMPH increased GABA from 0.05 ± 0.01 to 0.87 ± 0.09 and GLU from 0.48 ± 0.14 to 4.20 ± 0.34 (µM). The putative role of GABA transporter was investigated using nipecotic acid (NIP) (2, 4 and 8 mM), a selective blocker of GABA uptake. NIP, at the doses of 4 and 8 mM decreases the release of GABA induced by AMPH 70 % and 90 %, respectively. NIP (2, 4 and 8 mM) increased basal levels of GABA in a dose-related manner. However, NIP had no effect either on GLU basal levels or GLU AMPH-increased levels. These results show that AMPH increases extracellular concentrations of GABA specifically through the high affinity GABA transporter.

Supported by DGICYT PB93-0075 (Spain).

P1 23 ENDOGENOUS DOPAMINE INCREASES THE EXTRACELLULAR CONCENTRATIONS OF GLUTAMATE AND TAURINE BUT NOT GABA IN STRIATUM OF THE FREELY MOVING RAT: CORRELATIONAL STUDIES. I. Expósito, A. del Arco, S. Segovia and F. Mora. Dept. Physiology, Fac. Medicine, Complutense University, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 106, 1997.

Growing evidence is emphasizing the relevance of a neurochemical interaction between multiple neurotransmitter systems in specific circuits of the brain. In striatum, synaptic and volumetric interactions among GLU, TAU, GABA and DA have been described. The aim of the present study was to investigate the correlation between the increasing concentrations of endogenous DA and extracellular concentrations of GLU, TAU and GABA in striatum of the freely moving rat. For that, different doses of the specific DA-uptake blocker nomifensine (NMF) were used. Microdialysis was performed using an artificial cerebrospinal fluid at a flow rate of 2.5 µl/min. The amino acid and catecholamine content in the samples was analyzed by HPLC-fluorometric and electrochemical detection. Intrastriatal infusion of NMF (0.5, 1 and 2 mM) produced a dose-related increase in extracellular [DA]. Extracellular [DA] increased from 0.61 ± 0.09 nM (control values) to 5.18 ± 1.67 nM (0.5 mM), 17.31 ± 6.00 nM (1 mM) and 30.67 ± 3.00 nM (2 mM). At the following two higher doses (4 and 8 mM) NMF did not further increase the extracellular [DA] reaching to 31.58 ± 11.00 nM (4 mM) and 31.08 ± 5.00 nM (8 mM). A positive correlation exists between [DA] and [GLU] ($r = 0.83$, $p < 0.01$) and between [DA] and [TAU] ($r = 0.74$, $p < 0.05$). [DA] was not significantly correlated with [GABA] ($r = 0.62$). These results suggest that an interaction between DA, GLU and TAU exists in specific circuits in striatum.

Supported by DGICYT PB93-0075 (Spain).

P1 24 ROLE OF NITRIC OXIDE IN MODULATING THE RELEASE OF DOPAMINE, GLUTAMATE AND GABA IN STRIATUM OF THE FREELY MOVING RAT. G. Segovia and F. Mora. Dept. Physiology, Fac. Medicine, Complutense University, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 106, 1997.

The aim of the present study was to investigate the role of NO in modulating the basal and NMDA-induced release of dopamine (DA), glutamate (GLU) and GABA in striatum of the freely moving rat. Microdialysis experiments were performed with CSF at a flow rate of 2.5 µl/min. Samples were collected every 15 min. Catecholamine and amino acid content of samples were analyzed by HPLC with electrochemical and fluorometric detection. Intrastriatal infusion of NMDA (5 mM) for 15 min increased the extracellular [DA] (from 1.26 ± 0.12 nM to 15.99 ± 3.75 nM), [GLU] (from 0.74 ± 0.16 µM to 3.89 ± 1.02 µM), and [GABA] (from 0.20 ± 0.03 µM to 0.71 ± 0.15 µM). Perfusion of N-nitroarginine (NARG) (1-5 mM), an inhibitor of the synthesis of NO, produced no effects on basal [DA], [GLU] and [GABA]. However, the increase of extracellular [DA] produced by NMDA was potentiated by NARG (maximal increase: from 2.86 ± 0.61 nM to 35.46 ± 5.65 nM). No effect of NARG on NMDA-induced increases of [GLU] and [GABA] was found. Intrastriatal infusion of the NO donor 3-morpholino-sydnonimine (SIN-1) (10 mM) for 30 min increased the basal extracellular [DA] (from 0.58 ± 0.11 nM to 0.93 ± 0.38 nM) and decreased [GLU] (from 2.47 ± 0.52 µM to 1.46 ± 0.55 µM) and GABA (from 0.12 ± 0.01 µM to 0.09 ± 0.01 µM). However, perfusion of a non-fresh solution of SIN-1 (10 mM), which does not release NO, produced the same effects as the fresh SIN-1. These results suggest a role for NO in modulating the release of DA in striatum.

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ANTIBODIES TO THE DOPAMINE RECEPTOR SUBTYPES. Z. U. Khan, R. Martín, A. Gutiérrez, A. Peñafiel, A. Rivera and A. de la Calle. Dpto. Biología Celular, Universidad de Málaga, Málaga 29071 (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 107, 1997. P1 25

We have prepared specific polyclonal antibodies to the dopamine D_3 and D_4 receptors. In Western blot of rat membranes, anti-D_3 recognizes a 49 KDa single polypeptide band, however, anti-D_4 reacts with 41 KDa band. The molecular sizes of the immunoreactive bands are close to the predicted molecular weight of cDNA clones of dopamine D_3 and D_4 subtypes.

The immunoprecipitation of [^{3}H] YM-09151-2 binding sites of digitonin solubilized rat membranes were saturable with both antibodies. Anti-D_3 immunoprecipitated 42-48 % with 20 µl of antiserum and anti-D_4 51-56 % with 80 µl of antiserum.

The distribution of the dopamine D_3 and D_4 receptors in rat and human brains was analyzed by immunocytochemistry. Dopamine D_3 receptors were more abundant in cerebellum and hippocampus, whereas, dopamine D_4 receptors were present in cerebral cortex, hippocampus and caudate-putamen.

This work was supported by EC no. BMHl-CT94-1060 and DGICYT no. PB94-0219-C02-02 (Spain).

ACTIVIIY OF THE NADPH-DIAPHORASE ON THE HUMAN STRIATE CORTEX. R. Durán, L. R. F. Faro*, M. Alfonso, M. Arufe, B. Arias and C. W. Picanço-Diniz*. Dpto. Biología Fundamental, Facultad de Ciencias, Universidad de Vigo (Spain), and *Dpto. de Fisiologia, Centro de Ciencias Biológicas. Universidade Federal do Pará (Brasil). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 107, 1997. P1 26

The study of the cortical metabolic activity and the characterization of neuronal populations are possible using the histochemistry for NADPH-diaphorase (NADPH-d). Recently, it was found that this enzyme is a neuronal nitric oxide synthase (NOS), important because of the physiological actions of nitric oxide as second messenger. The results obtained by the application of a histochemical technique for NADPH-d in postmortem human brains show the presence of two different populations of neurons in every cortical layer: one group in which only the neuronal bodies are identified and other group with Golgi-like neurons. The activity of neuropile is quite similar to the old-world primates, showing a pattern of blobs and interblobs. These results characterize different NADPH-d positive neuronal populations in the human striate cortex, as well as a complex pattern of labelling of neuronal processes, important for the study of the cortical function.

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P1 27 LACK OF ROLE OF THE VASCULAR ENDOTHELIUM IN HISTAMINE-INDUCED RELAXATION OF HUMAN DORSAL PENILE ARTERY. S. Benedito, C. Martínez, S. Novella, R. Raposo, J. A. Delgado*, L. Resel* and A. García-Sacristán. Dpto. Fisiología Animal, and *Dpto. Urología, Hospital Clínico San Carlos (UCM) Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 108, 1997.

The present study was designed to evaluate the relative contribution of endothelium-derived substances in the response to histamine of human dorsal penile artery *in vitro*. These preparations were mounted as rings in a myograph for measurement of isometric tension. Histamine evoked a dilatation in precontracted preparations ($pD_2 = 6.57 \pm 0.13$; $E_{max} = 86.82 \pm 2.95$ %). Mechanical removal of the endothelium did not modify the relaxations elicited by histamine ($\Delta pD_2 = 0.31 \pm 0.14$; $\Delta E_{max} = 16.47 \pm 7.43$ %). The role of NO could be evaluated specifically by the use of the inhibitor L-NAME (3×10^{-4} M) and the precursor of NO synthase L-arginine (3×10^{-4} M). Neither L-NAME ($\Delta pD_2 = 0.27 \pm 0.11$; $\Delta E_{max} = 6.90 \pm 0.95$ %) nor L-arginine ($\Delta pD_2 = 0.32 \pm 0.04$; $\Delta E_{max} = 18.46 \pm 5.15$ %) produced any significant change on dilatation to histamine. The histamine response was also unaffected by indomethacin (3×10^{-6} M), a cyclooxygenase inhibitor ($\Delta pD_2 = 0.26 \pm 0.06$; $\Delta E_{max} = 10.46 \pm 4.29$ %). In conclusion, these results suggest that the mechanism by which histamine induces a vasodilatation in human dorsal penile artery appears to be largely endothelium-independent.

This study was supported by Grant No. 188/92-4103 from U.C.M.

P1 28 K_{ATP} CHANNELS IN THE PIG URETERAL NITRERGIC RELAXATION. M. Hernández, D. Prieto, R. L. M. Orensanz*, M. V. Barahona, M. Jiménez-Cidre@, L. Rivera, A. García-Sacristán and U. Simonsen. Dpto. Fisiología, Facultad de Veterinaria UCM and Dptos. *Investigación and @Urología, H. Ramón y Cajal, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 108, 1997.

The aim of the present study was to investigate the underlying mechanisms through which nitric oxide (NO) acts as an inhibitory neurotransmitter in the pig intravesical ureter. Ureteral strips (4 x 2 mm) were dissected and mounted for recording isometric tension in 5 ml organ baths with Krebs solution at 37 °C and bubbled with carbogen, pH = 7.4. The preparations were equilibrated under a passive tension of 2 g. NO was endogenously-released by electrical field stimulation (EFS) with rectangular pulses (0.5-10 Hz, 30 ms) with the adjusted current to 75 mA and exogenously-added as an acidified solution of $NaNO_2$ (pH = 2). Guanethidine (10^{-5} M) and atropine (10^{-7} M) were present through the experiment to block the adrenergic neurotransmission and muscarinic receptors, respectively. The relaxations to EFS or exogenous NO were reduced in the presence of methylene blue (Mb,10^{-5} M) and glibenclamide (Glib, 10^{-6} M), the inhibitors of guanylate cyclase and ATP-sensitive K^+ channels, respectively. Combined treatment of Mb and Glib did not exert an additional blocking effect versus those elicited by Mb alone. In contrast, the blockers of large and small Ca^{2+}-activated K^+ channels, charybdotoxin (3×10^{-8} M) and apamin (5×10^{-7} M), respectively, did not change the relaxations to EFS or exogenous NO. The present results support that NO relaxes the pig intravesical ureter through a guanylate cyclase-dependent mechanism which favours the opening of ATP-sensitive K^+ channels.

α AND β-ADRENOCEPTORS IN THE HORSE CORPUS CAVERNOSUM. P1 29
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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 109, 1997.

We studied the presence of α and β-adrenergic receptors in the corpus cavernosum (CC) of the horse and we characterized the α-adrenergic subtype. CC tissue was obtained from sexually mature penis horse. The penis were removed immediately after sacrificing the animals and transported to the laboratory in physiological saline solution (PSS) at 4 °C. The CC strips (0.2 x 0.2 x 0.7 mm) were suspended vertically in 30 ml organ baths containing PSS maintained at 37 °C and gassed with carbogen, pH = 7.4. Passive tension of 4 g was applied to the preparations and they were allowed to equilibrate at least for 60 min.

Noradrenaline and phenylephrine induced concentration-dependent contractions in CC preparations. BHT 920 had no effect. Phentolamine (10^{-8}-10^{-7} M) and prazosin (3 x 10^{-9}-3 x 10^{-8} M) produced a shift to the right of the dose-response curve of noradrenaline, while the α_2-antagonist, rauwolscine had no effect on the response to noradrenaline. Phenylephrine-evoked contractions of corporal strips were significantly inhibited by α_1-adrenergic antagonist prazosin. Isoprenaline relaxed precontracted CC preparations in a concentration-dependent way, the isoprenaline effect was blocked by propranolol (10^{-10}-10^{-9} M).

These results suggest that stimulation of β-receptors relax the horse CC, whereas contraction is mediated by postjunctional α_1-receptors which contribute to detumescence and flaccid state of penis.

RESPONSE OF CEREBELLAR CORTEX CELLS TO ACOUSTIC STIMULATION. P1 30
M. A. Pozo, M. E. de la Peña, R. Corripio and F. J. Rubia. Unidad de Cartografía Cerebral, Instituto Pluridisciplinar UCM, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 109, 1997.

The cerebellum receives inputs from the peripheral receptors and the cerebral cortex through well known precerebellar system. However, little seems to be known on acoustic afferents pathways to the cerebellum. The aim of this experiment is to analyze the frequency discharge of the cerebellum Purkinje cells to acoustic stimuli.

Adult rats were anesthetized with ketamine (100 mg/kg, i.v.), paralyzed with pancuronium bromide (2 mg/kg i.v.) and artificially ventilated. Electrical activity was recorded from the cortex of the lobuli VI and VII of the cerebellar vermis. Clicks and ramps of various duration were used as sound stimuli and presented through earphones at a carrier frequency of 1 KHz and 10 KHz and sensation level of 85 db.

We recorded from 21 units located at depths of 400-650 μm. These units showed spontaneous activity (complex spike: 1.1 Hz and simple spike: 20.8 Hz) and were unresponsive to clicks. Some of these (6 units) showed frequency inhibition after binaural ramps with 20 ms delay between ears. This sensitivity to the directionality of the sound implies that this region of the cerebellum may be involved in orientation of the head towards auditory stimuli from the environment.

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P1 31 ANTERODORSAL THALAMI NUCLEI AND CHRONIC STRESS IN RATS. M. Suárez, M. Maglianesi and N. Perassi. Instituto de Fisiología, Fac. Cs. Médicas y Cs EFN, U.N.C., Córdoba (Argentina). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 110, 1997.

The limbic thalami nuclei are involved in regulation of cortico- and medullo-adrenal function, in resting and stressful conditions. Experiments were carried out in order to evaluate the effect of anterodorsal thalami nuclei (ADTN) lesion on plasma ACTH and corticosterone (C), in stressed rats. Thirty days after lesion, basal values of plasma ACTH plasma adrenal C are significantly higher than those in sham lesioned ($P < 0.05$, respectively). Chronic stress (forced immobilization, 15 min/day, during 12 days and variable stress for 24 days) in sham operated animals produced a significant increase in ACTH ($P < 0.05$) and plasma C ($p < 0.05$), and a slight decrease in adrenal C, as compared to unstressed rats. In lesioned stressed rats (both groups), plasma ACTH was below that found in sham lesioned stressed rats ($p < 0.05$, respectively). Variations of plasma C concentration of stressed lesioned rats were not significant. The adrenal glands of stressed lesioned rats (both groups) showed a significant lower content of C than unstressed lesioned rats ($p < 0.005$). It is concluded that ADTN in rats, may pay a significant role in regulating the hypophyso-adrenal system.

P1 32 THE ACTIVITY STATES OF REPTILES. M. C. Nicolau, A. Gamundí, C. Rosselló, E. Sáez, M. Akaârir and R. Rial. Laboratori de Fisiologia. Dept. de Biologia F. i C. S. Universitat de les Illes Balears. 07071 Palma de Mallorca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 110, 1997.

The similitudes between mammalian and reptilian sleep has been repeatedly studied. However, up to now the results have been contradictory. This report is based on laboratory and field studies performed in lizards *Gallotia galloti, G. stehlini, Podarcis lilfordi, Iguana iguana* and *Agama agama.* Three main activity states have been found: a) activity, corresponding to behavioral thermorregulation and full behavioral display; b) diurnal rest, corresponding to a more or less relaxed posture, eyes either open or closed, slightly raised sensory thresholds and easy reversibility, and c) nocturnal sleep, characterized by hiding in secure places, loss of behavioral thermoregulation and limited reversibility. From the EEG point of view, the active state is characterized by a slow wave, high voltage power fraction to which spindles and fast rhythms can be superposed. The EEG of diurnal rest shows only the slow wave fraction with a slightly reduced amplitude and the nocturnal rest is similar to the diurnal one, but the low body temperature determines a deep drop in EEG amplitude. These activity states may have been equated to mammalian wakefulness and sleep, but it seems probable that the failure in distinguishing between diurnal and nocturnal (sleep) rest might be the main cause of contradiction between previous studies.

THE REPTILIAN EEG DOES NOT CHANGE DURING DIFFERENT ACTIVITY STATES. P1 33

A. Gamundí, M. C. Nicolau, C. Rosselló, G. Timoner, G. Alemany, and R. V. Rial. Laboratori de Fisiologia. Dept. de Biologia F. i C. S. Universitat de les Illes Balears, 07071 Palma de Mallorca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 111, 1997.

Correlations between activity and EEG are well known in mammals and birds. For instance, three activity states can be readily distinguished from EEG analysis. The EEG space and time differences reflect the neuronal activation of various behavioral states. In reptiles, however, only two clear conclusions can be drawn: 1) the EEG amplitude is dependent on body temperature, and 2) behavioral activation, unlike in mammals, consists of increased EEG amplitude and synchronization.

These low correlations might have been due to inaccurate EEG analysis, as time series analysis were not available until recently. In this report Fourier and RMS analysis were applied to *Gallotia galloti* lizard EEG, at controlled temperature, in the following states: 1) active with open eyes; 2) inactive with open eyes; and 3) inactive with closed eyes.

A significant reduction of EEG amplitude from states 1 to 3 was found but no difference between states was found in relative power corresponding to frequencies from 0.5 to 40 Hz. Thus, no differential activation of brain regions can be deduced from the reptilian EEG.

Therefore, reptiles only show a single activity state, in contrast with the three activity states known in mammals and birds.

POSTNATAL DEVELOPMENT OF CA^{2+} DEPENDENT K^+ CURRENT Ic IN CA1 RABBIT HIPPOCAMPAL PYRAMIDAL CELLS: A COMPUTATIONAL MODEL. P1 34

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The developmental changes of the voltage and calcium dependent potassium current Ic were studied with single electrode voltage-clamp (SEVC) method. We have analyzed its activation by computing the normalized conductance. Steady-state activation was well fitted with the Boltzmann equation $g_{norm}(V) = \{1+\exp[(V_{1/2} - V)/k]\}^{-1}$, where k is the activation slope and $V_{1/2}$ is the voltage value of the half-maximum potential for activation. We have found significant difference in k values ($t=4.85$, $p = 0.0014$) from immature and adult cells while there is not any significant change in the $V_{1/2}$. This result supports a developmental change in the operation of these channels. Three different mechanisms can account for these changes: 1) Modifications in the regulation of internal $[Ca^{2+}]$. 2) Changes in the channel distributions along development, and 3) an actual acquisition of sensitivity by Ic channels. We have developed a realistic computational model to discriminate among these hypothetical mechanisms. We conclude that the changes in the activation kinetics of Ic observed with SEVC could only be explained on the basis of a developmental change that occurs in BK channels. Previous works have reported such a channel maturation in spinal neurons (Blair and Dionne 1985, *Nature*, 315, p. 329). Further research based in single channel analysis would be required to check this result.

P1 35 FAST ALGORITHM FOR PHYSIOLOGICAL SIGNALS NONLINEAR ANALYSIS (FASA). E. J. Díaz-Calavia, G. Ezpeleta-Lobato, C. Varela-Rodríguez and P. Berraondo-López. Biofísica. Facultad de Medicina. Universidad de Navarra. 31080 Pamplona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 112, 1997.

Introduction. The Grassberger-Procaccia (G-P) algorithm is used in the analysis of physiological time series: ECG, EEG, etc. We present a much faster algorithm.

Methods. The cause of the slowness of the G-P algorithm is the sum of the modulus between the difference of one row vector and the rest of the vectors during the reconstruction of the attractor. Solution: 1) Reconstruction of the D-dimensional attractor (to obtain a matrix with D columns and (N2•τ) rows). 2) Calculate the arithmetic mean and the variance of each column. 3) Find the sum of the square difference between each row data and the mean. 4) Subtract from the last total the difference between each number of the i row and the mean. 5) Sum the data calculated before for each of the D columns and then obtain the value of the sum of squares of the matrix. 6) Subtract, from the square root obtained from the norm of the vectors, the value of r. We obtain a very fast algorithm which substitutes the expression between parentheses in the G-P formula. Following this way we calculate Cr, with identical value than the one calculated by G-P.

Results. The new algorithm, (FASA), is 35 to 85 times faster than the G-P for time series formed by 300 000 data (300 s of ECG, sampling to 1 kHz) and 5 000 data respectively. This new algorithm is faster for other series shorter than the ones mentioned above.

References. Grassberger, P., Procaccia, I.: *Phys. Rev. Lett.*, 50: 346 - 350. 1983.

P1 36 A SIMPLE METHOD FOR THE NON INVASIVE STUDY OF AUTONOMIC NERVOUS SYSTEM. D. González-Nieto, A. Valentín, J. Torregrosa, J. J. García-Seoane, and J. M. Ortiz. Departamento de Fisiología, Facultad de Medicina, Universidad Complutense. Madrid 28040 (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 112, 1997.

The changes in heart rate during respiration are mainly due to the sympathetic nervous system reflex activity. It is generally admitted that the afferent fibers come from lung stretch receptors and the efferent ones are vagal cardiac fibers. This has allowed the use of this reflex as a mean of ANS analysis; nevertheless the quantitative relationship between ANS activity and respiratory modulation of heart rate is not well known. We have designed a simple system to gather and analyze data in respiratory sinus arrhythmia using a one channel ECG differential amplifier which produces, by means of a level detector, a ± 9 V pulse synchronous with the registered derivation R wave. The amplifier and detector were designed using an electronic circuit design program (OrCAD V-4.10). The R-R interval was measured using an application written with a language (Microsoft QBASIC), using a pulse as data entry signal (RD) at a PC (IBM compatible) RS-232 serial port. The computer directs the experimental subject's respiratory rate using an analog graph and the estimation is made by means of the changes in the ECG amplitude synchronously digitalized with the pulse. The method's validity was confirmed with 15 healthy male and female volunteers studying the potency cross spectrum in the R-R interval series and the instantaneous ECG amplitudes. The main advantages of this method are its non invasive character and the reduction of design and assembly costs.

LONG-TERM RECOVERY OF SCIATIC NERVE FOLLOWING PROLONGED ELECTRICAL STIMULATION. W. Agnew, T. Yuen, D. McCreery and L. Bullara. Dept. of Neurological Research, Huntington Medical Research Institutes, Pasadena, CA (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 113, 1997. P1 37

This study presents the long-term effects of continuous electrical stimulation of the cat's sciatic nerve using stimulus parameters (50 Hz or higher) and at moderately high pulse amplitude which results in definite neural injury when the nerves are examined at one week after stimulation. Nerves stimulated intermittently, or at 20 Hz, with the same pulse amplitudes showed no damage. The objective is to determine the extent of functional and morphologic recovery as demonstrated by changes in the recruitment curve of the averaged evoked compound action potential and by light and TEM over a time course of 1 week to 3 months. At 1-3 weeks marked neural injury was present in the form of degenerating axons, edema and phagocytes. At 1-3 months after stimulation, there is a marked resolution of axonal injury with rare phagocytes and sparse remyelination. Studies are presently being conducted to quantitate the extent of both axonal injury and remyelination using a computerized morphometric system. These findings are of importance in establishing safe and effective guidelines for the use of functional electrical stimulation in the neurologically handicapped.

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NON-OPIATE-MEDIATED LEARNED HELPLESSNESS. N. A. Teixeira, D. G. Pereira, and A. H. Hermini*. Depto. de Farmacologia, Faculdade de Ciências Médicas, and *Centro de Engenharia Biomédica, UNICAMP, Campinas SP (Brasil). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 113, 1997. P1 38

Opiates have been implicated in learned helplessness (LH), a phenomenon known to be related to opiate stress-induced analgesia (SIA). In this study, we have investigated the role of opiates in the induction of LH and SIA under different conditions. Adult female Wistar rats were trained either by receiving 60 inescapable 1 mA footshocks (IS group, N = 106), or by confinement in the shock box (NS group, N = 84). Some of the animals were immediately tested for analgesia in a tail-flick test while the rest were used twenty-four hours later in a shuttle box experiment to examine their escape performance. The opiate antagonist naltrexone (0 or 8 mg/kg, i.p.) and the previous induction of cross-tolerance to morphine by the chronic administration of morphine (0 or 10 mg/kg, s.c., for 13 days) were used to verify opiate involvement. Analysis of variance revealed that only animals in the IS group demonstrated both hypoalgesia and an escape deficit, both of which were resistant to the procedures applied before the training session. However, the escape deficit could be reversed if the treatments were given before the test session. We conclude that induction of the known "interference effect" on escape performance is not opiate-mediated although its expression is opiate modulated. These results suggest that the demonstration of opiate involvement in LH may depend on the experimental parameters used.

FAPESP (grant No 92/3839-3 and 92/3816-3).

P1 39 MOTOR CONTROL BY THE SPINAL CORD: A TEACHING MODEL. A. Fuente, P. Varona and J. A. Sigüenza. Facultad de Medicina, Dept. Fisiología, Universidad de Salamanca, 37007 Salamanca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 114, 1997.

As a consequence of the necessary integration of new technologies in physiology teaching we have used a neural network simulator: XSim to develop a model for generating and controlling the several components of miotatic reflex also showing the functionality of involved structures. This response is produced by stretching fusimotor chains which are surrounded by Ia afferent fibers producing (a)activation of α-motor neurones, and accordingly reflex muscle contraction; and (b)γ-motor neurones that modulate this shrinkage. This scheme includes several interneurones that modulate and change the effector activity. Fusimotor stretching was simulated as a number which is passed to functions representing Ia activity. These functions represent both HkC E, uency related to length and dynamic response, showing changes due to velocity in which length changes had occurred. The result of this activity is afferent information to several motor units. Units representing α-motor neurones were connected to extrafusal muscle fibers and Renshaw cells. γ-motor neurone units were connected to intrafusal muscle fibers. Extrafusal muscle fiber units simulate changes in tension and length of the muscle, which is the main feed-back system to fusimotor structure. The computer program XSim runs under a graphical environment which permits the visualization of both global activity and activity from each of its components as if being seen using an oscilloscope. Also students are able to modify parameters or connections making queries of the type "what would happen if...". These features give us capacity to use it as a very useful learning aid. The model has an open structure to incorporate other functions reproducing activity from many different structures which, obviously, have an important role in motor control by the spinal cord.

P1 40 CONTRIBUTIONS OF INHIBITORY INPUTS TO MEMBRANE RESISTANCE IN DEVELOPING HYPOGLOSSAL MOTONEURONS. P. A. Núñez-Abades and W. E. Cameron. Dept. Neuroscience, University of Pittsburgh, Pittsburgh, PA (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 114, 1997.

Motoneurons of the hypoglossal nucleus innervate the tongue muscles and play an important role in respiration, deglution and vocalization. Using a slice preparation of the rat brainstem and intracellular recording, we have measured the changes in membrane properties of developing hypoglossal motoneurons (HMs) at different stages of postnatal development. The relative contribution of glycinergic and GABAergic transmission to the membrane resistance of a motoneuron was determined by blocking the postsynaptic receptors with 10 μM strychnine (n = 64), 20 μM bicuculline (n = 13) or a combination of both (n = 20). In all cases, the blockade of inhibitory transmission increased the input resistance (R_n) and membrane time constant (τ_m). Similar changes were measured when glycinergic or GABAergic synapses were blocked. The most interesting observation was that the increase resulting from both blockers was the same as either one of the individual blockers at 5-6 (n = 3), 13-15 (n = 4) and 19-30 days (n = 6). These data suggest that 1) inhibitory synapses contribute to the R_n and τ_m of developing HMs and 2) there is some kind of interaction at all ages between the blockers with their receptors or with a shared ionic channel for chloride.

Work supported by NIH grant (HD 22703).

IMMUNOHISTOCHEMICAL EVIDENCE FOR THE PRESENCE OF D4 DOPAMINE RECEPTOR (D4Dr) IN DOPAMINERGIC NEURONS OF THE SUBSTANTIA NIGRA IN HUMAN BRAIN. P1 41

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D4Dr has been mapped by immunohistochemistry in the rat brain where a widespread distribution was described (Defagot *et al.*, *Mol. Brain Res.*, 1996, In press). Also recently D4Dr has been identified in gabaergic neurons in primate brain (Mrzljak *et al.*, *Nature*, 381, 245-248, 1996). The goal of the present report was to further extend those studies to the human brain. By means of immunohistochemistry (ABC technique) and indirect immunofluorescence combined with conventional and confocal microscopy we analyzed the presence of D4Dr in human brain. The nervous tissue was obtained at autopsy from human subjects (23-77 years of age) with a lack of history of neurologic disorders. Our results demonstrate the presence of D4Dr in dopaminergic neurons of human substantia nigra (pars compacta). Also they indicate a possible decrease in the expression of D4Dr during aging.

THE *IN VIVO* DOPAMINE RELEASE FROM RAT STRIATUM IS ALTERED BY THE ADMINISTRATION OF METHYLMERCURY. P1 42

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Mercury is an important poluent agent in the environment and it produces neurotoxic effects on the brain. Neurological manifestations in animals include impairment of psychomotor tasks, which can be related to striatum neurotransmitter metabolism. To study the possible effects of methylmercury (MeHg) on the release of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) from striatum, we use an *in vivo* microdialysis technique coupled to HPLC with electrochemical detection. The chronic administration of an equivalent total dose of 6-7 mg/kg of MeHg induces significant increases in the striatal release of DA and/or its acidic metabolites, independently of the pattern of administration. The effects of MeHg on the release of DA and its metabolites seems to have a component which is dose-dependent, as well as to be an accumulative process.

Financial support: Vigo University (Spain) and FNMA and UPPa-PROPESP (Brasil).

P1 43 EFFECTS OF THE MARINE TOXIN DOMOIC ACID ON THE OF DOPAMINE AND ITS METABOLITES FROM STRIATUM OF RATS: A MICRODIALYSIS STUDY. B. Arias, R. Durán, M. Alfonso, L. R. F. Faro* and M. Arufe. Dpto. de Biología Fundamental, Facultad de Ciencias, Universidad de Vigo (Spain) and *Dpto. de Fisiologia, Centro de C. Biológicas. Universidade Federal Do Pará (Brasil). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 116, 1997.

The neurotoxin domoic acid (Dom) is a rigid analog of the excitotoxic amino acid glutamate. The *in vivo* effects of Dom on the release of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were investigated in freely-moving rats using microdialysis. Microdialysis probes were stereotaxically implanted in the striatum. The day after surgery, the dialysis probe was perfused with Ringer's solution. After a stabilization period, Dom (20 μM) was infused for 30 min. The substances (DA, DOPAC and HVA) were determined by reversed-phase High-Performance Liquid Chromatography (HPLC) with electrochemical detection. Perfusion of 20 μM Dom increased extracellular levels of DA in striatum. It had a similar but smaller effect on extracellular DOPAC levels, while there was no change in the level of HVA. The DA increase continued until 24 hours after the perfusion of Dom. These findings suggest a change in the striatal activity of dopaminergic neurons, which was improved by the Dom administration.

P1 44 HYPOTHALAMIC NEURON PROJECTION TO VAGAL PREGANGLIONIC LEVEL AND LITHIUM CHLORIDE SENSITIVE NEURONS WITHIN THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS. M. Carrasco, F. Portillo and J. J. Vallo. Depto. de Fisiología, Facultad de Medicina, Cádiz (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 116, 1997.

Lithium chloride (LiCl) is a compound that provokes dramatic disorders in the feeding behavior in the rat, such as conditioned taste aversion, anorexia, pica or emesis. It is well known that the paraventricular hypothalamic nucleus (PVN) is an important center of the autonomic control of the food intake. It has been described neurons within the PVN which are projecting to autonomic preganglionic levels related with the metabolism. We have used the retrograde fluorescent method combined with c-fos like immunohistochemistry (FLI) in order to describe these different populations of neurons within the PVN, i.e. parasympathetic preganglionic neurons related with the metabolism (HB) and LiCl sensitive neurons (LCS). We have used Diamidino Yellow as parasympathetic tracer and LiCl as stimulus for FLI. Thus HB neurons were found mainly in the ventral and lateral part of the PVN and LCS neurons were located in the magnocellular part of the PVN without relation with the fluorescent labelled neurons. These data suggest that a clear segregation exists within the PVN between HB and LCS neurons.

EFFECT OF THE LOW-POWER LASER IRRADIATION ON ABSTINENCE OPIOIDS SYNDROME. M. T. Labajos-Manzanares and M. Labajos-Claros. Biofísica, Facultad de Medicina, Universidad de Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 117, 1997. P1 45

Introduction: In this work we pretend determine if the treatment whit low-power laser is effective in order to diminish the stress produced in situation of abstinence in drogodependent subjects, given that this type of radiation increases the b-endorphine levels according to it have been demonstrated in previously published work.

Material and Methods: Experiments were performed on adult Wistar rats of either sex weighing 225-500 g from an esterile solution at 10 %. Animals were irradiated on the shaven cervical zone with two different kinds of low-power laser (HeNe and GaAs), depending on the experimental group considered. Urinary cortisol levels were determined after the induction opioids dependence with morphine chlorhydrate.

Conclusions: In experimental subjects multiple and single low- power laser irradiation was able to control the biochemical response stress induced by the opioids abstinence, showing more effective than the treatment with methadone as deduced for evaluation of urinary cortisol levels.

ADHESION MOLECULES EXPRESSION IN CULTURED HUMAN OSTEOBLASTS. C. Ruiz, C. Reyes-Botella*, M. J. Montes**, A. C. Abadía-Molina** and E. García-Olivares**. Dept. Enfermería (Sección de Fisiología y Bioquímica), Escuela Universitaria de Ciencias de la Salud. *Dept. Estomatología, Facultad de Odontología, and **Dept. Bioquímica y Biología Molecular (Sección de Inmunología), Facultad de Medicina, Universidad de Granada (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 117, 1997. P1 46

Bone is a highly organized tissue comprising a calcified connective tissue matrix and specific bone cells. We have isolated, characterized and maintained in culture osteoblastic cells from human bone section obtained during mandibular osteotomy.

Adhesion molecules are cell surface proteins that are functionally involved in cell-cell or cell-extracellular matrix interactions. Using flow cytometry we examined human cultured osteoblasts to determine the expression different adhesion molecules. We found that ICAM-1 (CD54), the HLA-DR antigen (which is considered by some authors as an adhesion molecule), CD44, CD80 and CD86 antigens (the latter two involved in antigen presentation) were expressed by cultured osteoblasts.

P1 47 MODULATION OF INWARDLY RECTIFYING POTASSIUM CHANNELS BY INTRACELLULAR pH IN HeLa CELLS. M. Díaz[1], G. Riquelme[2] and F. V. Sepúlveda[3]. [1]Dpto. Biología Animal, Universidad de La Laguna, Tenerife (Spain), [2]Dpto. Fisiología y Biofísica and [3]Dpto. Medicina Experimental, Centro de Estudios Científicos de Santiago, Facultad de Medicina, Universidad de Chile, Santiago (Chile). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 118, 1997.

Regulatory volume decrease (RVD) following hypotonicity-induced cell swelling is mediated in part by exit of KCl through specific ionic conductances. However, the underlying transduction mechanisms are still unknown, and the detailed characterisation of the possible channels involved is therefore important. HeLa cells have been shown to possess volume-sensitive Cl^-channels that become activated upon exposure to hypotonicity and Ca^{2+}-activated K^+ channels showing inward rectification. Here we investigate the possible involvement of pH in the regulation of the K^+ channels using the patch-clamp technique. Our results indicate that intracellular pH strongly affects potassium channel activity. Under symmetrical 145 mM KCl at pH 7.4, the normalized cell potassium conductances were 335, 372 and 563 pS/pF at 60, -60 and –120 mV, respectively. The corresponding values at pH 6.4 were 53, 146 and 210 pS/pF and at pH 8.2, 430, 268 and 233 pS/pF. The analysis of the results using inside-out patches showed that similar changes in pH did not affect the single channel conductance but markedly affected the channel open probabilitv, in a way that accounted for the observed changes in rectification of the macroscopic currents. These results indicate that intracellular pH changes might modulate the activity of K^+ channels during RVD.

Supported by Fondecyt (Chile) grant 1940408.

P1 48 PHARMACOLOGICAL EVIDENCE OF CHLORIDE CHANNELS IN THE ISOLATED MALPIGHIAN TUBULE OF *FORMICA POLYCTENA*. I. Vanschoonbeek, P. Steels and E. Van Kerkhove. Limburgs Universitair Centrum, Department MBW-Fysiologie, B3590 Diepenbeek (Belgium). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 118, 1997.

In *F. polyctena* K^+ is actively secreted into the lumen of malpighian tubules. Cl^- and water follow passively. Anion substitution experiments suggested a diffusional pathway may be important in Cl^- secretion at least in the presence of a high K^+ concentration (Vanschoonbeek *et al.*, 1995). To find out whether this diffusional pathway involves anion channels located in the cell membrane, the effect of Cl^- channel blockers was tested on fluid secretion.

Only water and ethanol soluble drugs were used, since DMSO at 0.1 vol % caused a significant increase in fluid secretion by itself (132 % ± 13 %, $p < 0.05$, $n = 10$), whereas ethanol (0.1 vol %) did not. A reduction in fluid secretion [control value 333 pl/min ± 40 SE (= 100 %) ($n = 67$)] was caused by DIDS, directly dissolved in saline, and by tamoxifen added to the solution from a 10 mM stock in ethanol.

Secretion significantly dropped to 56 % ± 9 SE ($n = 4$) and 50 % ± 14 SE ($n = 5$) in 10^{-6} M and 10^{-5} M DIDS, respectively and to 70 % ± 3 % SE ($n = 3$) and 44 % ± 8 SE ($n = 3$) in 10^{-6} M and 10^{-5} M tamoxifen. The effects were irreversible. No significant effect was obtained with 10^{-7} M of either drug. The results suggest that at least part of the chloride secretion occurs transcellularly through a diffusional pathway in the membrane.

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EFFECTS OF Zn^{2+} ON THE RESPONSE OF THE STRIATUM TO SENSORY-MOTOR CORTEX IN THE RAT. P1 49
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Melatonin inhibits the excitatory response of the striatum to sensory-motor cortex stimulation. The excitatory response of the striatum cortex is mediated by glutamate. In a series of studies, we have shown that this response may be mediated glutamate receptor of NMDA type. If the inhibition produced by melatonin had similar effects than those of NMDA antagonists, it is possible that melatonin effects are mediated by NMDA receptors; if melatonin and NMDA antagonist had accumulative effects, both compounds used different receptors.

Extracellular unit recording were made in 31 striatal neurons. Three microiontophoretic pipettes were attached to the recording electrode. First, basal response was recorded and peristimulus time histogram was performed. Then melatonin and Zn^{2+} were iontophorised. Both substances produced a significant attenuation of the excitatory response of the striatum to sensory-motor cortex. Melatonin produced attenuation in 64.3 % of the neurons, and Zn^{2+} in 64.5 % of the neurons. Melatonin is a natural compound and the possibility that melatonin may be used as NMDA antagonist has important implications.

INTERACTION BETWEEN CALCIUM IONOPHORE A-23187 AND MELATONIN IN THE RAT STRIATUM. P1 50
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In rat striatum, melatonin significantly inhibits the excitatory response of the striatum to sensory motor cortex stimulation. This inhibition may explain the antiparkinsonian and antiepileptic effects of melatonin. In other works, we have suggested that the inhibitory effects of aMT are mediated by Ca^{2+}.

In order to study if this ion is related to melatonin action, electrophysiological and microiontophoretical experiments were made in the rat striatum. Melatonin iontophoresis produced an inhibition of the excitatory response in 64.3 % of the striatal neurons evoked by sensory motor cortex stimulation. On the other hand, A-23187 iontophoresis produced a significant increase in the excitatory response. When melatonin plus A-23287 was ejected by iontophoresis together, the inhibition produced by melatonin disappeared, and the excitatory response returned to control levels.

These results suggest that the inhibitory effects of melatonin in the striatum are mediated by Ca^{2+}.

P1 51 PHARMACOLOGIC REVERSAL OF MEMORY DYSFUNCTION AFTER CHRONIC BRAIN ISCHEMIA IN AGED RATS. J. C. de la Torre, N. Nelson, and R. J. Sutherland. Division of Neurosurgery and Department of Psychology, University of New Mexico, Albuquerque, N.M. (U.S.A). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 120, 1997.

We reported that a combination of a free radical scavenger dimethyl sulfoxide (DMSO) and a glycolytic intermediate, 1,6- fructose diphosphate (FDP) can reduce sensory motor and neural damage following severe head injury in mice[1]. Each agent alone, did not modify the brain injury outcome. In the present study, aging rats were subjected to chronic brain ischemia by bilateral carotid artery occlusion (2-VO) or no occlusion (NO-VO). Twelve weeks after 2-VO, all rats showed memory impairment. DMSO-FDP was injected daily on week 13 for 7 or 14 days. Significant improvement of memory function was observed at both time points. On week 14 or 15, treatment was discontinued and rats were retested for memory function. All previously treated rats showed a return of memory impairment to pretreatment levels. No neuronal damage was observed between treated or untreated groups. These results suggest that increasing glycolytic activity and reducing free radicals in brain can reverse memory-induced deficits secondary to cerebrovascular insufficiency. This data may be relevant to age-related neurodegeneration and human dementia.

[1]de la Torre J. C: *Neurosurgery*, 37:273, 1995.

P1 52 INTRACEREBRAL NAVIGATION AND RECONNAISSANCE OF TUMORAL TEXTURES ASSISTED BY INTRASURGERY ECHOGRAPHY. A. Gutiérrez, C. López-Murciano, M. Arias, I. García-Fortea, A. Rengel, F. Martín-Guerrero and R. González-Carrascosa. Dpto. Neurocirugía. Hospital Regional, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 120, 1997.

We have studied different types of subcortical brain tumors using intrasurgery echography improved by means of a system of densitometric color analysis. This technique allows to locate in the surgical field the tumoral lesions without carrying out lesive maneuvers of detection, thus reducing the size of tissue corticotomy. The degree of tumoral removal was evaluated using control echography.

The technique has allowed us to evaluate and to detect the regions fixing high levels of contrast in the MR with Gadolinie, thus facilitating the recornnaissance of the tumoral texture. The method allows to locate and position in the operative field the tumoral region, analyzing the different grades of activity of the tumor in order to use a more radical exceresis in the zones with more activity. This methodology has also allowed us to define with a better precision the limits of tumors with a high degree of infiltration (interface tumor/edema and edema/normal tissue). In summary the Intraoperative Echography assisted by means of densitometric color analysis represents a valid system for intracerebral navigation that contributes with on line and not artefacted frames.

DENSITOMETRIC ANALYSIS OF THE BRAINSTEM IN THE POST-TRAUMATIC CEREBRAL EDEMA. P1 53

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 121, 1997.

In the present work we have studied the densitometric variations present in the brainstem of 48 patients that have suffered a severe head trauma and that died as a consequence. In this study we have evaluated brain CTs effected in the moment of the admission to the Hospital. Using a system of Densitometric Analysis with Color Conversion (DACC), we have verified the presence of hyperintense densitometric regions at the level of the lower brainstem which are correlated with a greater neurological deterioration of the patient. The main locations of these hyperintense densitometric zones have been observed at the level of the middle regions of the brainstem and in the vicinity of the IV ventricle.

The study shows a close relationship between the neurological status of the patient with edema and the presence of these densitometric lesions that, to our judgement, represent alterations in the irrigation of the brainstem, that topographically coincides with the regions involved in cardiorespiratory control.

THE NEURONAL DENSITY OF THE MAMMILLARY BODY IS IRREVERSIBLY DECREASED AT BIRTH IN RATS AFTER EMBRYONIC EXPOSITION TO ETHANOL. P1 54

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In man, the exposition to ethanol during the embryonic period leads to the Fetal Alcoholic Syndrome. We wanted to know if the hypothalamic mammillary bodies are particularly affected in this syndrome, as it is the case in the Korsakoff's syndrome (an amnesic syndrome occasioned by chronic alcoholism). The mammillary bodies are integrated in many different neuronal circuits (limbic, extrapyramidal, olfactory, of memory and behaviour), and their alteration may explain at least partly the neurological consequences of fetal alcoholism.

We measured the neuronal density (as the most objective value to compare different ages and groups) in the mammillary body of young rats whose mothers had been chronically exposed to ethanol, and compared it to the one obtained in control rats.

In all studied ages (postnatal day 5 to 21), the ethanol exposed rats display less neuronal density in the studied nuclei (medial mammillary nucleus, pars medial and pars posterior) than control rats. This decrease in cellular density is more conspicuous in the pars posterior. In controls, a maximal cellular density is reached around postnatal day (P)5; ethanol exposed rats fail to reach a similar density. In both groups the cellular density decreases until P 12, and then slightly increases until P 16, but the total cell number is unaffected.

In conclusion, the mammillary bodies of rats exposed to ethanol during gestation are affected at birth; and they fail to recuperate postnatally even if they grow in normal conditions.

P1 55 GLUTAMATERGIC PATHOPHYSIOLOGY AND CHRONIC NEUROLEPTICS.

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Neuroleptic drugs (e.g. haloperidol) are effectively utilized in the long term treatment of schizophrenia, but their use has been hampered by a high incidence of motor side effects. Various theories have been suggested in order to explain this drug-induced pathophysiology, including the slow onset of excitotoxicity in basal ganglia nuclei. In order to determine the possibility that overactivation of striatal glutamate pathways plays a role in the development of motor side effects, intracranial microdialysis was applied in an animal model of chronic neuroleptic effects. HPLC measurement of extracellular glutamate levels in rats showed that chronic (months), but not subchronic (weeks), typical neuroleptic administration increased striatal glutamate levels. This effect was not seen with administration of clozapine, a drug devoid of motor side effects. In addition enhanced glutamate levels were not noted in other brain regions. A model is proposed in which it is hypothesized that prolonged neuroleptic treatment leads to an increase in corticostriatal glutamate release as well as a reduction in glutamate reuptake transport.

P1 56 NEUROTENSIN RECEPTORS MEDIATE THE EFFECTS OF NEUROTENSIN AND NEUROMEDIN N ON SELF-STIMULATION OF THE RAT PREFRONTAL CORTEX.

R. Fernández, H. Chaatouf, R. Montes and J. M. R. Ferrer. Dpto. de Fisiología, Facultad de Medicina, Granada (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 122, 1997.

Intracortical microinjections of neurotensin (NT) and neuromedin N (NN) selectively decreased intracranial self-stimulation (ICSS) of the medial prefrontal cortex (MPC) in the rat. To elucidate whether this effect is mediated by NT receptors or by the formation of NT-dopamine complexes, we investigated the effects on ICSS of intracortical microinjections of NT (1-11), an NT fragment that forms extracellular complexes with dopamine but does not bind to NT receptors. We also studied the effects of peripheral administration of SR 48692, a selective antagonist of NT receptors, on the inhibition of ICSS produced by the intracortical administration of NT and NN. Male Wistar rats were bilaterally implanted with monopolar electrodes and 23 ga guide cannulae in the MPC. Microinjections of NT (1-11) at doses of 10, 20 and 40 nmol into the MPC did not change the basal ICSS rate of this area. The intraperitoneal administration of SR 48692 at doses of 0.08 and 0.16 mg kg^{-1} 30 min before the microinjection of 10 nmol of NT or 20 nmol of NN into the MPC, antagonized the inhibition of ICSS produced by the neuropeptides. These results demonstrate that the inhibitory effect of NT and NN on ICSS is mediated by NT receptors.

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GLUTAMATE MEDIATES THE NEUROTRANSMISSION OF THE GENICULO-CORTICAL PATHWAY IN THE RAT. P1 57

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The participation of glutamate in the neurotransmission of the geniculo-cortical pathway in the rat was investigated. Wistar rats of both sexes were used in this study. In a first set of experiments, we studied the effects of kynurenate, an antagonist of glutamate receptors, on visual cortex neurons monosynaptically excited by electrical stimulation of the dorsal lateral geniculate nucleus (dLGN). Microiontophoresis of kynurenate into the visual cortex selectively inhibited the excitatory response of these neurons to dLGN or the optic tract (OT) stimulation. The inhibition of kynurenate was dose-dependent. In a second set of experiments, we studied the effects of electrical stimulation of the dLGN and the OT on the release of amino acids in the rat visual cortex *in vivo*. Using the push-pull method, we perfused a discrete region of the visual cortex with artificial cerebrospinal fluid and the amino acids content of the perfusates was analysed by high performance liquid chromatography. Stimulation of either dLGN or the OT increased significantly glutamate release in the visual cortex. The rest of the amino acids studied did not show significant changes. These results strongly suggest that glutamate is the neurotransmitter of the geniculo-cortical pathway in the rat.

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ATRIAL TACHYARRHYTHMIAS AND THYROID HORMONES: THE REVERSE WAY. P2 1

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There are many studies about the influence of thyroid and adrenal hormones on cardiac conduction. However, the reverse way, that is, the possible influence of tachyarrhythmia on these hormones in previously euthyroid patients, has not been established yet. 43 patients were studied. At the time of admission, 33 of them presented atrial tachyarrhythmia lacking any thyroid pathology and their treatment did not include amiodarone. The control group was made up by 10 patients suffering sinus tachycardia with no thyroid pathology either. Levels of TSH, FT4 and Cortisol in both groups serum were determined by electrochemiluminiscence, being used as reference values: TSH 0.35-5.50 μIU/mL; FT4 0.80-1.50 ng/dL; Cortisol 4.30-22.40 μg/dL. Results showed that there were meaningful differences in TSH (being lower in the experimental group); FT4 did not change and finally, serum Cortisol increased in both groups with hardly any difference. The results point to the fact that first, atrium is more sensitive toward these hormones than the ventricle and second, the amount of circulating hormones is not a determining factor in cardiac conduction, but those hormones located in the cardiac tissue instead.

REGULATION OF THYROID HORMONES SECRETION BY GLUTAMIC ACID AND GLUTAMATERGIC AGONISTS IN FREELY-MOVING RATS. P2 2

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The present study was designed to investigate the role of Glutamic acid and its agonists on serum Thyroid Hormones (T3 and T4) levels in conscious rats. Male Sprague-Dawley rats weighing 250-300 g were used for all experiments. The animals received an intraperitoneal injection of Glutamic acid (20 and 25 mg/kg), Domoic acid (DOM) (1 mg/kg), Kainic acid (30 mg/kg) and NMDA (20 and 25 mg/kg). Permanent cannulation of the jugular vein was used to take continuous blood sampling (0, 30, 45 and 60 min) from the general circulation in the freely-moving rats. Thyroid hormones were measured by Enzymomunoassay. We found that serum concentrations of thyroid hormones increased in the treated animals, reaching values significantly higher than in control males. Glutamic acid and its non-NMDA agonists stimulate the release of thyroid hormones (20-30 %) more than NMDA agonists 30, 45 and 60 min after treatment. The results show that the effect of Glutamic acid on serum thyroid hormones is mediated by both receptor types.

P2 3 RELATIONSHIP BETWEEN SELF-ESTEEM, OTHER PERSONALITY TRAITS AND THE LYMPHOPROLIFERATIVE RESPONSE TO ACADEMIC STRESS. M. I. González-Quijano, M. Martín*, S. Millán** and A. López-Calderón**. Dptos. *Enfermería, Psicología Médica, and **Fisiología, Universidad Complutense, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 126, 1997.

Personality is considered as a source of individual variability that can be related to the differences in the response to stress. Our aim was to examine the effect of academic stress on the lymphoproliferative response to mitogens, and the influence of self-esteem and other personality traits on this response. Undergraduate male medical students were tested in November and March, periods without academic pressure and in June during final examinations. Lymphoproliferative response to phytohemagglutinin A (PHA) and blood pressure were measured. The following personality traits: anxiety, independence, cortertia and extraversion were evaluated by the Cattell Questionnaire and self-esteem by the Rosenberg Scale. The lymphocyte response to PHA was similar in November and March and significantly ($p < 0.05$) reduced in June. A negative correlation ($p < 0.05$) was found between self-esteem and the decrease in the lymphoproliferation caused by academic stress. The same correlation was found in relation to the extraversion. Blood pressure did not correlate with the personality traits analyzed, except in relation to anxiety which showed a positive correlation ($p < 0.05$) with systelic blood pressure. Our results indicate that self-esteem and extraversion can be considered as moderators of the immunological response to stress.

This work was supported by U.C.M. grant n° PR295/95-6090.

P2 4 NOREPINEPHRINE MODULATES CHEMOTAXIS OF LYMPHOCYTES FROM MICE. CHANGES WITH AGING. E. Ortega, J. J. García and M. de la Fuente*. Dept. Fisiología, Fac. de Ciencias, UEX, Badajoz and *Dept. Biología Animal, Fac. de Biológicas, UCM, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 126, 1997.

Primary and secondary lymphoid organs present noradrenergic inervation. In addition, lymphocytes present receptors for norepinephrine (NE). Thus, it is accepted that norepinephrine modulates lymphocyte function. However, the effect of NE on chemotaxis has not been elucidated. On the other hand, a decline of the lymphocyte function with aging has been reported. In the present work we evaluated the effect of NE on chemotactic capacity of lymphocytes from spleen, thymus and axillary lymph nodes; in young (22 weeks) and in old (72 weeks) mice. Chemotaxis was evaluated with the technique of Boyden. Our results show that NE decrease chemotaxis at 10^{-5} M, but increase it at 10^{-3} M in lymphocytes from young animals. However, after incubation of lymphocytes from old mice with all concentrations of NE evaluated (10^{-12}-10^{-3} M) we observed an increased, chemotaxis. We conclude that NE modulates chemotactic activity of lymphocytes in a different manner in young and in old individuals.

This work was supported by grant 96/1059 from FISss (Spain).

IN VITRO EFFECT OF MELATONIN UPON SUPEROXIDE ANION LEVELS AND SUPEROXIDE DISMUTASE ACTIVITY IN RING DOVE HETEROPHILS. P2 5

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A functional connection between the pineal gland and the immune system in mammals and birds has been established. Recently some findings concerning melatonin, the chief hormone of the pineal gland in vertebrate, as a free radicals scavenger and general antioxidant. This *in vitro* study investigates the possible antioxidant action of 100 μM of melatonin in heterophils isolated from adult male and female ring dove (*Streptopelia risoria*). Thus, the superoxide anion levels (O^-_2) and the superoxide dismutase (SOD) activity as indicator of the metabolic burst of heterophils after ingestion of inert particles (latex beads) was evaluated. The heterophils O^-_2 levels, measured by nitroblue tetrazolium (NBT) reduction test, was decreased after incubation in the presence of the hormone. In addition, melatonin look to stimulate the enhancement produced by latex beads on the heterophils SOD activity (U-525 nm), measured by spectrophotometric assay. In conclusion, our findings corroborate that the neurohormone melatonin could be considered as a antioxidant.

EFFECTS OF PERINATAL EXPOSURE TO (-)Δ^8 TETRAHYDROCANNABINOL-DIMETYL-HEPTYL (HU-210) ON THE IMMUNE AND ENDOCRINE SYSTEMS IN ADULT MALE RATS. P2 6

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Animal models have revealed that psychoactive cannabinoids induce characteristic behavioral, endocrinological and immune alterations in adult animals. However, little is known about the effects of perinatal administration of cannabinoids on the adult offspring. In the present study we examined the effect of the highly potent cannabinoid receptor agonist HU-210 during gestation and lactation on endocrine and immune systems of adult animals. For this purpose HU-210 was administered in a daily single oral dose (1, 5 or 25 μg/kg bw) from the fifth day of gestation until weaning. Animals were sacrificed on day 70 of life, and plasma PRL, GH, IGF-I and corticosterone were analyzed by RIA. Spleen lymphocytes were evaluated for their proliferative responses *in vitro* to T- and B-cell mitogens. Subpopulations of T-cells in blood, spleen and thymus were also examined. Hormone levels were not modified after HU-210 treatment. Splenic lymphocytes from HU-210 treated animals showed no significant differences in their proliferative responses to the mitogens concanavalin A or to lipopolysaccharide (LPS). However, proliferative response to LPS in blood was significantly increased. Lower dose of HU-210 decreased the number of $CD4^+CD8^-$ thymocytes and increased the percentage of $CD4^-CD8^+$ peripheral lymphocytes. The number of lymphocytes T helper in the spleen was diminished in rats treated with the highest HU-210 dose. In conclusion, maternal administration of HU-210 did not strongly alter the endocrine and immune systems of adult rats.

This work was supported by FIS 94/0299 and CICYT SAF 94-0465 (Spain).

P2 7 MONOAMINE TURNOVER IN THE TROUT (*Oncorhynchus mykiss*) PITUITARY DURING GONADAL RECRUDESCENCE. M. Aldegunde, R. Hernández-Rauda, J. Otero, I. Míguez and J. M. Míguez. Laboratorio de Fisiología Animal, Facultad de Biología, Universidad de Santiago de Compostela (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 128, 1997.

In fishes the pituitary is directly innervated by aminergic fibers, and it is particularly known that DA modulates GtH (gonadotropin) release. In previous works, we have shown a decreased DOPAC/DA ratio during gonadal recrudescence[1] in female rainbow trout. The aim of the present work was to determine the variations in catecholamine (NA, DA) and serotonin (5-HT) turnover in the pituitary of male and female trouts during different states of gonadal development. The levels of DA, DOPAC, NA, 5-HT and 5-HIAA were measured by HPLC-EC[1] and catecholamine turnover was determined using α-MpT (250 mg/kg, i.p.). Results showed a drop in the pituitary dopaminergic turnover (μg/g/h) during the late states of exogenous vitelogenesis as compared to early states of exogenous vitelogenesis. The turnover of NA in the pituitary was very low, suggesting a scarce functional implication in gonadotropin release, similarly to that observed for 5-HT. In males, the most important result was a possible increase in dopaminergic turnover, but no changes were observed in NA and 5-HT metabolism.

1. Hernández-Rauda, R., J. Otero, P. Rey, G. Rozas and M. Aldegunde. *Gen. Comp. Endocrinol.*, 103, 13, 1996.

P2 8 EFFECT OF 3,3',5-TRIIODO-L-THYRONINE, CORTISOL AND GROWTH HORMONE ON SALINITY TOLERANCE IN *FUNDULUS HETEROCLITUS*. J. M. Mancera and S. D. McCormick*. Dpto. Biología Animal, Facultad Ciencias Mar, Univ. Cádiz (Spain), and *S. O. Conte Anadromous Fish Research Center, Turners Falls, Massachusetts (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 128, 1997.

The capacity of 3,3',5-triiodo-L-thyronine (T3), cortisol and ovine growth hormone (oGH) to increase hypoosmoregulatory capacity in the euryhaline teleost *Fundulus heteroclitus* was examined. Fish acclimated to brackish water (BW, 10 ppt) were injected with a single dose of hormone, transferred to seawater (SW, 35 ppt salinity) 10 days post-injection and sampled 24 h post-transfer. Cortisol or oGH improved the ability to maintain plasma osmolality and to increase gill Na^+,K^+-ATPase activity. Treatment with T3 alone did not increase salinity tolerance or gill Na^+,K^+-ATPase activity. Cortisol plus T3 showed a cooperation for increasing gill Na^+,K^+-ATPase activity and hypoosmoregulatory ability. However, this interaction was not observed in fish treated with T3 plus oGH. The results confirm the role of cortisol and GH as seawater promoting hormones and suggest a role of T3 in seawater acclimation in *Fundulus heteroclitus*.

DIABETES INDUCED ENZYMATIC CHANGES IN RAT INTESTINE. I. M. Martínez, J. E. Campillo and M. A. Tormo. Dpto. Fisiología, Fac. Medicina, Badajoz (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 129, 1997. P2 9

Diabetes produces modifications in the intestine. We measured the activities of Lactate dehydrogenase (LDH), Phosphofructokinase-1 (PFK-1), total (T) and active (A) Pyruvate dehydrogenase (PDC) in the proximal and distal small intestine of normal (NR) and non-insulin-dependent diabetic (DR) rats. RESULTS: The concentration of proteins (mg/mL) in the complete small intestine of DR was lower ($p = 0.001$) than in NR (14.95 ± 0.58 and 19.65 ± 0.78). The enzyme values are expressed per g wet tissue weight (mean ± SEM):

	Intestinal segment	PFK- 1 (UI/g)	LDH (UI/g)	PDC_T (µmol Ac.CoA/min/g)	PD_A
N R	Proximal	12.40 ± 1.04	335.75 ± 20.58	91.00 ± 2.13	5.08 ± 2.13
	Distal	9.38 ± 1.05	165.63 ± 16.37[a]	11.83 ± 2.80	5.88 ± 1.59
D R	Proximal	10.25 ± 1.43	253.72 ± 22.33[*]	3.60 ± 1.04	2.31 ± 0.47
	Distal	5.09 ± 0.71[*a]	131.15 ± 12.60[a]	6.17 ± 2.35	5.14 ± 1.44

[*]$p < 0.05$ vs *normal* [a]$p < 0.05$ vs jejunum. n = 6, for each experimental group.

The protein concentration is less ($p = 0.001$) in RD than in RN. The LDH and PFR-1 activities are greater ($p < 0.05$) in the proximal than in the distal intestine in both NR and DR. The proximal intestine LDH activity is less ($p < 0.05$) in DR than in NR. The distal intestine PFK-1 activity is less ($p < 0.05$) in RD than in RN. *Acknowledgement:* Junta de Extremadura grant (Spain).

EFFECT OF THE ORAL ADMINISTRATION OF A DECOCTION OF FIG LEAVES UPON GLYCEMIA IN IDDM. M. D. Torres, F. Hawkins, A. Serraclara, E. Domínguez, C. Pérez and J. E. Campillo. Dept. Physiology, Faculty Medicine, Badajoz and Serv. Endocrinology, Univ. Hospital 12 Octubre, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 129, 1997. P2 10

The effect upon diabetes control of a decoction of fig. leaves (*Ficus Carica*) as a supplement to breakfast was studied in IDDM patients. 10 volunteers (6 men, 4 women) with IDDM were recruited, age 22-38 yrs, BMI: 20.8±3 kg/m^2, HbA 1c 7.6±0.9 % and mean duration of diabetes of 9±6.3 yrs. They were managed with their usual diabetes diet and twice daily insulin injection. Patients were given during one month a decoction of Fig leaves (FL) and during another a non-sweet commercial tea (TC), divided in two groups (n = 5) with random allocation and crossover design. A standard breakfast was given at the beginning and end of each month-run. C-peptide, pre- and 2 h postprandial glycemia, HbA 1c, cholesterol, lipid fractions and hematology data, were analyzed in each visit. Glycemic profiles (7 per day/week) were recorded by patients. Two patients had intolerance dropout. Postprandial glycemia was significantly lower during supplementation with FL (8.7 ± 4.22 mmol/l) vs CT (16.32±2.5) ($p < 0.001$) without preprandial differences (8.17±2.5 and 10.5±2.4 respectively). Medium average capillary profiles were also lower in the two subgroups of patients during FL (9.2±1.3, $p < 0.05$ and 8.7±7.9 mmol/l) vs CT (13.6±0.7 and 12.3±1.5 mmol/). Average insulin dose was 12 % lower during FL in the total group. The addition of FL to diet in IDDM could be of help to control postprandial glycemia. Acknowledgements: "Junta de Extremadura" grant (Spain).

P2 11 VARIATIONS IN SUBCELLULAR STRUCTURES OF THYROID RAT CELLS DURING POSTNATAL DEVELOPMENT. L. Vidal, G. Mata, C. Valverde, A. Peláez, C. Duarte and I. Pérez de Vargas. Dpto. de Morlología Normal y Patológica, Facultad de Medicina, Málaga, (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 130, 1997.

We investigated changes in secretory organelles from thyroid follicular cells, follicular epithelial cells and C cells, during postnatal development. The study was carried out on 11, 21 and 35 day old Wistar rats whose thyroid glands had been processed using EM techniques. Changes in secretory organelles were determined by stereoloical methods to establish morpho-functional correlations. In follicular epithelial cells all follicle parameters show very high values in 11 day old rats, decreasing by the 21st day, especially mitochondria, and recovers again by the 35th day. The results obtained show that follicular epithelial cells reach maturity early during postnatal development and present fluctuations in their activity during the period studied. C cells are in a growing phase in the 11 day old animals, with plenty of free ribosomes and RER but with little secretion. From the 21st day they are found to be in a mature phase, the Golgi apparatus has increased as has numerical density in secretory granula. Parameters in these organelles reach maximum values by the 35th day when C cell maturity becomes stable. The results seem to indicate that C cells mature later than follicular epithelial cells and their secretory activity increases in a progressive manner during the period studied.

P2 12 EIA SANDWICH FOR MEASURING SERUM AND PERITONEAL FLUID LH LEVELS IN WOMEN. M. Illera, J. C. Illera, C. Munro*, G. Silván, M. J. Illera and B. Lessey**. Departamento de Fisiología Animal, UCM, Madrid (Spain), *Dept. Health, Popul. and Reprod., UCD, and **Dept. Obst. &Gyneacol., UNC (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 130, 1997.

A rapid, sensitive, sandwich EIA was validated for measuring luteinizing hormone (LH) in peritoneal fluid (PF) and serum from fertile (n = 20) and infertile women (n = 20) with endometriosis for the purpose of developing a complementary diagnosis tool. Microtiter plates were coated with 100 µl (250 ng/ml) of a well-characterized monoclonal antiserum (518B7, Roser, UCD, USA) generated against bovine (LH) which was used as the capture antiserum in the solid-phase EIA. The purified gamma globulin fraction of a polyclonal antiserum raised in rabbits against human chorionic gonadotropin (hCG CR-121) was conjugated to sodium-periodate activated horseradish peroxidase (HRP), and used as the second antibody signal of the sandwich assay at a dilution of 1:1,500. Standards (100 µl of hLH, NIADDK-hLH-I-3, diluted in MOPS buffer) and samples (100 µl of human PF or serum) were incubated for 2 h with the solid phase antibody. Second antibody enzyme label (Anti-h-HCG:HRP, R76) was incubated for further 2 h. Substrate used was TMB. The least detectable concentration of hLH was 0.018 ± 0.06 ng/ml. Mean intra- and inter-assay coefficients of variation (%) were 6.5 and 9.8, respectively. Mean LH serum values from fertile and infertile women were 4.50 ± 0.60 and 11.54 ± 1.10 ng/ml, respectively and PF values were 5.76 ± 0.68 and 73.48 ± 5.51 ng/ml, respectively. This sandwich EIA for human LH offers a stable, rapid, and improved system for the measurement of serum and PF LH concentrations and demonstrates its ability as a complementary diagnosis tool for detection of endometriosis in women.

ANGIOTENSIN II BINDING SITES INTERNALISATION FROM PLASMA MEMBRANES IN ISOLATED RAT HEPATOCYTES. P2 13

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Angiotensin II (AII) provokes a rapid internalisation process of its receptor from plasma membrane in isolated rat hepatocytes. After 10 min stimulation with AII, plasma membrane lost about 60 % of ^{125}I-AII binding capacity. Internalisation process was blocked by phenylarsine oxide (PAO), while okadaic acid did not have a preventive effect on AII-receptor complex sequestration. These data suggest that AII-receptor internalisation would probably be independent of phosphorylation/dephosphorylation cycle of critical serine/threonine residues of the receptor molecule. To establish a relationship between the AII-receptor sequestration and the physical properties of the AII-binding sites, ^{125}I-AII-receptor-complex profiles were analysed by isoelectric focusing. In plasma membranes two predominant AII-binding sites, migrating to pI 6.8 and 6.5, were found. After exposures to AII, cells lost ^{125}I-AII-binding capacity to AII-receptor complex migrating at pI 6.8 which was prevented in PAO-treated cells. Pretreatment of hepatocytes with okadaic acid did not modify AII-receptor complex profiles, indicating that the binding sites corresponding to pI 6.5 and pI 6.8 do not represent a phosphorylated and/or non phosphorylated form of the AII-receptor. The results showed that AII-receptor-complex isoform at pI 6.8 represents a functional form of the AT_1 receptor subtype. Further studies are necessary to identify the AII-related nature of the binding sites corresponding to pI 6.5.

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THE PATTERN OF TESTOSTERONE REPLACEMENT INFLUENCES THE RECOVERY OF THE STIMULATORY EFFECT OF CLONIDINE (CLO) ON GROWTH HORMONE (GH) SECRETION IN ORCHIDECTOMIZED RATS. P2 14

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The effects of the agonists of alpha-2 adrenoreceptors CLO (150 μg/kg) and xylazine (8 mg/kg) on GH release have been analysed in neonatal, young and adult males at different times (3 days, 2 and 12 weeks) after orchidectomy. In addition, we have assessed the effects of different patterns of replacement with testosterone or α-diol. Our results show that: 1) orchidectomy abolished the stimulatory effect of clonidine and xylazine at all ages tested; 2) testosterone, but not α-diol, implanted at the moment of orchidectomy prevented the loss of CLO effect in orchidectomized males, but testosterone-delayed administration was unable to restore the effectiveness of CLO inducing GH release. We conclude that orchidectomy at all ages tested abolishes GH secretion induced by α_2 agonists, which suggests that the functionality of α_2-adrenergic receptors involved in the control of GH secretion is critically dependent on a permanent exposure to testosterone in males. The testosterone effects on CLO-induced GH secretion were exerted by itself or after hormone aromatization to estradiol.

P2 15 ROLE OF PROGESTERONE-INDUCED ACTIVIN PRODUCTION IN THE FSH SECONDARY SURGE OF THE CYCLIC RAT. M. Tébar, J. Th. J. Uilenbroek*, R. H. N. van Schaik*, A. Ruiz, F. H. de Jong* and J. E. Sánchez-Criado. Dpto. de Fisiología, Facultad de Medicina, Córdoba (Spain), and *Dept. of Endocrinology and Reproduction, EUR, Rotterdam (The Netherlands). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 132, 1997.

In the first experiment we studied the effect of an antagonist of LHRH (LHRHa) on proestrus on ovulation, serum gonadotropins and inhibin as well as inhibin/activin subunit mRNAs ovarian expression measured by *in situ* hybridization at early estrus. In the second experiment, the same parameters were analysed in LHRHa-treated rats injected with oLH, progesterone (P), the antiprogesterone RU486 or RU486 and oLH. Saline-injected rats had low mRNAs and serum levels of inhibin and the FSH surge on estrus. LHRHa prevented the fall in mRNAs and serum levels of inhibin and blocked the FSH secondary surge, while oLH reversed these effects. P increased mRNAs hybridization intensity when compared to LHRHa-injected rats and partially restored the surge of FSH. RU486 did not modify the effect of oLH on either mRNA or serum levels of inhibin, but blocked the FSH surge. These results indicate that, in the cyclic rat, the proestrous P secretion is not involved in the fall of ovarian inhibin synthesis and secretion. Furthermore, the action of P on another factor, possibly pituitary activin, seems to be necessary to stimulate the secondary surge of FSH in combination with the LH-induced drop of inhibin production.

P2 16 SYNERGISM BETWEEN FOLLICULAR AND LUTEAL PROGESTERONE IN 5-DAY CYCLIC RATS. J. E. Sánchez-Criado, A. Ruiz, M. Tebar and J. A. M. Mattheij*. Dpto. de Fisiología, Facultad de Medicina, Córdoba (Spain), and *Dept. of Human and Animal Physiology, Agricultural University, Wageningen (The Netherlands). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 132, 1997.

The length of the ovarian cycle in the rat is determined by the duration of progesterone (P) secretion from the corpora lutea (CL) during diestrus. On the other hand, the action of P secretion from the preovulatory follicles on proestrus is also responsible for the cycle length in 4-day cyclic rats. To study whether follicular and luteal P participate in the maintenance of 5-day cyclicity, we investigated the effects of the anti-P RU486 on estrous cycle length and on the serum concentrations of luteinizing hormone (LH) in 5-day cyclic rats and in 4-day cycle experimentally induced by the dopamine agonist CB154. Furthermore, serum concentrations of P on the day of ensuing ovulation were measured to see whether activation of the CL function after treatment with RU486 had occurred. Both 5-day and CB154-injected rats had a 3-day estrous cycle after RU486 on proestrus, while RU486 on estrus shortened by 1-day the estrous cycle length in 5-day but not in CB154-injected rats. Serum concentrations of LH increased and the LH surge decreased after RU486 in both cycle types. These results indicate that the actions of both follicular and luteal P synergize in maintaining the length of the estrous cycle in 5-day cyclic rats.

EFFECTS OF RU486 ON THE PRL SECRETION IN CYCLIC AND PSP RATS. P2 17

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Administration of RU486, a synthetic steroid with antiprogestagenic and antiglucocorticoid activity has been found to alter the secretion of prolactin (PRL) in cyclic and pseudopregnant (PSP) rats. The secretion of PRL is modulated by estrogens and progesterone (P), as well as by glucocorticoids (G). To investigate whether the effects of RU486 on PRL secretion in cyclic and PSP rats are the result of its anti-P or anti-G actions, cyclic and PSP rats were treated with RU486 and compared to those treated with an anti-P lacking anti-G action (Organon 31710). Both antiprogestagens were administered over 12 consecutive days starting on estrus (cyclic rats) or on the first day of PSP. Controls were cyclic and PSP rats injected with oil. Rats were decapitated at 0800 or 1800 h on day 13. Trunk blood was collected and serum levels of LH, E_2, P and PRL were determined by RIA. Results from these experiments indicate that G suppress, along with P, the E_2-stimulated PRL secretion. In fact, in the presence of the same serum levels of LH, Organon 31710 increased PRL secretion on day 13, and hence P production, both in cyclic (0800 and 1800 h) and PSP rats (1800 h) in a lesser extent than RU486 did. Conversely, the nocturnal PRL surge seems to be not dependent on G, but only on P.

PROLIFERATIVE ACTIVITY OF PREOVULATORY FOLLICLES AND NEWLY-FORMED CORPORA LUTEA IN CYCLING RATS FROM LATE PROESTRUS TO EARLY ESTRUS. P2 18

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Adult cycling rats were studied from 1600 h in proestrus to 0700 h in estrus in order to correlate the cyclic hormonal changes with the proliferative activity of preovulatory and postovulatory (i.e., newly-formed corpora lutea) follicles. The proliferative activity was studied by immunohistochemical demonstration of DNA-incorporated 5-Bromodeoxyuridine (BrdU). The preovulatory LH surge was found at 1830 h in proestrus, and this was coincident with an increase in FSH, PRL and progesterone concentrations, whereas 17β–estradiol and testosterone concentrations were decreased. From 0200 h in estrus onward there was a progressive decrease in the concentrations of the different hormones, except in those of FSH that continued to be increased. The proliferative activity of granulosa cells (GC) in large preovulatory follicles decreased in proestrus, reaching a minimum at 2100 h. However, a proliferative wave was found in the GC of preovulatory follicles at 0200 h in estrus and in those of newly-formed corpora lutea at 0700 h in estrus. These results suggest that the proliferative activity found from 0200 to 0700 h in estrus is critical for the establishment of the number of steroidogenic cells in the cyclic corpus luteum.

P2 19 EFFECTS OF N-METHYL-D ASPARTATE (NMDA) AND KAINIC ACID (KA) ON PROLACTIN (PRL) SECRETION IN PREPUBERTAL FEMALE RATS. L. Pinilla, D. González, M. Tena-Sempere, R. Aguilar and E. Aguilar. Dpt. of Physiology, Faculty of Medicine, University of Córdoba (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), **53** (1), 134, 1997.

Serum PRL concentrations and hypothalamic and pituitary dopamine (DA) and serotonin (5-HT) concentrations have been measured in female rats injected with NMDA, KA or the two blockers of NO synthase (NOS) N_w-nitro-L arginine methyl ester (NAME) and N_w-nitro-L arginine (NA). We have found that PRL release was inhibited 15 min after NMDA and KA administration, an effect probably mediated through the release of hypothalamic DA as shown by the higher pituitary DA concentrations seen after NMDA or KA administration. The inhibitory effect of NMDA was preceded by an increase in serum PRL levels, observed at 5 and 10 min after NMDA administration. NAME alone inhibited prolactin secretion, and both NAME and NA abolished the inhibitory effect of KA, but not that of NMDA. We conclude that administration of NMDA exerted a dual action on PRL secretion: initially DA release was inhibited leading to an increase in PRL secretion which in turn stimulated DA release and decreased serum PRL concentrations. KA also inhibited PRL secretion by releasing DA, an effect blocked by NO synthase inhibitors.

P2 20 ADJUVANT-INDUCED-ARTHRITIS IN RATS INDUCES AN INHIBITION OF GROWTH AND A DECREASE IN SERUM CONCENTRATIONS OF GH AND IGF-I. L. Soto, A. I. Martin and A. López-Calderón. Departamento de Fisiología, Facultad de Medicina, Universidad Complutense Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), **53** (1), 134, 1997.

Adjuvant-induced-arthritis is an experimental model of chronic activation of the immune system which is associated with several metabolic and endocrine modifications, including an activation of the adrenal axis. The aim of this work was to study the effect of chronic inflammation on the GH-IGF-I axis. Arthritis was induced in male Sprague Dawley rats by injection of complete Freund's adjuvant, impairment of body weight gain occurred in arthritic rats from day 14 after injection. On days 18 and 22 rats were sacrificed and serum levels of corticosterone, GH, IGF-I and hepatic concentration of IGF-I were measured by RIA. Arthritic rats had a significant increase in adrenal ($p < 0.01$) and spleen weights as well as in serum concentrations of corticosterone ($p < 0.05$). On the contrary, arthritis significantly decreased body and hepatic weights, serum concentrations of GH ($p < 0.05$) and IGF-I ($p < 0.01$), together with a decrease in hepatic IGF-I content ($p < 0.05$). These results suggest that adjuvant arthritis induces a delay in somatic growth secondary to an inhibition of the GH-IGF-I axis.

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DIFFERENTIAL EFFECTS OF INFANTILE AND JUVENILE ADMINISTRATION OF CYCLOSPORIN A (CsA) ON ADULT SEXUAL BEHAVIOR IN MALE RATS. P2 21

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We have examined the effect of CsA administration to immature male rats on adult sexual behavior and on the hypothalamic-pituitary-testicular function. Male Wistar rats were sc injected with CsA (5 or 15 mg/kg bw) or vehicle, from day 10 to 25 of life (infantile period) or from day 25 to 40 of life (juvenile period). Twenty four hours after the last injection or on day 90 of life, LHRH medial basal hypothalamic (MBH) content, LH, FSH and testosterone (T) plasma levels were determined. Adult animals were evaluated for their sexual behavior, motor activity and behavioral response to novelty. Infantile CsA-treated rats showed a sexual behavior impairment reflected in a prolonged mounting and ejaculation latencies and a reduced number of mounts, while the rats treated in the juvenile period do not show any alteration in their sexual behavior. This effect is not a consequence of an altered motor behavior or an increased reactivity to novelty conditions, as reflected in the normal scores obtained by both, infantile and juvenile CsA-treated animals in the open field test, and in the p-maze. Infantile CsA treatment decreases the MBH content of LHRH, FSH and T levels 24 h. after the last injection. In contrast, juvenile treatment do not modify LHRH content or plasma levels of LH, FSH and T 24 h after the last injection. Plasma T levels of adult rats were not altered by CsA, so the impairment of sexual behavior observed is not induced by decreased T in these animals. The data suggest that gonadal steroids and/or gonadotropin levels in early stages of sexual maturation determine the magnitude of male sexual behavior impairment induced by CsA.

This work was supported by FIS 94/0299 and CICYT SAF 94-0465.

THE MALE-PHEROMONAL INDUCED LHRH RELEASE IN YOUNG ESTROGENIZED PERSISTENT ESTRUS FEMALE RATS IS AN α-ADRENERGIC EFFECT. P2 22

M. M. Cabrera and O. A. Mora. Depto. Fisiología, Fac. de Medicina, UCM, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 135, 1997.

Pheromonal restoration of cyclic activity in aging and young estrogenized persistent estrus (PE) female rats is due to the activation of cyclic hypothalamic LHRH release by male urinary pheromones (*Biol. Reprod.*, 51, 920, 1994). For this study young estrogenized (2 mg of estradiol benzoate subcutaneously) PE Wistar female rats were used. They were divided in four groups with intraperitoneal injection of: I, saline; II, propranolol; III, prazosin, and IV, yohimbine. One hour after the injections, 300 µl of male urine were sprayed every 10 min for one hour at a distance of 2 cm from the nostrils. Rats from each group were decapitated immediately after the last dose of male urine and at 30, 60 and 120 min. The hypothalami were dissected, homogeneized and frozen at -25 °C until utilization for LHRH measure by a double antibody radioimmunoassay method. Hypothalamic content of LHRH in the groups I and II decreased at 30 min reaching to a minimum at 120 min from the urine treatment, while not significant variations were detected in the groups III and IV. These results indicate that the pheromonal effect on LHRH release is produced through the stimulation of α-adrenergic pathways originating in some nervous receptor in the nasal fosa, since the administration of α_1- and α_2-adrenergic antagonists blocks the LHRH release that can be seen after the delivery of saline or a β-adrenergic blocker.

Thanks to DGCYT grant no PB93/0467.

P2 23 SHORT TERM GROWTH: A CONTINUOUS OR A STEPWISE PROCESS? A. Rol de Lama, A. Pérez Romero, C. Ariznavarreta, J. Burmeister*, L. Grasedyck*, J. A. F. Tresguerres and M. Hermanussen**. Dept. Physiology, Medical School, Univ. Complutense Madrid (Spain), and **Aschauhof, Altenhof. *Praktische Mathematik, Univ. Kiel (Germany). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 136, 1997.

Our group started to investigate 3 years ago, the growth pattern of rats using an accurate, non-invasive, lower leg length measurement technique called Microknemometry. Obtained results showed the appearance of an oscillatory pattern with "minigrowth spurts". In order to analyze the growth curves a mathematical model was developed which searches for s-shaped structures screening individual series of daily length measurements, and defining each spurt by its amplitude, inflection point, slope and timing of maximal growth velocity. A total of 143 male and female rats, aged between 24 and 100 days of life were used. Mean amplitude of minigrowth spurts differed significantly between female and male rats, and tended to decline with increasing age. The slope was independent of age, but sex related. Partial GH deficiency induced with neonatal monosodium glutamate treatment, diminished the amplitude and increased the slope, being this effect partially reverted with GH. GH was unable to modify neither slope nor amplitude in normal males. Time between subsequent growth spurts was increased by age. The present study confirms that growth consists on multiple incremental bursts of approximately 4 days.

This work was supported by FISS number 94/0389 (Spain).

P2 24 PINEAL-RELATED CHANGES IN SEROTONIN METABOLISM IN THE SUPRACHIASMATIC, PREOPTIC AND ANTERIOR AND MEDIAL HYPOTHALAMIC NUCLEI. J. M. Míguez, I. Míguez* and M. Aldegunde. Dpto. de Fisiología Animal, Facultad de Biología y Farmacia*. Universidad de Santiago de Compostela (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 136, 1997.

The pineal hormone melatonin has been implicated in the regulation of several endocrine and circadian functions, and the hypothalamic serotonergic system pointed as a target for the integration of the melatonin signal. The aim of the present work was to examine the effect of pinealectomy and melatonin treatments on the metabolism of 5-HT in discrete nuclei of the hypothalamus, including the suprachiasmatic nuclei (SCN), the anterior hypothalamic nuclei (AHN), the lateral and medial preoptic nuclei (LPON; MPON), the ventromedial (VMHN) and dorsomedial (DMHN) hypothalamic nuclei, the paraventricular hypothalamic nuclei (PVHN) and the arcuate nuclei (ARCN). Pinealectomy decreased 5-HT content in the AHN and VMHN, and 5-HIAA content in the preoptic nuclei. Pinealectomy resulted also in a decreased 5-HT synthesis rate in AHN and PVHN, but it enhanced 5-HT synthesis and 5HIAA content in the SCN. The administration of melatonin for ten days to pinealectomized rats reversed most of pinealectomy effects. These data indicate differential effects of melatonin on areas containing serotonergic terminals, which may be relevant for the mode of action of melatonin and its behavioral and endocrine effects.

ALTERATIONS OF PREPUBERTAL RATS OOCYTE CELL CYCLE BY MATERNAL MELATONIN. P2 25

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In the cell cycle the following phases are recorded: M phase, includes the nuclear division (mitosis) and cytoplasmic division (cytokinesis); G_1 phase, with the biosynthetic activities at high rate; S phase, in which the cromosomes replicate; G_2 + M phase, end of the cycle.

A morphometric validation of cellular cycle from ovarian oocytes by flow cytometry was realized. Three groups of prepubertal female rats (25-, 30- and 34-days-old) were used: A) Control, B) offspring of pinealectomized mother rats, C) offspring of melatonin treated mother rats. RESULTS: At 25 days of age, significantly lower value ($p < 0.05$) of G_1 phase cells in the control group than in the experimental groups. Significantly higher values for S phase in the control group. At 30 days of age, similar values for the three phases of cellular cycle in the three groups studied. At 34 days of age, significantly higher values ($p < 0.05$) of G_1 phase cells in control group than in the experimental groups. G_2 + M phase showed significantly higher values ($p < 0.05$) in offspring of melatonin treated mother rats than in the other two groups. Being significantly lower the value of the control group as compared to the pinealectomized mother offspring value.

CONCLUSION: Maternal melatonin influences the ovarian oocyte development.

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INDUCED OVULATION IN ADULT GUINEA PIGS BY HUMAN MENOPAUSAL GONADOTROPIN. P2 26

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We are trying to establish embryo cryopreservation technique for various laboratory rodents including guinea pigs. For this purpose, we need an efficient method for collecting embryos. In this study, we attempted to induce superovulation in guinea pigs by human menopausal gonadotropin (hMG) since the combination of PMSG and hCG is not effective. Adult guinea pig females of Fub:Hartley strain, which showed at least two normal estrous cycles (~20 days/cycle), were used in this study. We made three daily subcutaneous injections of 5 iu/kg hMG (Pergonal®, Teikoku Hormone MFG Co. Ltd, Tokyo) to them on days 14, 15 and 16 after ovulation. Then, they were mated with males and embryos were collected by flushing oviducts and uteri on day 4 of pregnancy. Of 10 guinea pigs tested, we could collect embryos from nine guinea pigs and the number of collected embryos per animal was 5.4±1.6 (Mean±s.e.m.) ranging 0 to 17. It was significantly more than the ovulation rate at spontaneous ovulation (3.6±0.1, n = 96), based on the number of corpora lutea [*Reprod. Fert. Dev.*, 5:425-432, 1993]. Our data suggest that the hMG could be used as an effective agent to induce superovulation in guinea pigs.

P2 27 INSULINE ADMINISTRATION PREVENTS DIABETIC BONE LOSS IN THE RAT. J. Novalbos, E. Hdez-Barbáchano, J. I. San Román, A. Diez*, E. Martín*, M. Martín*, J. R. García-Talavera* and J. J. Calvo. Depto. Fisiología y Farmacología, Univ. Salamanca, and *Hospital Clínico Salamanca (Spain).J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 138, 1997.

Conflicting results have been reported about the protective effect of insulin in diabetic bone loss. The aim of our work was to study whether osteopenia can be stopped by insulin treatment in long term diabetes. Three groups of female Wistar rats weighing 150 g in the starting of experiment were used: Controls (C), poor insulinized diabetic (D), well insulinized diabetic (DT) rats. BMD, BMC and area were measured at 0, 15, 30, 60 and 90 days, in lumbar column (CL) and tibial diaphysis and epiphysis. At 15 days statistically significant differences ($p < 0.05$) were observed between DT and D groups. Area and BMD were increased in DT group. These values were similar to those of the C group. This tendency continue up to 90 days. At this time we observed the maximal differences in BMD (0.26±0.003, 0.21±0.01 and 0.25±0.01 in CL from C, D, and DT groups; 0.37±0.01, 0.28±0.01 and 0.36±0.01 in tibial epiphysis; 0.24±0.05, 0.19±0.004 and 0.24±0.004 in tibial diaphysis from the same groups). In summary we can conclude that an adequate treatment with insuline prevents the osteopenia induced by diabetes. Moreover, this effect can be early observed in a few days after the beginning of treatment.

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P2 28 FLUORIDE IS A PROTECTIVE AGENT FOR BONE LOSS IN DIABETIC RAT. E. Hdez-Barbáchano, J. Novalbos, J. I. San Román, A. Diez*, E. Martín*, M. Martín*, J. del Pino* and J. J. Calvo. Depto. Fisiología y Farmacología, Univ. Salamanca and *Hospital Clínico, Salamanca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 138, 1997.

A stimulating role has been suggested for fluoride on bone formation. The aim of this work was to observe the fluoride effect on the low turnover osteopenia associated to diabetes. Three groups of female Wistar rats weighing 150 g at the starting of experiment were used: Controls (C), poor insulinized diabetic (D), well insulinized diabetic (DT) rats; each of them was divided in two subgroups, depending on drink, tap water (A) or tap water containing fluoride (100 ppm) (F): CA, CF, DTA, DTF, DA & DTF groups. BMD, BMC and area were measured in lumbar column (CL) and tibial diaphysis. A month after the beginning of experiment, BMD for CL were 0.23±0.004, 0.24±0.003, 0.23±0.003, 0.23±0.003, 0.21±0.003 and 0.21±0.005 for CA, CF, DTA, DTF, DF and DA groups respectively. In the diaphysis BMD were 0.23±0.004, 0.22±0.003, 0.2±0.003, 0.19±0.003, 0.17±0.003 and 0.17±0.004 for the same groups. There were statistically significant differences ($p < 0.05$) between DA & DF groups vs CA, CF, DTA & DTF groups. This situation continues up to 60 days. At the 90 days there were statistically significant differences ($p < 0.05$) between DA and DF groups and these differences disappeared between DF group vs DTF & CF groups. The BMD for CL were 0.26±0.003, 0.27±0.004, 0.25±0.001, 0.25±0.001, 0.25±0.003, 0.21±0.01 for CA, CF, DTA, DTF, DF and DA groups respectively; in diaphysis 0.24±0.05, 0.24±0.003, 0.24±0.004, 0.23±0.004, 0.22±0.003 and 0.19±0.004 for the same groups. In summary, fluoride treatment, at indicated doses, stopped the osteopenia induced by diabetes, although this effect is only observed three months after the beginning of treatment.

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PERIPHERAL AND CENTRAL ANTIESTROGENIC EFFECTS OF LY117018-HCl IN THE FEMALE RAT. P2 29

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LY117018-HCl (a gift from Eli Lilly & Co., IN), a pyrrolidine analog of raloxifene, that behaves like an estrogen receptor modulator, has been shown to act peripherally as an estrogen antagonist with negligible agonist activity[1]. We have evaluated both central and peripheral effects of LY117018 in ovariectomized (OVX) rats in response to fourteen daily sc injection of estradiol benzoate (E2; 2 μg/rat), or the combination of E2 plus LY117018 (10 mg/kg). Peripheral effects of LY117018 were confirmed since it reduced uterine weight to the level of OVX-oil treated animals. Neither LHRH median eminences content, nor LH serum levels were affected by LY117018, indicating that the drug was unable to affect LHRH secretion. Interestingly, serum levels of FSH, as it occurred with uterine weight, were significantly reduced in rats treated with LY117018. Under the present design, LY117018 seemed to act both at peripheral and pituitary levels, where it had impaired both estradiol-induced increase of uterine weight, and pituitary response to LHRH, respectively, without affecting LHRH output. On the other hand, since LH and FSH secretion were differently affected by treatment with LY117018, this compound could be potentially useful as a pharmacological tool for analyzing estrogen interactions on gonadotropin regulation.

1. Byrant *et al. J. Bone Miner. Res.*, 10: T421, 1995.

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C-PEPTIDE DOES NOT ALTER CARBOHYDRATE METABOLISM IN ISOLATED MOUSE MUSCLE. P2 30

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C-peptide connects the α- and β- chains of insulin. C-peptide and insulin are released in equimolar concentrations from the pancreatic β-cell in response to secretagogues. In general, physiological concentrations of C-peptide have been shown to be biologically inactive with respect to carbohydrate metabolism in peripheral tissues. Evidence for a direct effect of C-peptide on carbohydrate metabolism in isolated human skeletal muscle strips was recently presented, wherein it was shown that a physiological concentration of C-peptide activated glucose transport and increased the content of glycogen. The C-peptide effects, although significant, were, however, small, possibly because the muscle strips were cut. Nevertheless, these findings raised the possibility that C-peptide activates glycogen synthase in skeletal muscle, and implicate C-peptide as a potentially important factor in the regulation of carbohydrate metabolism *in vivo*. Therefore, we investigated the effects of C-peptide metabolism in isolated mouse soleus muscle. C-peptide, at concentrations up to 1000 nM, had no effect on either [^{14}C]glucose incorporation into glycogen, glycogen synthase activity, or 2-deoxyglucose uptake. These data demonstrate that C-peptide has no direct effect on the measured parameters of carbohydrate metabolism in isolated mouse muscle.

P2 31 REGULATION OF THE STRIATAL-SPECIFIC GENE SE6C BY THYROID HORMONE. P. Vargiu, B. Morte, J. Falk*, A. Guadaño-Ferraz, P. Lorenzo, J. G. Sutcliffe* and J. Bernal. Instituto Investigaciones Biomédicas, 28029 Madrid (Spain) and *The Scripps Research Institute, La Jolla, CA (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 140, 1997.

The syndrome of neurological cretinism is due to severe deficiency of iodine intake during gestation, which leads to poor thyroid gland function and lack of thyroid hormone (TH). Prominent features of this condition are mental retardation and signs of striatal damage, such as spastic diplegia. To examine the possibility that TH controls the expression of striatal-specific genes we have studied the effects of hypothyroidism and TH administration on the expression of several striatal-enriched genes isolated by subtractive hybridization.

We found that the striatal-specific mRNA SE6C is low in hypothyroid animals and is increased by TH treatment during the postnatal period in the rat, but not in adult animals. The full-length SE6C cDNA was isolated and sequenced, and was found to encode a novel GTP-binding protein of still unknown function. To dissect the mode of action of TH we have isolated the 5'-flanking region of the gene. This region confers TH regulation to a heterologous reporter after cotransfection with TH receptors.

In conclusion, TH regulates the expression of at least one striatal-specific gene, at the level of transcription. Control of SE6C might mediate the motor manifestations of neurological cretinism.

P2 32 *IN VITRO* INCREASE OF HEART VENTRICLE FORCE AND TISSUE CYCLIC AMP (cAMP) INDUCED BY DIAZEPAM. E. Romero-Vecchione, J. Vásquez, M. A. Davoli and M. Lezama. Cardiovascular Studies Lab. Pharmacol. Dept. "JM Vargas" Sch. Medicine, UCV, Caracas 1010 (Venezuela). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 140, 1997.

The anxiolytic drug diazepam was tested in paced isolated rat right ventricle strips, maintained in Krebs sol. Contractions were recorded with a force transducer. Diazepam (1-30 mM) increased (10-30 %) basal contractile force. This effect was potentiated by 150 and 525 % with subeffective doses of theophylline (10^{-8} M) and isoproterenol (10^{-11} mM) respectively. Diazepam also increased tissue cAMP (radioimmunoassay) from 3.3±0.3 to 14.4±2.1 pM/g tissue ($p < 0.01$); atenolol (10^{-8} M) slightly blocked and flumazenil (1 mM) did not change this effect. In conclusion, diazepam heart effect was not mediated through β-adrenoceptors and probably involves peripheral myocardial benzodiazepine receptors.

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IDENTIFICATION OF FOUR INDEPENDENT K^+ CHANNELS IN RAT HEART. P2 33

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 141, 1997.

A controversy exists about if the described early outward K^+ current, I_{eo}, is a composite of a transient outward current, I_{to} and a delayed rectifier, I_k, or if it is carried through only one channel population. We have also seen another sustained current, I_s, not previously described. To study these currents, we used the whole-cell variation of the Patch-Clamp technique.

Results: Changes in external K^+ concentration produces only a little positive shift in I_{to} V_{half} of inactivation, but more pronounced in I_k, and augments the slope and amplitude of this last. Besides, I_s is not affected by these changes, so it is not a residual of the anomalous rectifier, I_{k1}. If we increase the external concentration of divalent cations, we see a 7 mV shift in I_k inactivation V_{half} and a 35 mV in the I_{to} one. On the other hand, there are no differences in the regional distribution of I_{k1}, I_k, and I_s current densities, whereas clear variations exists in the I_{to}.

In summary, we have a current, I_{to}, which interacts very few with K^+ ions, is strongly affected by divalent cations, and its current density varies among different cardiac regions. Another current, I_k, which interacts clearly with K^+ ions, is few affected by divalent cations and remains constant along the heart. A third one, I_s, which does not interact with K^+ or divalent cations and is homogeneously distributed in the heart. The last one, I_{k1}, is strongly affected by K^+ ions and is also homogeneously distributed.

ECG AND ELECTROLYTES IN IBERIAN AND DUROC-JERSEY PIGS BEFORE AND AFTER WEANING. P2 34

M. D. Rubio, P. Fernández, E. I. Agüera, B. M. Escribano, A. Muñoz and F. M. Catejón. Dep. Biology Animal. Sec. Physiology, Fac. Veterinary Medicine, Univ. Córdoba (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 141, 1997.

Maturational changes occurred in white-skinned pigs have been studied, establishing some electrocardiographic parameters for different ages, as standardized as possible, in spite of their difficulty (Ibañez *et al.* 1990; Rubio *et al.* 1993). The possible influence of weaning on electrocardiographic recording and on plasma ionic concentrations in dark skinned pigs was analyzed in this experiment with 25 Iberian pigs and 25 Duroc-Jersey (D), were studied before weaning (20 days old) and after weaning (45 days old), considering Heart rate (HR), Heart score (HS), dyastole/systole ratio (DS), and Ca^{++}, Na^+, K^+ and Cl^- concentrations. Heart rate mean values found before weaning (189.44 and 219.56 bpm in Iberian and Duroc, respectively) were similar to those reported in Landrace x Belgian White in the same conditions. Inversely, after weaning the heart rate remained higher (190.68 and 191.22 lat/min), even though, in the present experiment the pigs were weaned early HS mean values were higher in Iberian (0.040 and 0.041 s) than in Duroc (0.038 and 0.039 s) in both age groups. In relation to the DS ratio, both breeds showed significant differences after weaning, with an increase in the Duroc breed. These findings, together with the heart rate reduction, represents a better adaptation to in early weaning the latter bred. Alterations in ionic concentrations after weaning were similar in both breeds, with a decrease of Na^+ and a significant increase in K^+ ($p \leq 0.01$) and Ca^{++} ($p \leq 0.001$).

P2 35 MYOCARDIAL PRECONDITIONING BY 15 MINUTES OF ISOFLURANE ANESTHESIA. B. A. Cason, R. Slocum, K. Gamperl and R. F. Hickey. VA Medical Center, Anesthesiology Service (129), San Francisco CA, 94121 (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 142, 1997.

Introduction: Isoflurane is a volatile anesthetic which causes coronary vasodilation, in part, via opening of ATP-sensitive potassium channels (K-ATP). Because K-ATP channels are implicated in the phenomenon of myocardial ischemic preconditioning, we tested this hypothesis: Isoflurane administration, immediately prior to myocardial ischemia, can induce or mimic myocardial preconditioning.

Methods: Three groups of rabbits, anesthetized with propofol, were studied: a) control = no pretreatment (n = 5), b) ischemic preconditioning = 5 minutes of coronary occlusion followed by 15 minutes reperfusion (n = 4), and c) isoflurane pretreatment = 15 minutes of 1.1 % isoflurane administration, followed by 15 minutes of washout (n = 4). During isoflurane administration, propofol was temporarily discontinued in the isoflurane group, to allow control of blood pressure. Mean arterial pressure prior to the 30-min occlusion was not different among the groups. In each group, after pretreatment, the anterolateral marginal coronary artery was occluded for 30 minutes, followed by 3 hours of reperfusion. The area of infarction (AI) was defined by triphenyltetrazolium staining, and the area at risk (AR) by fluorescent microspheres, with areas measured by computerized planimetry. Data are presented as mean ± s.e.

Results: AI/AR was 33.4 ± 5.5 % in the control group, vs. 12.3 ± 0.74 %* in the ischemia-preconditioned group, vs 18.0 ± 4.2 %* in the isoflurane-pretreated group. (* = $p < 0.05$ by factorial ANOVA and Fisher PLSD tests).

Conclusion: Preadministration of isoflurane, prior to myocardial ischemia, reduces myocardial infarct size significantly, and mimics myocardial preconditioning.

P2 36 METABOLIC COMPONENTS OF THE ACIDOSIS FOUND DURING ACUTE HEMORRHAGE IN THE BLOOD OF RAT. V. Alfaro, J. Pesquero and L. Palacios. Dpto. Fisiología, Facultad de Biología, Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 142, 1997.

Metabolic acidosis is a common finding during hemorrhage in mammals and it is usually related to increases in blood lactate because of shock hypoxia. However, hemorrhage also produces changes in the main strong plasma ions and proteins which may also alter the acid-base status. We studied the effects of acute hemorrhage on the acid-base components of plasma applying Stewart's approach. 3 groups of rats, with a hemorrhage of 30 % total blood volume, were studied: one non-anesthetized (A) and two anesthetized (B, ketamine and C, urethane). We found a notable acidosis in groups B and C (pH ≈ 7.10) while group A preserved a normal blood pH through ventilatory compensation. Strong ion difference (SID) decreased 8.1 mEq/l in group A, 8.3 mEq/l in group B, and 11.3 mEq/l in group C. Increases in blood lactate were responsible for 42 % (group A), 57 % (group B) and 80 % (group C) of the total SID fall while the resting SID decrease was related to an imbalance of the $[Na^+]/[Cl^-]$ ratio in plasma. In conclusion, the acidosis found during acute hemorrhage in rat blood has two main metabolic components: an initial strong ion imbalance due to the acute blood loss followed by increases in lactate when tissue hypoxia appears.

EFFECT OF TICLOPIDINE ON EARLY HAEMOSTATIC ALTERATIONS INDUCED BY ENDOTOXIN IN RATS. P2 37

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Endotoxic shock produces blood coagulation disturbances and thrombocytopenia. The inhibition of platelet aggregation by ticlopidine, but not by aspirin, reduced the early hypotension in rats. Administration of endotoxin from *E. coli* (2.5 mg/kg iv), produced, in the first hour significative changes ($p < 0.001$) like the ones observed in disseminated intravascular coagulation: Thrombocytopenia (-58 %), lengthening of partial thromboplastic time (+42 %) and prothrombin time (+57 %), reduction of plasma fibrinogen (-43 %), and plasma fibrinolytic activity increase (+45 %). Three days pretreatment (9 mg/kg iv) with ticlopidine, an antiaggregating agent, significantly reduced the formerly described alterations. We conclude, that platelet aggregation plays a key role in early stages of endotoxic shock.

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MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF HUMAN DEFERENTIAL ARTERY. P2 38

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Segments of the prostatic part of the human deferential artery (HDA) were obtained from 12 men undergoing radical cystectomy or prostatectomy. Light microscopy revealed that HDA has an internal diameter ranging from 650 to 880 µm, a well developed tunica media composed of 6-8 layers of smooth muscle and a wall thickness to lumen ratio ranging from 1:1 to 1:2. The artery has also a well-developed internal elastic lamina. The major artery runs in the adventitia of the vas deferens and sends arterial branches through the muscle layer. These branches (150 to 180 µm in external diameter) are also of muscular type. Fluorescence microscopy (glyoxylic acid procedure) showed a perivascular green plexus characteristic of catecholaminergic-containing nerves. In isolated artery rings arranged in organ baths for isometric recording of tension, electrical field stimulation (20 V, 0.25 ms duration, for 30 s) induced frequency-dependent contractions that were abolished by tetrodotoxin (10^{-6} M) or prazosin (10^{-6} M). The maximum response to noradrenaline was about 140 % of the response to KCl (60 mM). The results indicate that the morphological features of HDA are in consonance with contractile response to adrenergic stimulation. Thus adrenergic activation of HDA could be of relevance in regulating blood flow to the vas deferens. Supported by DGICYT, FIS and "Generalitat Valenciana" (Spain).

P2 39 CORTICAL MICROCIRCULATORY AND HIPPOCAMPAL NEURODEGENERATIVE PATTERNS AFTER GLOBAL CEREBRAL ISCHEMIA IN THE GOAT. G. Torregrosa[1], M. D. Barberá[2], J. B. Salom[1], J. M. Centeno[1,2], M. Ortí[1,2], and E. Alborch[1,2]. [1]Ctro. Investigación, Hospital "La Fe", and [2]Dpto. Fisiología, Univ. Valencia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 144, 1997.

Transient global cerebral ischemia (5, 10, and 20 min duration) was induced by bilateral occlusion of the external carotid artery along with neck compression in goats previously instrumented for continuous monitoring of cortical perfusion (CP), intracranial pressure (ICP), electrocorticogram (ECoG), arterial blood pressure (ABP), and heart rate (HR). The various occlusions reduced almost completely CP, flattened ECoG, increased ICP and ABP, and did not modify HR. Release of the occlusions gave a rather heterogeneous cortical microcirculatory pattern: 11 and 6 out of 31 goats showed no initial hyperemia or delayed hypoperfusion, respectively. The remaining 14 goats showed both phases. Neither the magnitude of hyperemia nor that of hypoperfusion were related to the duration of ischemia. By contrast, neuronal loss in the CA1 subfield of hippocampus was evident 7 days after an ischemic episode of 20 min but not of 5 or 10 min. Therefore, the duration of the ischemic insult rather than the impairment of postischemic blood flow seems to determine the extent of neuronal damage.

Supported by FIS (grant # 95/1668), M.E.C. (J. M. Centeno) and G. V. (M. Ortí).

P2 40 CEREBRAL HEMODYNAMIC EFFECTS OF DOTARIZINE IN THE GOAT. E. Alborch[1,2], M. D. Barberá[1], G. Torregrosa[2], J. B. Salom[2], J. M. Centeno[1,2], and M. Ortí[1,2]. [1]Dpto. Fisiología, Universidad de Valencia, and [2]Centro de Investigación, Hospital "La Fe", Valencia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 144, 1997.

Dotarizine is a novel piperazine derivative characterized by its antagonistic actions on both 5-hydroxytryptamine receptors and Ca^{2+} transport. The effects of dotarizine were assessed in anesthetized goats instrumented for continuous recording of global cerebral blood flow (gCBF), cortical perfusion (CP), cerebral vascular resistance (CVR), arterial blood pressure (ABP), and heart rate (HR). Injection of increasing doses (1 μg - 5 mg) of dotarizine directly into the cerebroarterial supply of the goat produced transient and dose-dependent increases in gCBF, with no concomitant significant changes in ABP and HR. Therefore, transient and dose-dependent decreases in CVR were obtained. Such a cerebral vasodilatation was reflected in dose-dependent increases in CP, which time-course showed a slow-developing profile. For the highest dose tested (5 mg), gCBF increased by 72±4 %, CVR decreased by 42±2 %, and CP increased by 38±6 %. It can be concluded that dotarizine increases cerebral perfusion by a direct dilatatory action on the cerebroarterial vasculature.

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INHIBITORY ACTION OF AMITRIPTYLINE ON CONTRACTILE RESPONSES OF HUMAN MESENTERIC ARTERIES. P2 41

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We studied the effects of amitriptyline on isolated human mesenteric arteries obtained during abdominal operations (17 patients). Artery rings (4 mm long, 0.8-1 mm, external diameter) with and without endothelium were mounted for isometric recording of tension. In segments precontracted with norepinephrine (NE, 10^{-6} M), KCl (60 mM), or vasopressin (10^{-9} M) application of amitriptyline (10^{-7}-10^{-4} M) produced concentration-dependent relaxation. Removal of endothelial layer or incubation with tetraethylammonium (10^{-3} M), a blocker of Ca^{2+}-activated K^+ channels did not alter this relaxant response. This rules out the possible involvement of endothelium-derived relaxing or hyperpolarizing factors and Ca^{2+}-activated K^+ channels in the vasorelaxation induced by amitriptyline. The incubation with amitriptyline (10^{-7}-10^{-5} M) displaced the concentration-response curves to NE and KCl to the right and decreased the magnitude of the maximal contraction. These results indicate that amitriptyline could act as postsynaptic adrenoceptor antagonist and as direct inhibitor of muscle contraction. This relaxant mechanism involves an interference of amitriptyline with the entry of calcium through both voltage-dependent and receptor-operated Ca^{2+} channels.

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EFFECTS OF U46619, A TXA_2 ANALOGUE, ON CONTRACTILE RESPONSE OF RABBIT PULMONARY ARTERIES. P2 42

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Rabbit pulmonary artery rings, with and without endothelium, were mounted for isometric recording of tension. U46619 (10^{-10}-10^{-5} M) caused concentration-dependent and endothelium-independent contractions. The TP receptor antagonist SQ30741 (10^{-6}-10^{-5} M) displaced the curve to U46619 to the right and decreased maximal contraction. Electrical field stimulation (EFS) (1-4 Hz, 20 V, 0.25 ms for 30 s) caused contractions that were of greater magnitude in arteries without endothelium. U46619 (10^{-10}-10^{-9} M) diminished ($P < 0.05$) the EFS-induced contractions in arteries with endothelium. This inhibition did not change in the presence of SQ30741 (10^{-7}-10^{-5} M) but was abolished by indomethacin (10^{-5} M). In arteries without endothelium U46619 (10^{-10}-10^{-9} M) significantly potentiated the EFS-induced response but pretreatment with SQ30741 (10^{-7}-10^{-5} M) or indomethacin (10^{-5} M) completely reversed the potentiating effects. These results suggest an interaction between TXA_2 and neurogenic responses through activation of TP receptors located in the smooth muscle. This interaction may be mediated by the release of dilator prostanoids from endothelial cells and by the release of contractile prostanoids from smooth muscle cells.

Supported by DGICYT, FIS and "Generalitat Valenciana" (Spain).

P2 43 *IN VITRO* TRYPTOPHAN HYDROXYLASE ACTIVITY IN RAT BRAIN BASE ARTERIES IS NOT AFFECTED BY CERVICAL GANGLIECTOMY. A. L. López de Pablo, M. J. Moreno and E. J. Marco. Departamento de Fisiología, Facultad de Medicina. Universidad Autónoma de Madrid, 28029 Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 146, 1997.

Whereas there is increasing evidence that major cerebral arteries receive a serotonergic innervation of central origin, it is a matter of controversy whether these blood vessels are innervated by serotonergic fibers originating from superior cervical ganglia. In the present work, this possibility was explored in rat brain base arteries by studying the effect of cervical gangliectomy on tryptophan hydroxylase activity measured in cell-free extracts of these vessels. Superior cervical ganglia removal performed 15 days before the sacrifice of the animals did not significantly change tryptophan hydroxylase activity in brain base vessels when compared to sham-operated rats. No enzyme activity could be assayed in cell-free extracts of single ganglia or when three ganglia were pooled for each assay. When five ganglia were pooled, a low enzyme activity was detected. On the other hand, cervical gangliectomy decreased significantly noradrenaline levels in cerebral arteries when compared to sham-operated animals. These results suggest that the contribution of superior cervical ganglia to the overall serotonergic innervation of rat major cerebral arteries is either nil or very small.

Supported by a grant of "Fondo de Investigaciones Sanitarias" (F.I.S.) No. 95/0508 (Spain).

P2 44 VASOPRESSIN-INDUCED CONSTRICTION AND RELAXATION IN HUMAN RENAL ARTERIES. P. Medina, S. Roig, I. Noguera, S. Masiá, P. Chuan*, C. Domenech* and S. Lluch. Depts. of Physiology and *Surgery, Fac. Medicine, Valencia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 146, 1997.

We studied the effects of vasopressin on isolated renal arteries obtained from 15 patients (9 men and 6 women) undergoing nephrectomy for neoplasia. Paired rings, with and without endothelium, were mounted for isometric recording of tension in organ baths. Vasopressin produced concentration-dependent and endothelium-independent contractions (EC_{50} with endothelium $9.1x10^{-10}$ M vs EC_{50} without endothelium $2.7x10^{-9}$ M). The vasopressin V_1 receptor antagonist $d(CH_2)_5Tyr(Me)AVP$ (10^{-8} and 10^{-6} M) displaced the control curve to vasopressin 12 and 564-fold, respectively, to the right in a parallel manner. In contracted artery rings and previously treated with the V_1 antagonist $d(CH_2)_5Tyr(Me)AVP$ (10^{-6} M) vasopressin caused endothelium-independent relaxation. The relaxation to vasopressin was reduced significantly by indomethacin (10^{-6} M) and unaffected by the V_1-V_2 receptor antagonist $d(CH_2)_5Tyr(Me)ValdesGlyAVP$ or by N^G-nitro-L-arginine methyl ester (LNAME, 10^{-4} M). These observations indicate that vasopressin is primarily a constrictor of human renal arteries by V_1 receptor stimulation. Vasopressin causes dilatation of human renal arteries only if V_1-receptor blockade is present. This relaxation appears to be mediated by the release of prostacyclin and is independent of V_2 receptor stimulation or nitric oxide formation.

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ROLE OF PROSTAGLANDINS IN THE LONG-TERM RENAL EFFECTS OF ANGIOTENSIN II. M. T. Llinás, J. D. González, E. Nava, C. Moreno And F. J. Salazar. Depto. Fisiología, Facultad de Medicina, Murcia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 147, 1997. P2 45

The role of angiotensin II (Ang II) in the long-term regulation of renal function has been evaluated in many studies. However, the role of endogenous prostaglandins (PG) in modulating the renal effects induced by a long-term increment of Ang II is not known. In conscious chronically instrumented dogs, we have studied the renal effects induced by the i.v. administration of Ang II (5 ng/kg/min) during six consecutive days, with or without the simultaneous infusion of a cyclooxygenase inhibitor (meclofenamate, 5 μg/kg/min) during four days. The infusion of meclofenamate ($n = 6$) did not induce changes in arterial pressure (AP) and glomerular filtration rate (GFR) but induced a transitory decrease in sodium excretion (UNaV) and a decrease ($P < 0.05$) in renal blood flow (RBF). The administration of Ang II ($n = 5$) induced a significant increase ($P < 0.05$) in AP, and a decrease ($P < 0.05$) in RBF and UNaV but no changes in GFR. In the third experimental group ($n = 5$), meclofenamate was infused simultaneously with Ang II during four consecutive days and the changes in AP, GFR, RBF and UNaV were similar to those found during the administration of Ang II alone.

These results suggest that the renal effects induced by the long-term administration of a pressor dose of Ang II are not modulated by endogenous PG because these effects are not potentiated by the simultaneous infusion of a cyclooxygenase inhibitor.

ROLE OF NITRIC OXIDE AND PROSTAGLANDINS IN THE LONG-TERM CONTROL OF RENAL FUNCTION. J. D. González, M. T. Llinás, E. Nava, F. Rodríguez and F. J. Salazar. Dpto. Fisiología, Facultad de Medicina, Murcia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 147, 1997. P2 46

The objective of this study was to determine, in conscious dogs, if the long-term effects of nitric oxide (NO) synthesis inhibition on arterial pressure (AP) and renal function are potentiated by the simultaneous prolonged inhibition of prostaglandins (PG) synthesis.

The administration of meclofenamate (5 μg/kg/min) during four days ($n = 6$) did not induce changes in AP and glomerular filtration rate (GFR) but induced a decrease ($P < 0.05$) in renal blood flow (RBF) that was maintained even after a recovery period of three days. The inhibition of PG synthesis also induced a decrease in plasma renin activity (PRA) and a transitory decrease in sodium excretion (UNaV). The administration of L-NAME (5 μg/kg/min) during six days ($n = 6$) induced a significant increase in AP ($P < 0.05$) and a transitory decrease ($P < 0.05$) in RBF and PRA but no changes in GFR and UNaV. The simultaneous inhibition of NO and PG during four days ($n = 6$) induced an increase in AP and a decrease in RBF and PRA that were similar to those observed during the administration of L-NAME or meclofenamate. However, the simultaneous inhibition of NO and PG synthesis also induced a significant decrease in GFR and UNaV.

This study demonstrates that the administration of a cyclooxygenase inhibitor potentiates the renal effects induced by the chronic reduction of NO synthesis, suggesting that there is an important interaction between NO and PG in the chronic regulation of renal function.

P2 47 NITRIC OXIDE RELEASE DURING THE INTRARENAL INFUSION OF BRADYKININ. F. Rodríguez, E. Nava, C. Moreno, M. T. Llinás and F. J. Salazar. Dpto. Fisiología, Facultad de Medicina, Murcia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 148, 1997.

Previous studies have suggested that the renal effects induced by the administration of bradykinin (BK) are mediated by nitric oxide (NO). The objective of this study was to determine the changes in the renal NO production during the intrarenal infusion of BK in anesthetized dogs. The renal NO production was evaluated by measuring the concentration of $NO_2 + NO_3$ (NO_x) in urine and plasma obtained from the renal vein and femoral artery. The nitric oxide synthase activity was also determined in the renal cortex and renal medulla by measuring the conversion of ^{14}C-L-arginine to ^{14}C-L-citrulline.

The intrarenal infusion of BK (8 ng/kg/min; n = 5) during 2 h did not induce changes in arterial pressure, glomerular filtration rate and the concentration of NO_x in plasma obtained from the renal vein and femoral artery. This infusion of BK induced significant increments in renal blood flow (RBF), urinary sodium excretion (UNaV), urine flow rate (UV) and the urinary excretion of NO_x. However, these increments were transitory since RBF, UNaV and UV returned to control levels at the end of the BK infusion. The changes in RBF, UNaV and UV were correlated with those changes in urinary excretion of NO_x, during the administration of BK.

The results of this study suggest that renal effects of BK are transitory and further support the notion that renal effects of BK are mediated by NO.

P2 48 AMINOPEPTIDASE ACTIVITY IN ADRENALS OF LOW RENAL MASS MODEL OF HYPERTENSION. I. Prieto, A. Martínez, J. M. Martínez, M. J. Ramírez, F. Hermoso, F. Vargas, F. Alba and M. Ramírez. Unit of Physiology, University of Jaén, Jaén (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 148, 1997.

A local renin-angiotensin system has been described in adrenals. In addition, there exists research evidence for a relationship between aminopeptidase activity (AP) and the blood pressure. To investigate the behaviour of this activity in the adrenals of hypertensive rats, angiotensinase A activity (glutamyl-AP and aspartyl-AP), aminopeptidase M activity (alanyl-AP), aminopeptidase B activity (arginyl-AP), pyroglutamyl-AP, and cystinyl-AP were measured in the soluble and membrane-bound fractions obtained from one experimental animal model of renovascular hypertension: low renal mass rats (LRM). Aminopeptidase activities were measured fluorometrically using aminoacyl-2-naphthylamide as substrates. Alanyl, arginyl, cystinyl and pyroglutamyl soluble aminopeptidase activities exhibited significantly higher levels in LRM animals than in normotensive controls. However no differences were observed in membrane-bound activities for any activity tested. These results suggest a role for soluble aminopeptidase activity in the pathogenesis of LRM hypertension.

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PROTECTIVE ROLE OF MELATONIN AGAINST LPS-INDUCED MULTIPLE ORGAN DYSFUNCTION SYNDROME. P2 49

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Endotoxaemia caused by *E. coli* lipopolysaccharide (LPS) administration to rats produces a multiple organ dysfunction syndrome (MODS), which is partially dependent of an increase in both free radical production and iNOS activity. Melatonin displays an important antioxidant and free radicals scavenger activity, and it inhibits the activity of cNOS. In this work the role of melatonin to counteract the increase in lipid peroxidation (LPO) and in iNOS activity in rat liver and lung after LPS (serotype 0127:B8) administration to rats was assessed. The animals were grouped as follows: a) control; b) melatonin (10 mg/kg, i.p.); c) LPS (10 mg/kg, i.p.); d) LPS (10 mg/kg, i.v.); e) LPS (i.p.) + melatonin (i.p.), and f) LPS (i.v.) + melatonin (i.p.). The animals were injected between 08:00 and 10:00 h and they were killed six hours later. The obtained results show that LPS administration induces a significant increase in LPO in rat liver and lung. Moreover, a significant LPS-dependent increase in lung iNOS activity and in nitrites in both liver and lung were found. Melatonin administration counteract these effects of LPS in a dose-dependent manner. Interestingly, melatonin not only prevents the toxicity of LPS but also reverts these effects. The data support a role for melatonin on LPS toxicity and suggest that the indoleamine is able to modulate iNOS activity.

CHARACTERIZATION AND PURIFICATION OF THE NUCLEAR MELATONIN RECEPTOR. P2 50

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We have previously characterized, biochemically and pharmacologically, a melatonin high-affinity binding site in purified cell nuclei of rat liver. In order to further purify this nuclear protein, the follow steps were done: a) homogenization of liver tissue, purification of cell nuclei by sucrose gradient, and solubilization of nuclear proteins; b) affinity chromatography of this protein extract through an Epoxy-activated Sepharose 6B coupled to 6-hydroxymelatonin; c) SDS-PAGE gel electrophoresis with and without β-mercaptoethanol; d) Immunoblotting, and incubation with melatonin and treatment with antimelatonin antibody (G/S/704-6483, Stockgrand Ltd., Guildford, U.K.) followed by anti-sheep IgG-peroxidase conjugate (Sigma) incubation. Five protein bands were obtained and two of them, ranged between 63-67 KD, specifically bind melatonin. At the moment of this communication the sequencing of these bands is being done.

P2 51 K^+ TRANSPORT IN COLONOCYTES FROM THE CHICK: EFFECT OF ANISOSMOTIC BUFFERS. A. A. Ilundaín, M. L. Calonge and M. Cano. Depto. Fisiología y Biología Animal, Universidad de Sevilla, 41012 Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 150, 1997.

The transport of potassium has been studied in chicken colonocytes. K^+ uptake has been measured using Rb^+ as a tracer for K^+. The results revealed that net K^+ uptake is mediated by at least four mechanisms: i) a Na^+-dependent, ouabain-sensitive and bumetanide-resistant system (Na^+,K^+,ATPase); ii) a Na^+-independent, ouabain-sensitive and bumetanide-resistant mechanism, which is consistent with a K^+,ATPase; iii) a Cl^-- and Na^+-dependent, bumetanide-sensitive, ouabain-resistant mechanism ($Na^+/Cl^-/K^+$ cotransporter); and iv) a bumetanide- and ouabain-resistant mechanism. Cells incubated at 0 °C when rewarmed took up K^+ against a concentration gradient by mechanisms that were ouabain-sensitive. One of them was Na^+-dependent (Na^+,K^+,ATPase) and the other was Na^+-independent (K^+,ATPase). The separate transfer routes for K^+ (Rb^+) above mentioned were significantly increased by hypertonicity of the external media, except the bumetanide- and ouabain-resistant pathway that was decreased. Hypo-osmotic buffers do not significantly modify the movement of potassium across the membrane.

We are grateful to the DGICYT (N° PB92-0690) for the financial support (Spain).

P2 52 NITRIC OXIDE IN HYPOTHALAMUS HYPOPHYSAL-ADRENAL AND RENIN-ANGIOTENSINE SYSTEMS IN RESPONSE TO STRESS BY IMMOBILIZATION AND TO THE SUPPLY OF BACTERIAL LIPOPOLYSACCHARIDE (LPS). J. V. Soria, M. C. Muñoz, I. Túnez, R. Polonio and P. Montilla. Depto. Bioquímica y Biol. Molec. Fac. Medicina, U. Córdoba, Córdoba (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 150, 1997.

Several experimental approaches have put forward a possible role of nitric oxide (NO) as mediator in neuroimmune response through the hypothalamus-hypophysal-adrenal (HHA) axis as well as in the dynamic of secretion of the renin-angiotensine-aldosterone system (RAA).

This communication deals with the simultaneous changes of ACTH, corticosterone (B), interleukin-6 (IL-6), and active renin (AR) in the plasma and the nitrite/nitrate ratio in response to the immobilization (SI) and LPS injection in a control group of male rats plus another group subjected to ingestion of the NO-synthase inhibitor L-NAME, an L-arginine analogue. L-NAME (Sigma) was supplied dissolved in drinking water at a concentration of 70 mg/100 ml, corresponding to 80 mg/kg weight/animal/day. SI handling was maintained for 1 h, while LPS injection (100 µg/kg, Sigma) preceded in 2 h animal sacrifice and blood sample collection. ACTH, B, AR and IL-6 were determined by RIA with specific equipment from Izasa.

The immobilization increased very significantly ACTH, B and IL-6 values in the control group, with no changes in AR. L-NAME treated subjects showed a decreased response of HHA, IL-6 secretion, and nitrite/nitrate ratio, though AR levels increased remarkably. In this group, a partial inhibition of HHA and IL-6 response against SI and LPS was also observed. These treatments however did not modify AR increases caused by the L-arginine analogue under basal conditions.

These data suggest that NO has a positive modulating effect on HHA activity and the neuroimmune response, and a negative one on the release of renin. This different behaviour both under conditions and under stress suggests that NO triggers different modulating mechanisms in each endocrine system.

PATTERNS OF VENTRICULAR REPOLARIZATION IN ANDALUSIAN HORSES. P2 53

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Anomalies in the repolarization phase of the resting ECG have been suggested to indicate myocardial disease. Rose and Davis (1978) considered the presence of abnormal T waves one of the most significant findings in the ECG of racehorses in cases of limited performance potential. However, the criterion of abnormality have only been analyzed in Thoroughbred and Standardbred (Steel, 1963; Evans and Polglaze, 1994). Positive T waves in the unipolar and bipolar chest leads and positive or biphasic T waves in Lead I were observed in the cited breeds as being abnormal. This study focuses on the establishment of the most frequent T wave morphology in healthy Andalusian horses, taking into account the amount of abnormal waves according to the criterion followed in the aforementioned equine breeds. Eleven horses showed simple negative T waves in Lead I although in one horse a biphasic (negative/positive) morphology was observed. In Lead V1I (exploring electrode located at 5 cm dorsal to the midline and 5 cm caudal to the olecranon in the left hemithorax), eight horses presented simple negative waves. In the four remaining horses, the most frequent morphology was the biphasic (negative/positive), with a predominance of negatives. On the contrary, in Lead V1D (exploring electrode located at 5 cm dorsal to the midline and 5 cm caudal to the olecranon in the right hemithorax), only six subjects showed a simple positive T wave. In the other six animals, a biphasic (positive/negative) wave was established, having positive voltage in 5 cases and negative in one case. Finally, and in relation to Lead V10, seven Andalusian horses showed simple negative waves, four positive waves and one biphasic positive/negative wave. In conclusion, the number of supposedly abnormal T waves is quite high in Andalusian horses, in spite of their good state of health.

MAMMALIAN AT_1 AND AT_2 RECEPTOR ANTAGONISTS FAIL TO BLOCK THE PRESSOR RESPONSE TO ANG II AND ITS INHIBITORY EFFECT ON THE NASAL SALT GLAND OF WHITE PEKIN DUCKS (*Anas platyrhynchos*). P2 54

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We investigated the nasal salt gland function and blood pressure in white Pekin ducks following i.v. injections of Angiotensin II and III. Nasal salt gland secretion started and continued during a left ulnar vein (LUV) infusion of a 1000 mosmol kg^{-1} NaCl (0.32 ml kg $bw^{-1} \cdot min^{-1}$). There was a positive linear correlation between the $[Na^+]$ and $[K^+]$ in the nasal fluid ($r^2 = 0.84$; $p < 0.001$). When 1 nmol kg bw^{-1} of Asp^1-Val^5-Ang II was injected into the LUV during saline infusion there was a transient but pronounced (2 minutes) decrease in the rate of nasal fluid secretion. This response (the decreased flow rate) was not blocked by prior administration of either 20 mg kg bw^{-1} of the AT_1 receptor blocker losartan (DuP753) or the AT_2 receptor blocker PD 123319. Injections of 1 or 5 nmol kg bw^{-1} of Val^4- Ang III into the LUV had no measurable effect on salt gland secretion, thus indicating that the Asp^1 is required for the biological response.

Intravenous injections (LUV) of either 1 or 5 nmol kg bw^{-1} of Asp^1-Val^5-Ang II were followed by significant increases in mean arterial (brachial) blood pressure (MABP). Only about 30 % of this pressor response was observed following injections of the same doses of Asn^1-Val^5-Ang II. The LUV injection of 10 mg kg bw^{-1} of losartan or CGP 48933 (a non-heterocyclic AT_1 receptor antagonist) or PD 123319 failed to block the significant increases in MABP which always followed the injection of Asp^1-Val^5-Ang II. There were no measurable increases in MABP following i.v. injections of 1 or 5 nmol kg bw^{-1} of Ang 1 (1-7) or Val^4-Ang III.

P2 55 ALTERED *IN VIVO* CATECHOLAMINE RELEASE IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) OF THE STRESS-ADAPTED RATS. V. S. Sergeyev, V. I. Petrov and I. A. Grigoriev. Research Institute of Pharmacology, Medical Academy, Pavshikh Bortsov sq. 1, Volgograd, 400066 (Russia). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 152, 1997.

The effect of adaptation on basal and stimulated noradrenaline (NA) release in the PVN of the rats was examined by *in vivo* microdialysis. Microdialysis probes were inserted into the PVN of control and fourteenth days stress-adapted Wistar rats (SAR), and perfused with a modified Ringer solution. Following four basal 30-min collections, transmitter release was stimulated with 100 mM K^+ for one collection. After re-equilibration, blood pressure was raised 60 mmHg for 30 min by phenylephrine (PHE) infusion (1-1.3 mg/kg) then a 2-h recovery period followed. Dialysates were injected to HPLC-ECD. Basal extracellular NA was found to be similar in control and SAR. Basal DOPAC was significantly greater in adapted compared to control rats ($p < 0.05$). K^+ depolarisation induced a significant increase in NA concentrations in both groups ($p < 0.005$), however the NA response to K^+ stimulation was significantly higher in the SAR ($p < 0.05$). K^+-induced decreases in DOPAC and HVA were seen in both groups. Following PHE infusion, a modest delayed reduction in NA levels was seen. PHE-induced hypertension was associated with decreased DOPAC and HVA in control ($p < 0.05$) and SAR ($p < 0.05$), respectively. These results indicate that stress-adaptation is associated with changes in dopaminergic and noradrenergic activity in the PVN of the rats.

P2 56 EFFECTS OF ACTOPROTECTIVE PREPARATION BROMANTAN ON SEX GLANDS. T. V. Khamidova, A. A. Spasov, L. I. Bugaeva, L. N. Yozhitsa and I. S. Morozov. Pharmacol. Res. Institute. Volgograd, 400066 (Russia). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 152, 1997.

Bromantan is an actoprotective agent widely used in clinics. The reproductive effects of bromantan, given at doses 30 mg/kg and 200 mg/kg body weight (b.w.), have been studied in rats. The agent was given orally to male rats for 9 weeks. Female rats were treated at the same doses for 2 weeks. Treated males were mated during 2 weeks with treated and untreated females; and treated females were mated with untreated males. The increased b.w. of males was seen in higher-dosed group. The average b.w. of females was increased in all treated groups. Several parameters of motor activity in males and females were different from controls. Motor-exploratory activity was decreased. Emotional reactivity was induced by bromantan. The results indicate that bromantan can act on functional parameters of spermatozoa. Epididymal sperm counts were decreased (23-28 %) at no dose related effect. The morphological research shown that it had not adverse effects on testicular and epididymus. Withdrawal 9 weeks treated males had decreased index of spermatogenesis (7 %, $p < 0.05$). In this mate the rate of prenatal mortality was decreased by bromantan administration. The time of mate was not effected by bromantan. In current study it is demonstrated that chronic bromantan exposure, possibly have effects on human sexual function.

DISTANT CHRONIC EFFECTS OF BROMANTAN ON THE REPRODUCTION IN RATS AND DEVELOPMENT OF THEIR OFFSPRINGS. I. N. Yozhitsa, A. A. Spasov, L. I. Bugaeva, T. V. Khamidova and I. S. Morozov. Pharmacol. Res. Institute. Volgograd (Russia). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 153, 1997. P2 57

Bromantan is a new immunopotentiator with psychoactive properties. Experiments were conducted to determine whether this compound had any distant adverse effects on the reproduction in rats and development of their offsprings. Sexually mature rats weighing 180-200 g were used. Bromantan orally in doses of 30, 150, 600 mg/kg/body weight were given daily for 16 days to female rats and 60 days to male rats. Animals were mated as follows: 1st treated male with untreated female; 2nd treated female with untreated male; 3rd (control) untreated female with untreated male. Results revealed that fertility of treated female and male was not decreased in a dose-dependent manner. Bromantan had no adverse effects on the maternal weight gain, food consumption and length of gestation. In the newborn from the dams treated with 30 and 600 mg/kg of bromantan, birthweight was increased ($p < 0.05$). The percentage of stillborn rats did not differ significantly in all versions of mating. The percentage of rat pups that died between birth and weaning did not differ as well. Bromantan had different effects on the sex ratio of newborn in all treatment groups and doses. Learning and memory in passive avoidance test were not different from those of control one at eight weeks of age. In conclusion, bromantan at 30, 150, 600 mg/kg produced no clinical signs of toxicity on the offspring postnatal development.

EFFECTS OF K_{ATP} CURRENT ACTIVATION DURING MYOCARDIAL HYPOXIA AND ISCHEMIA: A SIMULATION STUDY. J. M. Ferrero Jr., J. Sáiz, M. Montserrat, V. Torres, J. M. Ferrerro and N. V. Thakor. Lab. Integrado de Bioingeniería, Universidad Politécnica de Valencia, 46020 Valencia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 153, 1997. P2 58

We have formulated a detailed mathematical model of the ATP-sensitive K^+ current (K_{ATP} current) and incorporated it into the Luo-Rudy model of the cardiac ventricular action potential in order to theoretically study the effects of K_{ATP} current activation during myocardial hypoxia and ischemia. The model reflects the dependencies of K_{ATP} current on ionic concentrations and intracellular nucleotide levels. Different computer simulations of the action potential were carried out under normal and pathological conditions. The results show that only a slight degree of K_{ATP} current activation causes drastic changes in action potential duration (APD): when only 0.6 % of the total cell population of K_{ATP} channels are opened, the APD is reduced to 50 % of its normal value. We conclude that K_{ATP} current activation could be the main cause of the experimentally observed action potential shortening during metabolically impaired situations in cardiac tissue.

COMPARATIVE EFFECT OF RAW AND EXTRUDED PEAS ON BLOOD CHOLESTEROL LEVELS IN GROWING RATS. P3 1

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The legume seed components such as tannins, phytates, and especially amino acid profile of seed proteins may be implicated in the serum cholesterol lowering effect. The aim of this report was to asses the effects of pea protein from either raw (RP) or extruded (EP) peas on blood cholesterol levels in young normocholesterolaemic rats. Male Wistar rats (n=10) ware fed diets similar in all respects with the exception of dietary protein source being RP, EP or casein (C) for 15 days. After experimental period rats were killed by decapitation. Trunk blood was collected and serum was separated. Total and HDL cholesterol were measured enzymatically. β-lipoprotein (VLDL+LDL) cholesterol was calculated by sustraction of HDL cholesterol from total cholesterol. Weight gain and feed efficiency ratio were lower in both RP and EP-fed rats compared to those fed casein ($p < 0.001$). There were not differences in relative feed intake among experimental groups. No differences in serum total cholesterol concentration were observed in rats fed RP and EP compared with C group. Serum HDL was elevated in rats fed EP compared with those fed casein ($p<0.05$). Not statistical differences were observed between RP and C rats. Seram LDL and LDL/HDL ratios decreased significantly either in RP ($p < 0.05$) and EP ($p < 0.01$) groups compared with casein fed animals. Results indicate that protein from RP and EP are both effective in lowering blood LDL cholesterol concentrations.

DIFFERENTIAL β3-ADRENOCEPTOR INVOLVEMENT IN THERMOGENESIS ON OBESITY AND DIABETES RAT MODELS. P3 2

F. .Milagro, B. Berraondo, J. A. Martínez and M. P Fernández-Otero. Dept. Physiology and Nutrition. University of Navarra. 31008 Pamplona. Spain. J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 155, 1997.

The sympathetic nervous system plays a key role in the regulation of thermogenesis mediated, at least in part, by β3-adrenoreceptors. The aim of this experimental trial was to study the effects in energy metabolism of Trecadnne®, a molecule with putative β3-adrenergic receptor affinity (Wassermann-Chiesi), in a cafeteria diet-induced obesity model and in an alloxan-induced diabetic model. Tissue oxygen consumption and respiratory quotient were carried out with a Clark-type oxygen electrode and with conventional methodology, respectively. Oral Trecadrine® treatment (1 mg/kg) for three days to diabetic rats increased the respiratory quotient (+11 %; n.s) as well as white and brown adipose tissue oxygen consumption (+50 %; $p < 0.01$, and +182 %; $p < 0.01$, respectively). On the other hand, oral Trecadrine® administration (1 mg/kg) for 35 days to obese rats, reduced the respiratory quotient (-27%; $p < 0.05$) and increased white and brown adipose tissue oxygen consumption (+33 %; $p < 0.05$, and +18 %; $p < 0.05$, respectively). Respiratory quotient data in diabetic rats points out to a major carbohydrate participation in the energy metabolism, probably by an improvement in mediated-β3-adrenoceptor glucose utilization. However, respiratory quotient values in obese rats indicates an increase in lipid oxidation. Oxygen consumption data in both models, suggest that Trecadrine® shows a possible lipolytic effect and an enhancement: in thermogenesis in both white and brown adipose tissue, respectively.

P3 3 INFLUENCE OF AN HIPERLIPIDAEMIC-DIET ON DIVERSE BIOCHEMICAL PARAMETERS. C. Pérez, J. R. Canal, J. E. Campillo, A. Romero, M. D. Torres. Depto. Fisiología, Facultad de Medicina, Universidad de Extremadura, Badajoz (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 156, 1997.

Diet is the main exogenous factor influencing plasma lipids. Its importance is enhanced by its being a modifiable factor. We studied the effects on diverse biochemical parameters of a sudden deprivation of a long-term ingestion of an LCT emulsion. A group of ten 4-month old female Wistar rats who had drunk a 5% LCT emulsion since birth were formed into two groups: Group A (n = 5) drank water; Group B (n = 5) continued drinking the 5% LCT emulsion. The animals were fed a standard diet at all times. The values of weight, and plasma levels of triglycerides, total cholesterol and the transaminases AST and ALT just before the, separation into groups were 292+34 g, 1.72±0.29 mM, 2.77±0.36 mM, 104±8 mU/mL and 52±7 mUlmL. (One month after the separation, these values in Group A were 301±31 g, 2.55±0.96 mM, 2.48±0.31 mM, 104±11 mU/mL and 53±8 mU/mL, and after 2 months were 302±28 g, 3.73±1.73 mM, 2.79±0.41 mM, 124±40 mU/mL and 63±11 mU/mL, the values in Group B were 313±43 g, 1.23±0.28 mM, 2.66±0.23 mM, 137±13 mU/mL and 59±14 mU/mL (after 1 month) and 318±48 g, 1.33±0.36 mM, 2.74±:0.13 mM, 113±24 mU/mL and 69±9 mU/mL (after 2 months). The sudden deprivation of the chronic ingestion (4 months, since birth) of a triglyceride-rich emulsion may therefore provoke differences (non-significant) in weights, a triglyceridaemia rise ($p < 0.05$), and a fall in AST and ALT levels ($p < 0.05$).

Acknowledgements are due to "Fondo Social Europeo-Junta de Extremadura" (Spain).

P3 4 MELATONIN ENTRANCES GLUTATHIONE PEROXIDASE ACTIVITY IN SEVERAL STROGENIZED CHICKEN TISSUES. M. T. Agapito, M. I. Pablos, A. B. Ríos, I. Redondo, R. Gutiérrez and J. M. Recio. Depto. Bioquímica, Biología Molecular y Fisiología, Facultad de Ciencias, Universidad de Valladolid, 47005 Valladolid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 156, 1997.

Melatonin, the pineal neurohormone, has been described as an antioxidant *in vivo* and *in vitro*. This hormone is able to increase the activity of several antioxidant enzymes such as GSH-Px. On the other hand, in avian species, it is known that estrogens mobilize the stored iron and may act as an antioxidant in the redox chemistry of iron. So, the aim of this study was to determine the effect of melatonin injections on GSH-Px activity in estrogenized chicks. Three week old chicks were estrogenized (a single injection); half of this animals were injected with melatonin twice per day during four days. Control animals were injected only with saline. Sacrifice were performed the first, second, third and seventh day after estrogen injection. Glutathione peroxidase activity was determined in brain, kidney, liver, lung and gut. Results are in the next table.

Glutathione peroxidase activity in several chicken tissues expressed as U/mg protein; C = Control (n = 5); E = Estrogenized (n = 5); and E+M = Estrogenized + Melatonin (n = 5).

Day	1st			2nd			3rd			7th		
	C	E	E+M	C	E	E+M	C	E	E+M	C	E	E+M
Brain	42.51	33.7	63.26	42.5	152.2[a]	101.5[a,b]	42.5	48.13[a]	105.1[a,b]	42.5	42.5	60.5
Kidne	193.5	244.2	370.9[a,b]	193.5	274.3	476.6[a,b]	193.5	445.2[a]	719.5[a,b]	193.5	859[a]	1744[a]
Liver	126.2	204.5[a]	535.4[a,b]	126.2	318.1[a]	541.4[a,b]	126.2	351.5[a]	136.4[a,b]	126.2	438.5[a]	121.8[a,b]
Lung	46.9	72.9[a]	237.5[a,b]	46.9	83.25	166.7[a,b]	46.9	94.4[a]	253.3[a,b]	46.9	149.5[a]	402.6[a,b]
Gut	144.5	130.8	210.2	144.5	28.5[a]	117.8[a,b]	144.5	123.5	281.8[a,b]	144.5	158.3	456.1[a,b]

Statistical: SD is not included but ANOVA's test was done. a, $p < 0.05$ vs control; b, $p < 0.05$ vs estrogenized.

RELATION BETWEEN RNA CONTENT AND AGING IN NEURONS OF DORSAL LATERAL GENICULATE NUCLEUS. P3 5

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We have carried out a study on the morphology and RNA contens in neurons from the dorsal lateral geniculate nucleus during ageing. Male albino rats aged 3, 18, 24 and 30 months old were used. The morphological study consisted of calculating somatic and nuclear size by means of a semi-automatic image analyser. According to our observations, size does not vary in a significant way between the 3rd and the 24th month. However, between the 24th month and the 30th we encountered somatic and nuclear hypertrophy, estimated at 32.8% and 35.6%, respectively. The histochemical study aimed at calculating RNA content in neuron nucleus and cytoplasm. Mean RNA content was deduced from the product of RNA concentration, obtained by cytophotometric measurements, and nuclear or cytoplasmic area. No significant differences were found between the 3rd and the 18th month in the nuclei, although a decrease in RNA concentration (18.73%), but not in RNA content, was detected at the 24th month. RNA content is in fact higher between the 24th and the 30th month. In neuron cytoplasm no variations are registered between the 3rd and the 18th month or the 18th-24th months, but RNA content and concentration increases between the 24th and the 30th month. These increments could well be due to compensatory hypertrophy phenomena occurring in the surviving neurons of this period.

HYPEROSMOTIC STRESS INDUCES AN ATTENUATE VASOCONSTRICTIVE EFFECT ON AGED MICE ARTERY. P3 6

G. M. Rocha, M. J. Forster, P. B. Raven. Dept. of Integrative Physiology, University North Texas Health Science Center, 76107 (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 157, 1997.

These experiments were designed to observe if Na^+/H^+ exchange is involved in arterial response of the aged mice. Tail arteries of aged (24-30 months old) and control young (3 months old) B6D2F1 mice were submitted to a hyperosmotic stress or to a_{-1} adrenergic receptor mediated arterial reactivity. Isolated arteries about 300 µm in diameter were cannulated and pressurized to 40 mmHg with the intraluminal flow arrested. Measurements of the arterial internal diameter were performed in vitro by a video dimension analyzer system. Phenylephrine (10^{-1} to 10^{-5} M) was used to induce a-1 adrenergic receptor mediated constriction; mannitol (10 to 200 mM) was used to increase the osmolar range of saline solution in the vessel-bath. Phenylephrine dose-dependently induced comparable vasoconstriction in aged (n = 5) and young (n =3) mice arteries. Hyperosmotic stress, induced vasoconstriction in both aged (n = 3) and young (n = 6) mice arteries. However, the hyperosmotic-induced vasoconstrictive effect was significantly attenuated in aged when compared to young mice arteries ($t = -3.74$; $p < 0.05$). These results suggest that the attenuated vasoconstrictive response of the aged mice tail artery due to hyperosmotic stress are independent of Na^+/H^+ ion exchange at the smooth muscle that occurs with a_{-1} receptor coupled vasoconstriction.

P3 7 EFFECTS OF TREADMILL RUNNING TO EXHAUSTION ON PLASMA REPRODUCTIVE HORMONES AND ADRENAL PERCURSORS ACCORDING TO PLASMA VOLUME CHANGES IN SEDENTARY SUBJECTS. A. M. Diego-Acosta, F. Guirado, V. Fernández-Pastor and M. Ruiz*. Dpto. Fisiología, and *Dpto. Bioquímica. Facultad de Medicina, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 158, 1997.

Reports on the effect of progressive exercise to exhaustion on the sexual endocrine system are scarce, even more considering the concomitant changes in plasma volume (YP). We intend to study the behavior of selected sexual hormones of the pituitary-gonadal-adrenal axis during exercise test and recovery. Eight men and seven women, all sedentary, carried out a graded treadmill test to exhaustion. To determine plasma reproductive hormone levels, venous samples were taken. All hormone assays were determined by RIA. In women's group were not found significative changes in FSH and LH The progesterone (P) levels increased significantly (15 min) at the recovery phase. In man's group FSH decreased during and after exercise while LH not change. The testosterone (T) increased significantly at the 3 min recovery phase. Adjustements according to % VP changes point out that T increase is consequence of hemoconcentration. Raised P is not dependent of decrease % VP. PRL is highly correlated with P suggesting a inhibitor effect on pituitary gland of both hormones. Androstendione is highly correlated with P values suggesting that its increase would be accounted by pituitary-adrenal and/or simpathetic-adrenal stimulation axis more than pituitary-ovary stimulation axis. It is concluded that the response to exercise of the suprarenal gland is sex dependent and is necessary to evaluate the hemoconcentration occurring during and in the recovery phase of exercise.

P3 8 MITOCHONDRIAL FUNCTIONALITY, PHYSICAL EXERCISE AND DIETARY FAT IN RATS. M. Mañas; J. L. Quiles, J. R. Huerta, J. Mataix. Instituto de Nutrición y Tecnología de Alimentos y Departamento de Fisiología de la Universidad de Granada (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 158, 1997.

Physical exercise contributes many benefits to the organism but also can increase free-radical production. Dietary fat may influence lipid peroxidation. The present work tests the hypothesis that both physical exercise and dietary fat may interact on mitochondrial functioning in relation to peroxidative damage. Two groups of rats were fed diets differing only in the type of dietary fat, monounsaturated (oleic acid) and another polyonsaturated (linoleic acid), dividing into 4 subgroups according to physical exercise: one sedentary subgroup and three others submitted 8 weeks to exercise varying with respect to the time of death (24 h after the last test, at maximum effort, and 30 min after maximum effort). The results indicate that rats ingesting monounsaturated fat, as opposed to polyunsaturated, may derive considerable benefits in relation to physical exercise, mitigating the phenomena of lipid peroxidation and improving mitochondrial functioning in the liver and skeletal muscle.

EVALUATION OF FITNESS IN HORSES: FUNCTIONAL INDICES. B. M. Escribano, M. D. Rubio, A. Muñoz, E. I. Agüera, R. Santisteban and F. M. Castejón. Dept. Animal Biology, Sect. Physiology. Fac. Veterinary. Univ. Córdoba (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 159, 1997. P3 9

Functional indices, arising from the relationships between velocity, heart rate (HR) and lactate, permits a physical evaluation of an individual and the establishment of a training program in order to improve fitness. A modified Conconi-test developed for human athletes were performed in harness horses. Andalusian horses were subjected to an increasing-intensity trial (4, 6, 8, 10 and 12 m/s.), covering different distances in each exercise load (400, 600, 800, 1000 and 1200 m.). The heart rate was recorded by means of HR-meters and plasma lactate was determined at rest and at finishing each velocity level. V_{LA2} and V_{LA4} (velocities at plasma lactate of 2 and 4 mmol/1), V_{150} (velocity at a heart rate of 150 bpm), HR_{LA2} and HR_{LA4} (heart rate at lactate concentrations of 2 and 4 mmol/l.) and finally, LA_{150} (lactate at a heart rate of 150 bpm) were calculated. The results showed the great interindividual fitness differences. Practical conclusions about the fitness level of each horse could be drawn from considering the aforementioned indices as aerobic (V_{LA2}, V_{150}, HR_{LA2} and LA_{150}) or anaerobic (V_{LA4}, HR_{LA4}). In this way, values from 5.5 to 6.5 and 9.4 to 10.5 m/s for V_{LA2} and V_{LA4} respectively, belonging to three horses (2, 4 and 8) revealed a better physical state in these animals in comparison with the other individuals of the experiment.

RESPONSE OF HEMATOLOGICAL AND METABOLIC PARAMETERS TO ACUTE PHYSICAL EXERCISE IN FASTING. A. Aguiló, E. Castaño, J. Oliver, N. Serra and A. Pons. Dept. Biología Fonamental i C.S., Universitat de les Illes Balears (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 159, 1997. P3 10

The response of different hematological and metabolic parameters to acute physical exercise has been determined in ten sporting men. The selection of the sporting men has been based on age and hours of training per week. The acute physical exercise (bicycle ergometric test), composed of a 30 W load increase every 3 minutes with direct determination of the oxygen consumption, respiratory parameters and electrocardiogram, was carried out in the morning after overnight fasting. Blood was collected both before the test and immediately after the maximum level of oxygen consumption. This study was made with the approval of the ethics commission of Son Dureta General Hospital (Palma de Mallorca).

The blood was analysed to determine the blood cell count, hematocrit, hemoglobin, corpuscular volume, corpuscular hemoglobin, hemoglobin corpuscular concentration, glucose, urea, creatinin, uric acid, triglycerides, total cholesterol, HDL cholesterol, total protein, bilirrubin and sodium.

The hemoconcentration and increase in the number of lymphocytes observed are in accordance with bibliographic references. The water loss produced during the acute physical exercise is reflected in a statistically significant increase in the hematocrit, hemoglobin levels and total protein. This rise is similar to that of sodium in blood, nevertheless it is smaller than the increase of glucose and creatinin. On the other hand, urea, uric acid, triglycerides, cholesterol, HDL cholesterol and bilirrubin maintain their level in blood in spite of hemoconcentration.

P3 11 AMMONIA AND LACTATE KINETICS IN BLOOD DURING EXERCISE IN HYPOBARIC-HYPOXIA. H. Casas, M. Casas, V. Fonces, J. Ibáñez, J. L. Ventura, F.A. Rodríguez, R. Rama, G. Viscor, L. Palacios and T. Pagès. Unitat d'Hipobària, INEFC-UB, Barcelona, and Departament de Fisiologia, Facultat de Biologia, Universitat de Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 160, 1997.

A comparison was made between changes in blood ammonia concentration and simultaneous blood lactate measurements during endurance and intensive exercise on the cycle ergometer under hypobaric hypoxia conditions (4,000 m, P_B= 462 torr, P_{O2}= 97 torr). Eight endurance trained subjects performed a two-step load exercise test under normobaric and hypobaric conditions, the first step was performed for 25 min below their individual anaerobic threshold (–10% $\dot{V}_{O2}$max) and the second step lasted 5 min at an intensity above the IAT (+10% $\dot{V}_{O2}$max). Maximal aerobic capacity was previously determined by an incremental cycle ergometer exercise test.

Blood ammonia progressively increased along the exercise, whereas lactate suddenly rose only at a work load above the individual anaerobic threshold. Under hypobarichypoxic conditions, blood ammonia and lactate levels were significantly higher when compared to sea level conditions at the same exercise intensity.

We conclude that blood ammonia concentration revealed some metabolic sensitivity in response to hypobaric-hypoxic conditions at different work load intensities.

P3 12 EFFECT OF INTERMITTENT EXPOSURE TO HYPOBARIC HYPOXIA AND EXERCISE ON HUMAN PHYSICAL PERFORMANCE. M. Casas, H. Casas, T. Pagés, J. L. Ventura, A. Ricart, J. Ibáñez, L. Palacios, F. A. Rodríguez and G. Viscor. Unitat d'Hipobària, INEFC-UB, Barcelona, and Departament de Fisiologia, Facultat de Biologia, Universitat de Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 160, 1997.

Six members of a high-altitude expedition (UPC-Everest '95) were exposed to intermittent hypoxia and exercise over 18 days in a hypobaric chamber. Simulated altitude was progressively increased from 4,000 up to 5,500 m over periods of time from 3 up to 5 h. All subjects combined passive exposure with 30 up to 80 min of low-intensity exercise in a cycle ergometer in each of the sessions. Before and after the hypobaric exposure protocol, subjects performed a set of physiological and physical capacity tests including a maximal incremental test in the treadmill and an experimental test to determine their cardiorespiratory and metabolic adaptation to a simulated altitude of 5,000 m at rest and during moderate exercise. A significant improvement in their maximal aerobic power ($\dot{V}_{O2}$max) and endurance capacity (ventilatory and lactate thresholds) was observed. Moreover, their hematological parameters (Hct, RBC count, Hb, and blood viscosity) significantly increased, indicating a higher oxygen transport capacity of the blood. We conclude that middleterm intermittent exposure to moderate hypobaric hypoxia and exercise improves human aerobic physical performance and altitude adaptation.

EFFECTS OP THE PHYSICAL EXERCISE ON FACTOR VIII RELEASE. A. Rodríguez-Jerez, C. García-Lucerga, J. A. Aznar, A. Iradi, M.ª Salvador and F. Querol. Unidad de Investigación en Fisiología del Ejercicio Físico y el Deporte. Universidad de Valencia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 161, 1997. P3 13

The lack or accused decrease of factor VIII (f. VIII), synthosized protein in the reticuloendothelial system, is the cause of hemophilia A, disease that produces hemarthrosis and whose expensive treatment requires the administration of this highly parified factor. Our objective is to determine if short duration and submaximum physical exercise, increases the plasmatic f. VIII levels, in order to accomplish the same experience in subject with hemophilia A.We have determined f. VIII and CPK in plasma, the cardiac frequency, and ECG before and after the accomplishment of the Ruffier-Dickson test, in 19 healthy and sedentary subjects of both sexes (11 women and 8 men), and between 21-24 years old.

Obtaining as a result that the plasmatic f. VIII concentrations before the exercise in women was of 55 % ± 19 and of 65% ± 19 in men, with no statistically significant differences found among them. After accomplishing exercise in women the results were of 78 % ± 18 and in men of 86 % ± 14, with no statistically significant differences found among them either. There are differences ($p < 0.005$) among the results of before and after the exercise in the group of women as well as in that of men.

In conclusion the short duration and submaximum physical exercise increases significantly the f. VIII concentration in the plasma of normal subjects without training. The increase in the f. VIII concentration produced by the physical exercise is similar in both sexes.

MITOCHONDRIAL VOLUMES IN SOLEUS MUSCLE FIBRES OF CONTROL AND ANEMIC RATS. J. R. Torrella, M. Casas, H. Casas, V. Fouces, J. Palomeque, G. Viscor. Departament de Fisiologia, Facultat de Biologia, Universitat de Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 161, 1997. P3 14

Male Sprague-Dawley rats (300-350 g) were randomly assigned to two groups of 7 animals. One group was used as control (C) and anemia (A) by blood withdrawal was provoked to the other. Soleus muscle was excised and processed for transmission electron microscopy treating images by the suitable software (ΣScan, Jandel Scientific). Mitochondrial volume was determined by the "point counting" method in three different zones of each muscle fibre: pericapilar (pc), sarcolemmal sl) and sarcoplasmatic (sp).

Significant differences in mitochondrial volumes were found between pericapilar (C= 12.7 ± 0.2; A=10.0 ± 0.3), sarcolernmal (C=10.1 ± 0.4; A =7.5 ± 0.4) and sarcoplasmie (C=7.9 ± 0.3; A=5.9 ± 0.4) zones, when data from animal groups were tested independently. Moreover, it has been verified that anemic rats had significant lower values than control animals in all the sampled zones. Nevertheless, a common proportional pattern of mitochondrial zonal distribution within the fibre was revealed.

P3 15 ANTIOXIDANT DEFENSES AND DIABETES MELLITUS: EFFECTS OF TRAINING. M. J. Cuevas, J. E. Caño, P. S. Collado and J. González-Gallego. Dept.of Physiology, Pharmacology and Toxicology and Institute of Physical Education, University of Le6n (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 162, 1997.

Diabetes mellitus is a commonly occurring disease that produces numerous pathological complications and profound alterations in intracellular metabolism. A complete analysis of alterations of the enzymatic components of antioxidant systems would be important in ultimately determining the possible role of radical-scavenging systems in the pathology of diabetes. Diabetes was induced in male Wistar rats by the intraperitoneal administration of streptozotocin (60 mg/kg body weight). The training program consisted of gradually increasing time (from 10 min/day to 60 min/day), speed (10 m/min to 22 m/min) and degree (from 0 % to 5 %). Diabetes did not modify either hepatic glutathione concentration or TBARS and glutathione peroxidase activities (citosolic and mitochondrial activities). However glutathione S-transferase and SOD activities were significantly lower in diabetic than in control rats. The association of training and diabetes significantly increased hepatic glutathione concentration and caused a decrease in the hepatic TBARS in rapport to diabetic animals. However this association didn't improve either SOD or glutathione Stransferase activities.

P3 16 DISCRETE CHANGES IN THE THERMORREGULATORY SET POINT DURING SLEEP. R. V. Rial, I. Barjau, M. C. Nicolau, A. Gamundí, M. Akârir and C. Rosselló. Laboratori de Fisiologia. Dept. de Biologia F. i C. S. Universitat de les Illes Balears. 07071 Palma de Mallorca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 162, 1997.

Neurones of the hypothalamic anterior preoptic region are in charge of the thermoregulatory function. They are modulated in turn by environment, activity, light, body position, circadian time, etc., working together in a complex manner. As a result, small variations in the set point of the physiological thermoregulatory mechanisms do occur. The mammalian physiological control, however, is proportional, with continuously varying parameters. The behavioral thermoregulation is much older, in evolutive terms, and it only works in an on-off form, with a non continuous variation of thermal losses and gains. The thermoregulation is slightly impaired during Slow Wave sleep, although the body temperature remains well controlled. During REM however, the body turns poikilothermic and paradoxical thermoeffector responses are usual.

To gain evidence about the evolution of sleep, the tympanic temperature of normal sleeping subjects has been continuously recorded during sleep, with a precision of 0.01 °C. Several segments of the temperature-time plot showed clear discontinuities which can only be due to discrete and very rapid changes in the thermoregulatory set point.

These results support the hypothesis of a primitive thermorregulatory mode during sleep.

ADAPTATION OF L-LYSINE UPTAKE BY THE CHICKEN JEJUNUM TO DIETARY L-LYSINE. P3 17

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We have measured the L-lysine influx by the jejunum of chickens fed a standard diet (containing 48 g L-lysine/kg crude protein) or a diet supplemented with L-lysine (containing 68 g L-lysine/kg crude protein). The experiments were carried out in 6-wk-old Label chickens. Fluxes were measured in everted intestinal segments mounted on rods and incubated for 1 min in a well stirred medium. ^{3}H-polyethyleneglycol-4000 was used to correct L-lysine uptake for adherent fluid. Results show that: a) In control birds, total L-lysine influx may be separated into two components: one passive, and the other mediated and Na^+-independent with a Vmax of 0.41 nmol/mg tissue min; b) Supplementation with L-lysine had no effect on either the morphometric parameters (surface area and wet weight of the jejunum) or the monosaccharide transport capacity; c) In supplemented chickens, the mediated uptake of L-lysine had a Vmax of 1.1 nmol/mg tissue min, significantly higher than control birds, and Na^+ sensitive. We conclude that feeding chickens with an L-lysine-enriched diet enhances the intestinal absorption of L-lysine. This adaptive response may involve an increase in the capacity of the mediated pathway without affecting the uptake of L-lysine by unspecific processes.

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CALCIUM SIGNALLING AND INTRACELLULAR pH IN SINGLE PANCREATIC ACINAR CELLS. P3 18

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In several cell types a relationship between Ca^{2+} and intracellular pH (pH_i) has been described. We investigated the effects of Ca^{2+}-mobilizing agonists in pancreatic acinar cells loaded with the pH probe BCECF, using a digital microfluorimetric system. Stimulation of acinar cells with CCK-8 (10 nM and 20 pM) induced a transient acidification followed by a slow and gradual recovery. When cells were superfused with ACh (10 µM) a similar pattern was observed, although the acidification was smaller compared to the response to CCK-8. Several manoeuvres increasing the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) (ionomycine 1 µM, thapsigargin -TPS- 10 µM or Ca^{2+} influx after thapsigargin or CCK-8 induced Ca^{2+} depletion) lead to cellular acidification. This indicates that a mere increase of $[Ca2^{2+}]_i$ is enough to reduce the pH; of those cells. The CCK-8 evoked acidification is completely dependent on the release of Ca^{2+} from intracellular stores, since it is abolished by pretreatement with TPS. When cells were bathed in a medium containing $LaCl_3$ 1 mM in order to inhibit the plasma membrane calcium ATPase (PMCA), the acidification observed after CCK-8 stimulation was blocked or strongly reduced compared to the response obtained when cells were stimulated with CCK-8 alone. Together, those results show that the transient acidification produced by CCK-8 is mainly due to Ca^{2+} mobilization and the subsequent Ca^{2+}/H+ exchange via the PMCA.

Supported by DGICYT PB94-1416-C02-02 (Spain).

P3 19 TRANSPORT OF UNCONJUGATED BILIRUBIN (UCB) BY RAT CANALICULAR LIVER PLASMA MEMBRANE. J. E. Bayón, L. Pascolo*, F. Cupelli*, D. Ostraw**, C Tiribelli* and J. González-Gallego. Dept. Physiology, Pharmacology and Toxicology, Univ. León (Spain); *BBCM Dept., Univ. Trieste, Italy and **GI Division, Nortwestern Univ. Medical School, Chicago, USA. J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 164, 1997.

Several organic anions (OA) are transported across the canalicular plasma membrane by active, ATP-dependent mechanisms. Though canalicular excretion of UCB *per se* has been regarded as marginal, both normal and Gunn rat bile contain significant concentrations of UCB. *Aims:* to determine if UCB is transported across the canalicular plasma membrane of the hepatocyte, and whether this mechanism is ATP-dependent. *Results:* UCB is transported into the canalicular plasma membrane vesicles (cLPMV) into an osmoticsensitive space by an ATP-dependent mechanism; is saturable with respect to both UCB (Km = 26.8 nM) and ATP (Km = 2.4 mM). These Km values are each within the physiologic concentration range of the substrate. UCB transport by cLPMV is not stimulated by, ADP, AMP AMP-PCP, and is reduced significantly known inhibitors of ATPase activity. Thus, ATP hydrolysis is necessary for the stimulation of UCB transport by ATP. ATP-dependent, as well as basal UCB transport by cLPMV is the same in both normal Wistar rats and TR^- rats deficient in cMOAT. The translocator responsible for the ATP-dependent canalicular transport of UCB remains to be determined, though the complete inhibition by DPC suggest that an ecto-ATPase may be involved.

P3 20 MECHANISM OF TAUROCHOLATE TRANSPORT INTO BRUSH-BORDER MEMBRANE VESICLES FROM CHICKEN RECTUM. J. Bolufer, R. Coleto and C. M. Vázquez. Dpto. de Fisiología y Biología Animal, Facultad de Farmacia, Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 164, 1997.

Taurocholate transport was studied in brush-border membrane vesicles isolated from chicken rectum using a rapid filtration technique. The purity of the brush-border membrane vesicles was checked by the finding that the specific activity of sucrase (a brush-border membrane enzyme marker) was severallold greater in vesicles compared with corresponding values in mucosal homogenate. The functional integrity of isolated brush-border membrane vesicles was evaluated by the uptake of D-glucose, which stowed transient increase in the presence of Na^+. A Na^+-dependence of taurocholate uptake was showed in brush-border membrane vesicles prepared from rectum, snnce taurocholate transport was transiently increased (accumulation) in the presence of a Na^+ gradient between the external medium and intravesicular medium. The magnitude of the accumulation was 1.8-2-fold. The use of taurochenedeoxycholate in the incubation medium reduced Na^+-dependent taurochelate transport (90 % inhibition). Those results are consistent with the presence of a Na^+-dependent bile salt transport in brush-border membrane vesicles obtained from chicken rectum.

INTESTINAL Na^+-DEPENDENT TRANSPORT OF GALACTOSE AND PHENYLALANINE IS INHIBITED BY CYTOCHALASIN. P3 21

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Cytochalasins are well-known cytoskeleton inhibitors by their disrupting actions on actin microfilaments. Previous results in rat intestine with Ussing-type chambers have shown that cytochalasin E (cyt E) increases mannitol paracellular permeability and inhibits mucosal to serosal flux of galactose. This last effect requires the presence of cyt E in the mucosal medium. In the present work, the effect of cyt E on Na^+-dependent Phe and galactose transport in rat intestine is studied *in vitro*. Uptake of 2 mM galactose by intestinal everted rings after 30 min incubation is clearly inhibited by cyt E. The effect enhances with the concentration from 5 to 20 µM. In Na^+-free medium, no inhibitory effect of cyt E on galactose uptake is observed. In everted sacs, where cyt E is only present in the mucosal medium, galactose uptake by the luminal surface of the tissue and transference from mucosal to serosal are also inhibited. Similar results have been obtained with phenylalanine, indicating that cyt E inhibits also Na^+-dependent transport of the amino acid. Na^+, K^+-ATPase activity in enriched fraction of basolateral membrane is not affected by 20 µM cyt E, which suggests that transmembrane gradient of Na^+ is not modified. The results seem to indicate that cyt E inhibits Na^+-sugar and Na^+-Phe cotransport which could be related with their direct effect on cytoskeleton.

5-HT RECEPTOR SUBTYPES INVOLVED IN THE SEROTONIN ACTION ON L-LEUCINE INTESTINAL ABSORPTION. P3 22

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Serotonin (5-hydroxytryptamine, 5-HT) mediates a wide range of physiological functions by activating multiple receptors. The aim of the present study was to determine the 5-HT receptor subtypes involved in the serotonin inhibition of L-leucine transport across rabbit jejunum in vitro. A number of agonists and antagonists were used to characterize the receptors through which serotonin inhibits this intestinal transport. The results show that $2.5x10^{-6}$ M 5-HT inhibits the amino acid intestinal transport by about 20 %. The 5-HT receptor agonists, α-methyl-5-HT (5-HT_2), 2-methyl-5-HT (5-HT_3) and zacopride (5-HT_4) at concentrations 2.5x10-6 and $2.5x10^{-5}$ M produced 10-30 % inhibition on L-leucine intestinal transport. 5-carboxyamidotryptamine (5-HT_1) did not produce any inhibition The 5-HT antagonists, GR 113808A (5-HT_4) at $2.5x10^{-6}$ M and ritanserin (5-HT_2) and ondansetron (5-HT_3) at $2.5x10^{-5}$ M completely blocked the effect of 5-HT. However, methiothepin (5-HT_1) did not produce any effect on serotonin action in the intestinal transport of amino acid. It can be concluded that 5-HT_2, 5-HT_3 and 5-HT_4 receptors could mediate inEibition of L-leucine transport across rabbit jejunum.

P3 23 *IN VITRO* EFFECT OF FLUOXETINE ON 5-METHYLTETRAHYDROFOLATE TRANSPORT. E. Urdaneta, M. J. Liberal, I. Idoate, A. Díez, M. Muñoz and J. Larralde. Dpto. de Fisiología y Nutrición, Facultad de Farmacia, Universidad de Navarra (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 166, 1997.

The intestinal absorption of folates involves the transport of pteroylmonoglutamyl forms. Transport occurs mainly by a saturable proton dependent process. Pteridine drugs like sulfasalazine, methotrexate and triamterene competitively inhibit the intestinal transport of folic acid. Diphenylhydantoin also impairs folic acid absorption. The aim of the work is to study the effect of fluoxetine (FLX) on 5-methyltetrahydrofolate (5-CH3H4PteGlu) absorption.

Experiments were made at 37° C in jejunum of male Wistar rats (170-200 g) that had been fasted. 0.5 μM 5-CH_3 H_4PteGlu and FLX ranging 0.01-0.1 mM were used. Methyltetrahydrofolate uptake in small jejunal pieces was reduced by FLX. For 0.1 mM FLX, substrate uptake was 65 % of the control value. Jms and in a lesser extent Jsm were also reduced by the drug. The inhibition in uptake and fluxes was observed when the incubation medium pH was 5.5 while when it was 7.4 the action was negligible. Time course uptake of 5-CH_3H_4PteGlu by brush border membrane vesicles was diminished by FLX in a $pH_{out}<pH_{in}$ gradient. No effect was observed in vesicles when pH_{out} and pH_{in} were 7.4. Altogether these results seem to indicate that FLX interacts with the luminal folate carr ier. As FLX is a widely used antidepressant human biopsies are now being tested.

P3 24 DIGESTIVE ENZYME ACTIVITY IN *DENTEX DENTEX*. A FIRST APPROACH. G. Cardenete, A. Garzón, M. C. Palma. Dpto. Biología Animal y Ecología. Universidad de Granada (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 166, 1997.

The knowledge of the digestives capacities of a specie is essential in order to feed it with commercial purposes. In this context, we undertake the study of some digestive enzyme activities (total proteolytic activity and amylase) in three fractions of the digestive tract of a sparid fish: *Dentex dentex,* a species of great interest for Mediterranean aquaculture. In the case of proteolytic activity (measured at four pH values: 1.5, 3, 7 and 8.5) the stomach seems to play a secundary rol, showing only a light activity of enzymes like Pepsin (pH 3). The highest proteolytic activity belongs to enzymes like Trypsin-Chymotrypsin (maximum activity at pH 8.5), especially in the first half of the gut, that includes the pyloric ceca. In general, the pattern of proteolytic activity corresponds with a carnivorous fish, being the values of "Trypsin" activity similar to the obtained in seabream of the same weight.

On the other hand, amylase activity found in all fractions studied was wery low in comparison with other carnivorous fishes; even when *dentex* was fed with a pellet of higher carbohidrates content than the basal diet. This fact would indicate that this species presents lower capacity for digest carbohidrates than other species of cultured fishes.

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DIFFERENTIAL HAEMATOLOGICAL ANALYSIS IN DIURNAL AND NOCTURNAL BIRDS IN THE SOUTH-EAST OF SPAIN. P3 25

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The purpose of this study was to bring new data on haematology in wild birds, in view of the limited bibliography about Spanish species (González, 1991; Heredia *et al.*, 1991). The data were obtained in Granada (South-East of Spain) in a total of 28 birds of 11 different species (9 diurnals and 2 nocturnals). Red blood count (RBC), Haematocrit value (Hc), Haemoglobin (Hb), White blood count (WBC), and total plasma proteins (TPP) were analyzed. Likewise, volumetric indices (MCV, MCH and MCHC) were calculated. Mean values of RBC (2.75 x 10^6/mm^3), Hc (38 %), VCM (146.6 fl.) and PPT (2.73 g/dl.), both diurnals and nocturnals, were similar to those documented by other auhors in the same bird species but in non autochthonous ones (Snyder *et al.*, 1981; González, 1995). On the contrary, Haemoglobin (8 g/dl.), and indicas ainsing from haemoglobin (MCH and MCHC) showed a slighthy lower values than those reported by other authors. White blood count in this study was higher (72000/mm^3) than those found in the Jerez Zoo in the same species. Kruskal-Walls test, mude in order to detect the possible differences between diurnal and nocturnal species, only revealed significant differences ($p = 0.04$) in RBC (3.06 and 2.13 x 10^6/mm^3, respectively).

INHIBITORY EFFECT OF CCK ON INTESTINAL Na^+-GALACTOSE TRANSPORT *IN VITRO*. P3 26

A. Barber, A. Gordobil, S. Sánchez, M. Cobos, A. Fortuño, M. Pérez. Dpto. de Fisiología y Nutrición. Universidad de Navarra, Pamplona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 167, 1997.

Cholecystokinin (CCK) is a hormonal peptide wilh several functions in the gastrointestinal tract and in the central nervous system by interacting with two distinct receptors, CCK-A and CCK-B. In guinea pig isolated ileum longitudinal muscle myenteric plexus, CCK-8S and CCK-4 elicit different contractile responses, preferably mediated by CCK-A and CCK-B receptors respectively. In the present work, the effect of CCK-8S and CCK-4 on galactose transport in rat jejunum has been investigated in vitro. Everted rings were used and sugar accumulation after 20 min incubation at 37°C in saline solution was measured. The uptake of 2 mM galactose was clearly diminished by CCK8S at concentrations between 10-5 and 10-8 M. CCK-4 also inhibited galactose uptake at 10-7 M and higher concentrations. The non-mediated sugar uptake, assessed in the presence of 2 mM phlorizin, was not modified by 10-6 M CCK-8S or CCK-4, which indicates that these peptides inhibit the transport system. When 10-6 M devazepide, a CCK-A receptor antagonist, is added to the incubation medium, the inhibitory effect of CCK-8 on galactose uptake was reverted, not being observed any effect on the inkibition due to CCK-4. Present results seem to indicate a possible modulation of intestinal sugar active transport by CCK.

P3 27 CHANGES IN THE ILEAL DISACCHARIDASE ACTIVITIES IN NEWBORN RATS AT 21 DAYS POSTPARTUM: EFFECTS OF MATERNAL ETHANOL CONSUMPTION. M J. Cano, E. Tavares, M.L. Murillo and O. Carreras. Dpto. Fisiología y Biología Animal. Fac. Farmacia, 41012 Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 168, 1997.

Effects of maternal ethanol consumption on disaccharidase activities (maltase, lactase and sucrase) in newborn rats at 21 days postpartum were carried out. The animals were randomized into three groups: CR (rats which consumed only water and chow diet), ER (rats which consumed ethanol in tap water) and EF (rats which consumed ethanol and folic acid supplemented diets). The experimental animals were exposed to an induction period to ethanol, which consisted of administering increasing quantities of ethanol to tap water up to a maximum of 20 %. This 20 % consumption was then maintained for one month. Subsequently they were mated to obtain the 1st progeny. Folic acid supplemented diets were given from mating until the end of the lactation period. At the end of this period, ileal disaccharidase activities were determined by the method of Dahlqvist and protein content by the Lowry method. Specific activity (U/g of protein) of sucrase in ER increased ($P < 0.02$) compared to CR. EF showed a significant increase m maltase and sucrase ($P < 0.001$) specific activities with respect to CR. EF against to ER showed only a significant increase in sucrase activities ($P < 0.02$). The enzymatic activities do not decrease with ethanol in tap water, so the low body weight found in rats at 21 days postpartum could be due either to a low milk production in alcoholic mothers, or to a less intestinal length and protein content in the newborn rats. (DGICYP PB93 1192).

P3 28 ISOLATION AND PRIMARY CULTURES OF BOVINE HEPATOCYTES IN ORDER TO USE *FOR IN VITRO* STUDIES. M. E. Rodríguez, F. Vega, M. T. Lorenzo and J. A. Pazo. Depto de Fisiología, Facultad de Veterinaria. Universidad de Santiago de Compostela (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 168, 1997.

Isolation and culture of liver cells (hepatocytes) are a valuable *in vitro* research technique with numerous applications, particularly in the fields of physiology, biochemistry, pharmacology and toxicology. The purpose of our investigation is the isolation and culture of bovine hepatocytes during a long time for our posterior studies.

Bovine hepatocytes were isolated and cultured by a modification of the method of Pazo *et al (Rev. Exp. Anim.* 1993, 4 2: 135). The isolation is a two steps perfusion method using different concentrations of collagenase. The caudate lobe was used for perfusion. Cell viability determined by Trypan Blue exclusion, was aproximately 80±7 % and the yield averaged $250 \times 10^6 \pm 25 \times 10^6$ of total hepatocytes.

This method allowed the maintaining of primary cultures of bovine hepatocytes during more than 72 hours, approximately. After this period of culture the viability decreased up to 50 %.

EFFECTS OF EPOMEDIOL ADMINISTRATION ON THE BILIARY SECRETION OF LIPIDS IN THE RAT. P3 29

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Epomediol (1,3,3-trimethyl-2-oxabicyclo (2,2,2) octan-6,7-endo,endo-diol) (EPO) is a terpenoid compound active in increasing bile flow and some enzymatic activities of liver plasma membranes in the rat. We have investigated the effect of EPO administration on bile flow and biliary secretion rate of bile compounds such as bile salts, cholesterol and phospholipids. We have also studied the key enzymes that maintain cholesterol homeostasis included HMG-CoA reductase (HMG-CoA-R) the rate-limiting enzyme in cholesterol biosynthetic pathway an cholesterol 7α-hydroxylase (C7-OHase), the rate-determining enzyme in the bile acids biosynthetic pathway.

EPO significantly enhanced bile flow with an increased in the bile salts and cholesterol secretion rate but there were not changes in the secretion of phospholipids. This uncoupling between cholesterol, phospholipids and bile acids secretion can be due to changes in the lipidic composition of the membrane. EPO administration also induced a not specific increase of HMG-CoA-R, C7-OHase and some different cytoctrome P450 dependent hepatic monooxigenase activities.

EFFECTS OF NITRIC OXIDE ON NERVE-MEDIATED SECRETORY RESPONSE IN RAT PANCREAS. P3 30

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There is evidence that non-cholinergic, non-adrenergic nerves may be involved with pancreatic enzyme secretion, one of which is nitrergic nerve. This study investigates the distribution of nitrergic nerves and its effect on nerve-mediated secretory responses in the exocrine rat pancreas. Nitric oxide (NO) synthetase (NOS) immumoreactive nerves are well distributed in the pancreas. Several NOS-positive nerve cell bodies were observed in the acinar spaces while NOS-immunopositive fibres surround pancreatic acinar cells and tend to follow the course of the blood vessels. The nerve cell bodies and fine fibers lie close to the basal surface of pancreatic acinar cells. Basal amylase secretion was 6.94±0.4 U ml (100 mg tissue)$^{-1}$. Activation of intrinsic nerves by electrical field stimulation (EFS; 50 v, 1 ms, 10 Hz) resulted in marked amylase output 25.58 ± 2.78 (n = 4) U ml (lOO mg tissue)$^{-1}$ above basal level. Pretreatment of tissue with sodium nitroprusside (SNP; 10^{-3} and 10^{-4} M) resulted in a decrease in amylase secretion. In the continuous presence of SNP the EFS-evoked secretory response was markedly decreased compared to EFS alone. SNP also caused a reduction on resting and stimulated intracellular Ca^{2+} levels in pancreatic acini. The results indicate the presence of nitrergic nerves in the rat pancreas and that NO decreases both amylase secretion and intracellular Ca^{2+} concentrations in pancreatic exocrine cells.

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P3 31 EFFECT OF EXTRACELLULAR ATP ON pHi OF RABBIT PARIETAL CELLS.
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It has been recognized that extracellular ATP exerts influence on a variety of physiological processes including gastric acid secretion, in which we have found that ATP inhibits acid secretion stimulated by histamine in rabbit parietal cells, and the presence of P2y purinoceptors has been suggested. Upon stimulation of the gastric parietal cell, the secretion of protons is accompanied by an accumulation of base equivalents in the cytosol and a compensatory efflux across the basal-lateral surface to maintain pHi. There has been some controversial about the magnitude of changes in pHi as the parietal cell moves from a resting to a stimulated state. The aim of this work has been to quantify pHi in resting vs. stimulated parietal cells and to determine the modifications of the compensatory mechanisms induced by extracellular ATP in both these conditions. The pHi was estimated from the change of fluorescence of the trapped pH-sensitive dye BCECF. During histamine (0.1 mM) treatment pHi of the parietal cells changes only slightly (control: 7.232± 0.039; histamine: 7.220±0.038;, n=5). In resting parietal cells, extracellular ATP (0.1 mM) caused a small but sustained pHi decrease (7.148±0.044) and a similar effect was observed in parietal cells stimuted by histamine (7.164±0.049)(n=4). However, the precise mechanism underlying this effect mains to be established.

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P3 32 EGF STIMULATION OF p125 FOCAL ADHESION KINASE ($p125^{FAK}$) AND PAXILLIN TYROSINE PHOSPHORYLATION ON RAT PANCREATIC ACINAR CELLS.
C. Camello, J. A. Tapia and L. J. García. Departamento de Fisiología, Universidad de Extremadura, 10071 Cáceres (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 170, 1997.

EGF, a potent mitogen in mamy cell types, exert effects tbroogh receptors that possess am intrinsic tyrosine kinase activity. The present study examines the effects of EGF in rat pancreatic acini on the tyrosine phosphorylation of $p125^{FAK}$ and paxillin. Cell lysates were incubated with anti-phosphotyrosine mAB (PY20), then analyzed with anti-$p125^{FAK}$ mAB, anti-paxillin mAB or PY20 mAB. EGF at 2.5 min increased tyrosine phosphorylation of multiple proteins with apparent molecular masses of 180, 135, 105, 95 and 75 KDa. A maximal increase in tyrosine phosphorylation of both $p125^{FAK}$ and paxillin was detected within 1 and 2.5 min with a 2.8+0.3-fold increase in $p125^{FAK}$ and 1.7±0.2-fold for paxillin. EGF caused a maximul effect at 10 nM. Pretreatment for 2 h with GF109203X (3 μM), a selective inhibitor of PKC, had no effect on the response to EGF. However, pretreatment for 30 min with wortmanimm (1 μM), an inhibitor of PI3-Kinase, caused a significant decreased in the phosphorylation of $p125^{FAK}$ and paxillin by EGF whitout any effect in the autophosphorylation of the EGF-receptor tyrosine kinase. These findings demonstrate that in rat pancreatic acini EGF causes rapid tyrosine phosphorylation of both $p125^{FAK}$ and paxillin. This stimulation is dependent, at least in part, of the PI3-Kinase, activation but not of the PKC activation.

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INFLUENCE OF THE TYPE OF DIETARY FAT (OLIVE AND SUNFLOWER OIL) UPON (GASTRIC ACID SECRETION AND SERUM GASTRIN LEVLES IN MAN. P3 33

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The effects of adaptation to two diets differing in the type of dietary fat on the gastric acid secretory response to food, and on the circulating levels of gastrin were exarnined in humans. The study wes performed in 18 cholecystectomized subjects previously submitted to a 30-d adaptation period to diets containing olive (Group O) or sunflower oil ((Group S) as the fat source. During the experiments, physiological simulation was achieved by ingestion of oleic acid- linoleic acid-eriched liquid mixed meals. These resulted in an immediate rise in gastric pH. In Group S, the return to the premeal value was completed within 60 min, and a further decline to values significantly lower than the basal ones was observed at the end of the study, period. In contrast, ingestion of the meal containing olive oil attenuated and prolooged the pH decrease after the meal. Gastric acid concentration values reflected the pH ones. The effect of olive oil on gestric acid secretion involved the suppresion of serum gastrin, which may be due to the release of some factor capable to inbibit its secretion from the G cells. Our results indicate that olive oil may be very useful in the nutritional therapy of gastrointestinal diseases requiring a limitation of acid secretion.

PLASMA PEPTIDE YY AND PANCREATIC POLYPEPTIDE IN DOGS AFTER LONG-TEM ADAPTATiON TO DIETARY FATS QF DIFFERENT DEGREES OF SATURATION : OLIVE AND SUNFLOWER OIL. P3 34

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Mangrel dogs from weaning to 6 months of age were fed on one of two diets that differed only in the type offat content (olive or sunflower oil) to compare plasma levels of peptide YY (PYY) and pancreatic polypeptide (PP) in the basal period and in response to food. Fasting blood concentrations of both peptides were significantly higher in the olive oil goup. Food intake was not followed by significant changes in PYY or PP levels, although some rises were observed. On the other hand, plasma PYY reached significantly greater values throughout the postprandial period in the dogs fed on the diet containing olive oil, whereas no differences were recorded between the groups as far as PP is concerned. These results explain the attenuated pancreatic secretory acttiviity observed previously by us in this animal species[1]. This mechanism may be responsible, at least in part, for the adaptation of pancreatic secretion to the quality of dietary fat.

1. Ballesta, M. C., Mañas, M., Mataix, F. J., Martínez-Victoria, E. and Sciquer, I., Long-term adaptation of pancreatic response by dogs to dietary fats of different degrees of saturation: olive and sunflower oil. *Br. J. Nutr.*, 64, 487-496 (1990).

P3 35 CHARACTERIZATION OF NEURAL PATHWAYS MODULATING GALLBLADDER MOTILITY IN DOG. S. Alcón, M. J. Pozo, P. J. Camello, G. M. Salido, C. Scarpignato*. Department of Physiology, UEX, Cáceres (Spain) and *Institute of Pharmacology, University of Parma (Italy). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 172, 1997.

Neurons containing different neurotransmitters have been found in guinea pig, dog, and human gallbladder (GB). While the role of endogenous CCK and cholinergic neurons in the physiological control of GB motility is well established, few data on peptidergic, nitrergic and adrenergic pathways are available. Isolated gallbladder strips were obtained from anaesthetized dogs and submitted to transmural electrical field stimulation (EFS). EFS caused a frequency-dependent contraction which was almost completely (90 %) blocked by tetrodotoxin (1 μM). Pretreatment of the strips with atropine (1 μM) transformed the EFS-induced contraction into a relaxation, which was abolished by propranolol (10 μM) and reduced (70 %) by the NO-synthase inhibitor, L-NAME (10 μM). VIP (10 μM) and somatostatin (10 μM) antagonists were unable to affect both EFS-induced contraction and (atropine-reversed) relaxation. Those data show for the first time that EFS activates both excitatory and inhibitory neurons in dog GB. While excitatory neurons are cholinergic, the inhibitory ones seem to be adrenergic and nitrergic. Conversely from other animal species VIP and somatostatin appear not to be involved in modulation of gallbladder motility in the dog.

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P3 36 CCK ENHANCES CHOLINERGIC TRANSMISSION IN THE GALLBLADDER THROUGH STIMULATION OF CCK-A RECEPTORS. M. J. Pozo, S. Alcón, P. Frati, G.M. Salido and C. Scarpignato*. Department of Physiology, UEX, Cáceres (Spain) and *Institute of Pharrnacology, University of Parma (Italy). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 172, 1997.

Both intrinsic cholinergic neurons and CCK are known to play a role in the physiological regulation of gallbladder (GB) motility. However, the interaction between the neural and hormonal components is not yet completely understood. Isolated gallbladder strips were obtained from white male guinea-pigs and submitted to transmural electrical field stimulation (EFS). EFS caused a frequency-dependent (5-40 Hz, 350 mA) contraction which was equally susceptible (75-80 %) to neural (TTX 1 μM) and cholinergic (Atropine 1 μM) blockade. Subthreshold concentration (0.1 nM) of CCK-8 significantly increased (by 25 %) the EFS-induced contraction while loxiglumide, a selective CCK-A receptor antagonist, reduced it in a concentration-dependent fashion, its maximal effect (27 % inhibition) being seen at 100 μM. This inhibition, however, was not observed when the CCK-A antagonist was added to the bath after cholinergic blockade. Loxiglumide, at concentrations *per se* unable to modify EFS-induced contractions, blocked the potentiating effect of CCK-8. These results suggest a facilitatory effect of CCK on ACh release from presynaptic nerves and indicate this action to be mediated through CCK-A receptor stimulation.

5-HT_4 RECEPTORS MEDIATE THE MMC-LIKE PATTERN INDUCED BY 5-HT IN SHEEP. P3 37

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The effects induced by 5-HT on gastrointestinal myoelectric activity in conscious sheep were recorded through electrodes chronically implanted and analysed by computer. The i.v. administration of 5-HT (0.5, 1, 2, 4 and 8 μg/kg/min, 5 min) induced an antral inhibition concomitant with a duodenal activity front that migrated to the jejunum, followed by a period of intestinal inactivity. This pattern closely resembled to the observed during phases III and I of a spontaneous migrating myoelectric complex (MMC). The 5-HT_1 antagonist methiothepin (0.1 mg/kg), the 5-HT_2 antagonists ritanserin (0.1 mg/kg) and ketanserin (0.3 mg/kg), the 5-HT_3 antagonists granisetron (0.2 mg/kg) and ondansetron (0.5 mg/kg) as well as the 5-HT_4 antagonist GR-113808 (0.2 mg/kg) did not modify the gastrointestinal myoelectric activity, whereas it was inhibited by atropine (0.2 mg/kg) or hexamethoninm (2 mg/kg). When each antagonist was injected 10 min before 5-HT (2 or 4 μg/kg/min, 5 min), only GR-113808, atropine and hexamethoninm were able to block the 5-HT-induced actions, without them being affected by the other agents. Those results show that 5-HT initiates a gastrointestinal MMC-like pattern in sheep through 5-HT_4 receptors. This pattern is mediated by cholinergic neural pathways involving moscarinic and nicotinic, receptors. However, our results do not indicate a role for 5-HT_1, 5-HT_2 and 5-HT_3 receptors in those actions.

INTESTINAL ABSORPTION OF LIPIDS AFTER MASSIVE INTESTINAL RESECTION. P3 38

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Massive intestinal resection leads to short intestine syndrome characterized by steatorhea, malabsorption, undernourishment etc., depending on size and location of the resection, but the adaptation of the resting intestine is not well known. As the worst symptoms of the syndrome are related to lipidic absorption, this stu dy aims to analyze *in vitro* the absorption of palmitic acid in segments of the remaining parts of the intestine in dogs submitted to 90 % intestinal resection between jejunum to colon, inclading the ileococal valve. Ten days after surgery, the mucosal part of remaining intestinal segments were incubated in micelles of ^{14}C palmitic acid, monoleine and tauorodeoxycholic acid. At different time intervals, the mucosal portions were digested to determine the accumulated radioactivity. Non operated and sham operated animals were also studied.

Resected animals showed slightly higher absorption rates than sham operated group, but the last ones also had a significantly higher absorption than non operated controls. In conclusion, the effects of abdominal surgery alone caused a short term increase in lipidic absorption. This effect would mask direct adeptive responses of the remaining intestinal mucose which could need a longer time interval to reach a full development.

P3 39 EFFECTS OF AN *IN VIVO* PARAQUAT TREATMENT ON CYTOTOXICITY, GLUCONEOGENESIS AND PARAMETERS RELATED TO OXIDATIVE STRESS IN RAT LIVER. M. P. Fernández-Otero, M J. Moreno, A. Berjón, J. C. Arenas-Vidal and G. Galobart. Dpto. de Fisiología y Nutrición, Universidad de Navarra, Pamplona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 174, 1997.

Paraquat is a widely used herbicide. Previous studies of our group have shown that *in vitro* paraquat treatment caused hepatotoxicity, as well as inhibition of gluconeogenic hepatocyte capacity. The aim of the present study was to elucidate the effects of an *in vivo* paraquat treatment on liver toxicity, gluconeogenesis, as well as on some pro-oxidant and antioxidant parameters. For this purpose, male Wistar rats (300 g b.w.) were treated with a single oral dose of paraquat (70 mg/kg). Studies were performed in isolated hepatocytes from 48 h-starved rats and the herbicide was given 24 h before cell isolation. Paraquat treatment induced a significant decrease in relative liver weight. Hepatocytes from treated rats showed a higher release of LDH to the incubation medium. A significant decrease in hepatocyte gluconeogenic glucose production was observed in paraquat-treated rats when fructose and alanine (20 mM) were used as substrates, while gluconeogesis from pyruvate, lactate and glycerol (20 mM) remained unaffected. A significant increase in both liver lipid peroxidation and oxygen consumption as well as a significant decrease in GSH levels were observed in paraquat-treated rats. The results indicate that paraquat given by oral gavage is hepatotoxic. In addition, our data suggest that the alterations caused by the herbicide on liver gluconeogenesis might be secondary to the alterations in hepatocyte membranes induced by the lipid peroxidation.

P3 40 MECHANISM OF ACTION OF BRADYKININ IN THE ISOLATED PERFUSED RAT PANCREAS. IS THERE A NOVEL PATIIVVAY?. M. Muñoz, J. H. Sweuy and G.E. Mann. Vascular Biology Research Centre, Physiology Group, Biomedical Science Division, King's College London (U.K). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 174, 1997.

In the present study we have investigated the mechanisms mediating the effects of bradykinin (BK) on vascular tone in the isolated perfused rat pancreas. Pancreta were surgically isolated from anaesthetised (60 mg/kg Sagatal I.P.) male Sprague-Dawley rats and perfused (1.6-1.8 mVmin g) *in vitro* with a Krobs-Henseleit bicarbonate medium containing Dextran T70 (5 %), bovine serum albumin (0.25 %) and L-arginine (100 μM). Vascular tone was raised with the thromboxane mimetic U46619 (10^{-7} M) or elevated K^+ (40-70 mM), and vasodilator responses to close-arterial bolus injections of BK (1 nmol) were examined. BK injection induced a rapid and transient decrease in perfusion pressure which was abolished by the B_2 receptor antagonist HOE 140 (1 μM). This response is not mediated by NO release, since it was not affected by NO-synthase inhibitors (L=NAME or L-NMMA, 1 mM), methylene blue (10 μM) or disruption of the endothelium. The response was unaffected by indomethacin (10-100 μM) or by NGDA (10 μM), excluding the participation of prostacychn. A BK-induced EDHF release, as a mechanism for BK-induced arterial relaxation, was ruled out by the perfusion with high K^+ or tetrabutylammonium (3 mM). Desferioxamine (0.2 mM), WEB (100 nM), and VIP and CGRP antagonists were without effect. Taken together, the data suggest the presence of a novel and distinct pathway for BK action in the isolated perfused rat pancreas.

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EVALUATION OF THE TEEM-100. F. Drobnic, P. Galilea, V. Pons and J. Riera. Depto. Fisiología y Valoración Funcional, Centro de Alto Rendimiento, 08190 S. Cugat del Vallés (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 175, 1997. P3 41

The aim of this study was to compare a portable gas analyser system (Aerosport TEEM 100™) with an internationally validated ergoanalyser system (Jaeger Oxycon Champion™) at different stages of work load. The usefulness of the effort tests has been widely endorsed in some medical specialities, as cardiology, work medicine and sports medicine. In pneumology everyday is more evident that it is necessary to train new professionals capable not only to guide tests to perform investigation studies, but also to diagnose more completely and definitely respiratory pathologies by these tests. One of the biggest problems we have to face is the high cost of the basic hardware to lead this kind of tests. Another problem, not so critical, but worthwhile, is the space occupied by this hardware. Continuous technological advances enter in the respiratory departments new hardware of shorter size, but more accurate and with a minor cost. One of the more important problems of these new respiratory systems was nowadays the difficulty to analyse accuretely carbon dioxide. The new TEEM-100 portable ergoanalyser system is equipped with an oxygen analyser and a carbon dioxide analyser. The validation of this equipement had been conducted by other laboratories, although the number of studies is limited. The possibility that the data subministered by the TEEM-100 were of the same quality than the data of other recognised analysers, is an interesting point to admit this hardware in clinics, teaching and work physiology laboratories.

DIAGNOSIS OF BRUCEL.LOSIS BY POLYMERASE CHAIN REACTION IN HUMAN PATIENTS. M. I. Queipo-Ortuño, P. Morata, P. Martas* and J. D. Colmenero*. Departamento de Bioquímica y Biología Molecular. Universidad de Málaga and *Unidad de Enfermedades Infecciosas. Hospital Regional Carlos Haya. Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 175, 1997. P3 42

Background: Brucellosis diagnosis is frequently difficult. Because of its ability to detect very small number of germs, the polymerase chain reaction (PCR) is being applied to the diagnosis of a growing number of infectious diseases.

Objective: To evaluate a peripheral blood-based PCR assay for the diagnosis of human brucellosis.

Method: Peripheral blood of 26 patients with brucellosis was collected. We extractad DNA of the specimens and amplified a specific pathogenic DNA sequence of 223 bp. Amplification was done on all DNA extracts following the protocol by Baily *et al.* with slight modifications. Primers were based on the gene encoding a 31 kDa outer membrana protein.

Results: Of the 26 study patients, seventeen (65.4 %) had a positive blood culture and 9 (34.6 %) were diagnosed by serologic tests. Polymerase chain reaction was positive in 22 patients (84.6 %), seven of whom had negative blood culture (77.7 % of patients with negative blood culture). All the healthy control subjects were negative.

Conclusion: Our peripheral blood-based PCR assay for Blucella has a high sensitivity and specificity and may represent a promising advance in the diagnosis of brucellosis.

P3 43 A MODEL OF THE THERMAL RESPONSE TO COLD-WATER WETTING. M. Wolf and R. Garner. Physiol. Dept., U. of So. Carolina. Columbia, SC 29208 and the FAA., CAMI, Okla. City, OK (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 176, 1997.

A model was developed of transient changes in metabolic heat production and core temperature for humans subjected to cold conditions. It was modified to predict thermal effects of the upper parts of the body being sprayed with water from a system desigued to reduce the smoke effects of an airplane fire. Temperature changes were computed at 25 body segments in response to water immersion, cold-air exposure and windy conditions. Inputs to the temperature controller were 1) temperature change siguals from skin segments and 2) an integrated signal of the product of skin and head-core (hypothalamic) temperature changes. The controller stimulated changes in blood flow to skin and muscle and heat production by shivering. Two controller parameters were adjusted to obtain good predictions of rectal-temperature and heat-production experimental data in head-out, water-immersion (0- 28 °C) studies in humans. A water layer on the skin, whose thickness decreased transiently by evaporation, was added to describe the effects of the water-spray system. Because the layer evaporated rapidly in a very cold and windy environment, its additional cooling effect over a 60-min exposure period was minimal. The largest additional decrease in rectal temperature due to the water layer was < 1 °C which was in normal conditions where total decreases were small.

P3 44 THE ANTIPLATELETS AGGREGANT TICLOPIDIN: A THERAPEUTIC OPTION IN THE SEPTIC SHOCK? N. Martínez de Lima and J. Pimentel de Oletta. Dpt. of . Physiological Sciences. Vargas Medical School.Central University of Venezuela (V enezuela). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 176, 1997.

The incubation of Endotoxin(E) (150 µg/ml) with platelets (P) reproduce in rats, actions of the E iv (2,5 mg/kg) such as fall of the PAM in 48 % and thrombocitopenia of 55 %, suggesting that the P are important in the genesis of the trombo-hemorragics complications of the septic patient. Our objective was to seek an antiplatelets agent that it would be capable of inhibiting the interaction E-P to studied the influence of these cells in the patogenic of the endotoxic shock. For this was proven inhibitors on the platelets aggregation (PA) induced by E in an agregometer Chrono-Log.The PA induced by E of *E. coli* 0127:B8 (Difco) in rats showed particular characteristics occur 2 min after adding the E. the greater aggregation (30 %) is seen between 2.5 and 10 µg/ml of PRP. is not produced in citrated PRP it is not inhibited either by ASA or pentoxifilin and it is with high dose of ticlopidin (Tc) *in vitro*. The effect *ex vivo* of Tc in the PA with E. was evaluated with 3 treatment plans of 9 mg/kg/day by 1, 2 and 3 days, being obtained inhibition in 84 % ($P < 0.001$) alone with 3 dose. The administration of 9 mg/kg of Tc 1 h before E increases the survival from 3 5% to 70 %. These findings put the Tc as election drug to study the beneficial actions that could be derived from the blockade of E-P interaction and could lessen complications as the CID.

(CDCH, N° 10.1797.86).

EFFECT OF THE TICLOPIDINE ON THE ANATOMOPATHOLOGICAL CHANGES AND THE SURVIVAL OF RATS SUBMITED TO ENDOTOXIC SHOCK. P3 45

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The treatment of rats with 3 doses of 9 mg/kg/day with ticlopidine (Tc), produced a reduction (84 %) of the aggregation induced by endotoxin (E) (5 µg/ml) effect not shared by others antiaggregants. Our objective was explored if Tc produces protective effect in rats submitted to endotoxic shock. It was determined the mortality rate produced by 2,5 mg/kg of E of *E. coli* 0127:B8 and were studied the changes macro- and microscopics of the thoraco abdominal visceras 1 h after E iv and was compared with a group treated with 3 doses of Tc 9 mg/kg/day. The E produced the death of the 65 % of the rats 95 % presented respiratory difficulty and rales during the first hour, with haemorragics zones, intensive and diffused hiperhemia, and edema of the small intestine, loss of the haustra. The liver showed intensive congestion with dotted granular diffused in its surface and the lungs were found congestive changes. The microscopy demonstrated injury of the indestinal hairinesss with infiltration of MN and necrotic material in the lumen. The treatment with previous Tc to E increased the survival from 35 % to 91 % ($p < 0.001$). No animal prsented injuries either macro or microscopic in the visceras. These tesults suggest that the Ticlopidine is an effective weapon therapeutic in the endotoxic shock. (CDCH10179786).

REGULATION OF Na^+-LINKED TRANSPORT SYSTEMS IN CHICKEN COLONIC LUMINAL MEMBRANE. P3 46

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The mechanisms of Na^+ transport in chicken colon are dependent on the dietary and/or hormonal states of the animal. The activity of Na^+-glucose cotransporter and Na^+-H^+ antiporter have been determined in brush-border membrane vesicles (BBMV) isolated from the colon of chickens maintained either on a low and a high salt diet or on a diet with or without water. The plasma level of aldosterone increased in response to low salt intake from 10±1 pg/ml to 207±19 pg/ml and in response to dehydration to 70±12 pg/ml. Both a low salt diet and water deprivation increased the activity of the apical Na^+-H^+ antiporter (60 and 46 % increase respectively). The activity of the Na^+-glucose cotransport decreased (65 % decrease) in the animals maintained on a low salt diet and increased (25 % increase) in the animals deprived of water. The molecular mechanisms by which the salt content of the diet and the water deprivation control the activity of the Na^+-glucose cotransporter and of the Na^+-H^+ antiporter are under investigations.

We are grateful to the DGICYT (N° PB92-0690) for the financial support.

P3 47 Na^+-H^+ EXCHANGE AND PH_i REGULATION IN CHICKEN COLONOCYTES. M. L. Calonge, M. C. de la Horra and A. A. Ilundáin. Depto. Fisiología y Biología Animal, Universidad de Sevilla, 41012 Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 178, 1997.

Colonocyte pH (pH_i) was measured with BCECF. The proton ionophore FCCP reduced basal pH_i, indicating that cytosolic [H^+] is not at electrochemical equilibrium across the membrane. External Na^+ removal decreased PH_i and reinstatement of Na^+ returns pH_i towards its control value. The initial rate of pH_i recovery from an acid load was Na^+dependent (K_m for Na^+, 24 mM), inhibited by EIPA (IC_{50}, 0.18 , µM) and increased as the pH_i decreased from 7.2 to 6.6 (Hill coefficients, 1.88). An outwardly directed proton gradient transiently stimulated $^{22}Na^+$ uptake into colonic BBMV. EIPA and amiloride inhibited pH gradient-driven Na^+ uptake (IC_{50} of 4 µM and 32, µM, respectively). The K_m for Na^+ of pH gradient-driven Na+ uptake was 6.8 mM. The Hill coefficient of the relationship between the initial rate of pH-driven Na^+ uptake and the intravesicular pH was 0.70. It is concluded that a Na^+-H^+ exchanger is involved in pH_i homeostasis in chicken colonocytes and that these cells possess at least two types of Na^+-H^+ antiporters with different sensitivity to EIPA and different kinetic parameters.

P3 48 COMPARATIVE STUDY OF THE ERYTHROCYTE MEMBRANE RESISTANCE TO LYSINE LAURATE. M. P. Vinardell, M. A. Vives and M. R. Infante*. Unidad de Fisiología, Facultad de Farmacia, Barcelona and *Depto. Tensioactivos, CID, Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 178, 1997.

Detergents and other surfactants penetrate biological membranes and induce several effects, as lysis. It is generally believed that lysis results from an interaction between surfactants and lipids of the membrane. The lipid composition of erythrocyte membranes presents variations depending on animal species and has been considered an important factor influencing their susceptibility to damage. We have studied the hemolytic activity of the lysine salt of the fatty lauric acid on human, rat, guinea pig, rabbit and chicken erythrocytes. After incubation of the erythrocytes with different concentration of lysine laurate, we have determined the percentage of hemolysis by comparing the absorbance (540 nm) of the supernatants with that of control samples totally hemolysed with distilled water. Mean corpuscular volume (MCV) of the different erythrocytes was determined from the hematocrit and the erythrocyte number by counting in a Bürker camera. The resistance of the erythrocytes to lysine laurate varied with the animal species, depending on their size. Smaller erythrocytes presented lysine at lower laurate concentrations in the similar way as the effect of bile salts, which was attributed to the peculiar phospholipid composition of erythrocyte from some animal species (Lipids, 28, 999, 1993).

MELATONIN COUNTERACTS THE RUTHENIUM RED CYTOTOXICITY. P3 49

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 179, 1997.

Ruthenium Red (RR) is a highly toxic inorganic complex for the cell. RR produces an increase in free radical production impairing normal cellular functions. Melatonin is now considered a potent antioxidant and free radical scavenger. Thus, we consider it worthwhile to assess the protective role of melatoninin RR-treatedr ats. Four groups of animals were done: control, RR (30 μg/kg, i.p.), melatonin (10 mg/kg, i.p.) and RR + melatonin. All the drugs were injected at 12:00 h, and the animals were killed two hour later. The degree of lipid peroxidation (LPO) in plasma membranes; changes in the glutation peroxidase (GPx) activity, and changes in mitochondrial metabolism through the variations in the oxidative complexes of the electronic transport chain were measured in brain, kidney and liver. The results show that RR injection significantly inhibits GPx activity without effects on membrane LPO. Melatonin was able to counteract the effect of RR on GPx activity, decreasing the control level of LPO. Regarding mitochondrial metabolism, RR causes a significant inhibition of the I and IV oxidative complexes, with less effect on II-III complexes. Melatonin counteracts all these effects of RR. Moreover, melatonin was also able to significantly increase the basal activity of IV oxidative complex. These data suggest an antioxidant activity for melatonin into the mitochondria, and support a role for this indoleamine in the modulation of mitochondrial metabolism.

PROTECTIVE ROLE OF LEGUME INCLUSION IN A SATURATED FAT-RICH DIET. P3 50

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The type, amount and distribution of macronutrients in the diet may affect fat metabolism and produce some lipid disturbances. The aim of this study was to investigate the potential beneficial effects of the inclusion of whole seeds from field beans in a diet rich in saturated fat and cholesterol. Male Wistar rats were divided in three groups (n = 10): control (C) diet; hypercholesterolemic (H) diet containing casein, saturated fat and cholesterol; and legume (HL) diet containing *Vicia faba,* saturated fat and cholesterol. Liver cholesterol increased in the H diet (80 times) and in the HL diet (69 times) fed animals as compared to controls; however, a reduction in the activities of HMG-CoA reductase (cholesterogenesis) and Hormone-Sensitive Lipase (lipolysis) were observed in those animals fed on the H diet (about –79 %), which were par~ally recovered (–53% and –49%, respectively) afrer legume intake. Lipoprotein lipase activity (triglyceride hydrolisis) was similar in the control and HL groups, while the values in those animals fed on the H diet were reduced (– 55%). These results suggest that *Vicia faba* intake may have a protective role in a model of hypercholesterolemia, where the protein and/or other components may participate.

P3 51 T WAVES 1N THE EQUINE ELECTROCARDIOGRAM: EFFECTS OF TRAINING. R. Santisteban, A. Muñoz, M. D. Rubio, S. Agüera, B. M. Escribano and F. M. Castejón. Dept. Animal Biology, Sect. Physiology, Fac. Veterinary, Univ. Córdoba (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 180, 1997.

It is known that the performance of a daily exercise induces changes in the cardiac morphology, such as the development of eccentric and circumferential growth in the left ventricular wall, as well as a certain degree of dilation of the ventricular cavity. These adaptations would lead to some modifications in the characteristics of the myocardial depolarization and repolarization processes. Horses - species belonging to the II Category according to the Purkinje fibres penetration in the myocardium - have been the object of numerous studies about this subject (Steel, 1983; Evans, 1991; Evans and Polglaze, 1994; Muñoz *et al.*, 1995a,b). However, the results have been controversial, especially those regarding T wave, whose lability has been accepted in the horse. The purpose of this study was to categorize T wave training-related changes. A total of 168 resting ECG were recorded, arising from 12 healthy Andalusian horses, in two controls: at the beginning of the experiment and at the end of 3 months of training. Leads I, aVF, V10, VID, V1I, V3D and V3I were analysed. The most sigmficant changes were detected in the five precordial Leads. In V1D, V1I, V3D and V3I, an increase in the percentage of simple waves in relation to a decrease in biphasic waves was found. T wave duration, even though it showed a trend towards the reduction did not reach statistical significance. The T wave voltage reduction should be emphasized. For instance, V3D, which started from 0.552 mV before training, reached 0.299 mV after training. Part of this change might be attributed to the Heart Rate decrease, although, even considering HR as covariable, ANOVA revealed significant differences.

P3 52 A COMPARISON OF ELECTROCARDIOGRAPHIC FINDINGS AND EXERCISE TOLERANCE IN HORSES BEFORE AND AFTER TRAINING. F. M. Castejón, A. Muñoz, M. D. Rubio, E. I. Agüera, B. M. Escribano, R. Santisteban. Dept. Animal Biology. Sect. Physiology. Fac. Veterinary, Univ. Córdoba (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 180, 1997.

The main aim of this study was to analyze the relationships between T-wave electrocardiographic characteristics and the results of an exercise tolerance test. Seven healthy Andalusian horses performed a SET (standardized exercise test) with four exercise levels (approximate velocities of 4.17, 5.55, 6.94 and 8.33 m/sec.) of 1.000 m each Heart rate was recorded and blood samples were obtained at rest and after each exercise load, determining the plasma lactate concentrations. The following functional indexes were calculated: VLA2 and VLA4 (exercise velocities performed at lactate of 2 and 4 mmol/1), VLA2 and VLA4 (heart rate at lactate of 2 and 4 mmol/l) and LA150 (lactate at heart rate of 150 beats/min.). Electrocardiographic study was made with the horses standing at rest and in the stable environment. Seven Leads were recorded: I, aVF and V10 (Hamlin's semiorthogonal system), V1D, V1I (exploring electrode located at 5 cm caudal to the olecranon in the right and left hemithorax) and V3D and V3I (exploring electrode situated at the right and left articulation humeri). Both the ECG study and SET were performed at the beginning (Pretraining) and at the end of a three-month training-period (Post-training). The most significant correlations were found in the post-training control and in Leads I, V1I and V3L Strong positive correlations between T wave duration and voltage and VLA2, VLA4, HRLA2, HRLA4 and negative with LA 150 ware detected. No correlation between the percentage of the different morphologies and functional indexes was observed. Therefore, it could be concluded that, T wave voltage and duration might be of great importance in equine fitness assessment, according to their relationship with exercise tolerance parameters.

DEGREE OF EXERCISE, ANTIOXIDANT VITAMINS INTAKE AND OXIDATION. P3 53

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The oxidation levels depend on the degrce of physical exercise. Our goal is to reveal the difference between oxidation levels according to the degree of exercise and the antioxidant vitamins intake. We have developed a model to compare the oxidative level as measured by MDA, between sportsmen and non sportsmen. The results were stratified considering the intensity of exercise and vitamin intake. Results: Sportsmen revealed lower statistical MDA levels than non sportsmen (x:2.188 SD:0.759 vs. x:1.486 SD:0977, $p < 0.01$). However, when we stratified by levels, those corresponding to higher exercise levels showed a more significative mean values than the ones corresponding to light or moderate exercise levels. Beyond a threshold, the protective role of antioxidants intake associated to exercise does not correlate to the doses of vitamins or to the degree of exercise. We observed higher values of MDA in the group performing strong exercise, including those individuals receiving higher doses of antioxidants vitamins.

Hartmann, A. *et al.:* Vit. E prevents exercise induced DNA damage. *Mutat. Res. Apr.*, 346 (4): 195-202, 1995.

Kanter, M.M. *et al.:* Effects of antioxidants vitamin mixture on lipid peroxidation at rest and postexercise. *J. App. L.-Physiol. Feb.*, 74 (2), 965-9. 1993.

POSITIVE EFFECTS OF THE ATHLETICS TRAINING AND COMPETITION IN DOWN SYNDROME. P3 54

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In this comunication we have attempted to show that the development of an athletics training program is a suitable approach for helping young adults with Down syndrome (DS) achieve a greater degree of integration and normalitation as the result of an improvement of the physiologycal conditions.

A group of about 20 young people with DS trained 2-3 times a week with a local athletic club. Prior to starting the athletic program a medical/sport check up was performed in order to safeguard the medical aspects of the programs.

Participating in competitions is fundamental, not only because it requires development of physical fitness, but also because it helps achieve a sense of belonging to a group and has a specially positive effect on the personality of those athletes with DS. Results were usefully from, i) an educative and sport point of view, ii) at the personal level, there was an increase in self esteem which has improved their physical conditions and iii) at the social level, facts like: the normalisation and acceptance of a group with such obvious phenotype, to treat them without privileges or overprotection in training, the inscription in the events, a normal participation in competitions, to have a warm arrival and the collection of trophies, if any, mean a global educative achievement to accept the diversity.

P3 55 ALCOHOL-INDUCED INTESTINAL ALTERATIONS. E. Rivada, E. García, P. Salinas, and I. Fernárdez. Dpto. de Morfología Normal y Patológica, Facultad de Medicina, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 182, 1997.

Absorption alterations, together with intestinal motility deficiencies, constitute the most frequent intestinal symptoms and signs in alcoholics. In this paper, we have tried to reveal the morphometric injuries responsible for these physiological intestinal alterations. To this end, we used tinctures such as the trichromate of Masson to reveal the structures of the intestinal villi in three subject groups: normal animals, acute alcoholics and chronic alcoholics. The method used was image analysis. After analysing the apex of the cells of mucous membrane in different areas of the villus, we found that the results were same as those in the apex of acute and chronic alcoholics, although there was a significant statistical difference with the controls. The crypts were damaged in the case of the chronic alcoholics and unchanged in the acute alcoholics and the controls. Due to this, the chronic alcoholics had fewer villi with a grater distance between them. In studying the neurones of the plexus of Meissner, we found higher maximum and minimum values in both the perimetric and diametric areas for the acute alcoholics than for the chronic alcoholics and the normal animals.

These results show: 1) a deficiency in the absorption surface; 2) alterations in the cellular regeneration; 3) ballooning or swelling of the neurones of the enteral nervous system, the neuronal sufferance index. This could be responsible for the described alterations in intestinal alcoholic pathology.

P3 56 MORPHOMETRIC ALTERATIONS QF THE ENTERAL NERVOUS SYSTEM IN DISTINCT MODELS OF ISCHEMIA AND MESENTERIC ISCHEMIA-REPERFUSION. P. Salinas, E. García, M. Ribeiro, E. Rivada and I. Fernández. Dpto. de Morfología Normal y Patológica, Facultad de Medicina, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 182, 1997.

The aim of this paper was to study the morphometric changes of the enteral nervous system and, more precisely, those of the Plexus of Auerbach by carrying out both an ischemia-perfusion of the upper mesenteric artery and vein in Wistar rats.

To this end, 13 groups were used in which both the mesenteric artery and vein were clamped -ischemia and ischemia perfusion- during 30, 60 and 90 minutes. All the results were subjected to both a morphometric analysis using an image analyser and an ANOVA multi-variant statistical study.

In both the arterial and venous ischemias we found that with the 30 and 60 minutes clamping periods, there was increase of morphometric values resulting in a phenomenon of reversible ballooning. After 90 minutes of pure ischemia the neuronal size diminished, this being a phenomenon representative of serious neuronal damage. In carrying out the reperfusion in the same groups, contrary to what happened in the group of pure ischemia, we noted an increase in neuronal size after 90 minutes.

These results lead us to believe that there is less neuronal damage to the enteral nervous system with the reperfusion and, therefore, less damage to free radicals, which is what happens with other elements of the intestinal wall more sensitive to the perfusion than the pure ischemia.

THE OSTEOPENIA ASSOCIATED TO LIVER CIRRHOSIS (LC) IS REVERTED BY LOW DOSES OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I). P3 57

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The pathogenesis of osteopenia occurring in LC is ill understood and lacks effective therapy. IGF-I treatment has been recently suggested to stimule bone formation in malnutrition states. The aims of this study were: to characterize bone disorder developed in CCl_4-induced LC; and to evaluate whether IGF-I is effective in this condition. LC was induced in male Wistar rats by ip injection of CCl_4 (8 weeks) and it was confirmed histopathologically in all cases. Rats with LC were randomly divided in two groups (n=12): CI+IGF which received IGF-I (sc 2 µg/100 g bw/d, during 21 days) and CI which received placebo. Healthy sex-and age-matched rats were used as controls (CO, n=12). On the day 22nd animals were sacrificed and bone morphometry, histopathology, densitometry (g/cm^2), radiographs and bone composition were analyzed in long bones (femur and tibia). Data from CI and CI+IGF groups are expressed as the difference (in %) with respect to values in CO group (considered to be 100 %). Posterior-anterior and latero-medial diameters were similar in all groups. However, as compared with controls, CI rats showed significant reductions in bone weight (–17.5 %, $p<0.001$) and total bone density (–9.5 %, $p<0.001$). In rats from CI+IGF these parameters improved significantly (–9.5 %, $p<0.05$; and –4.9 %, $p<0.05$ vs controls, respectively), as compared to CI group ($p<0.05$, all). These findings were also confirmed by X-ray. Histology showed a reduction in cortical bone suggesting an increased resorption around medullar cavity. However, the bone protein to mineral ratio was similar in all groups. In conclusion, rats with CCl_4-induced LC develop an IGF-I-sensitive osteopenia characterized by loss of bone mass with preserved bone composition. Since osteoporosis seems to be the predominant form of osteopenia in patients with LC, IGF-I should be viewed as a possible therapy for this disorder.

THE DECREASED JEJUNAL TRANSPORT OF D-GALACTOSE AND MORPHOLOGICAL ALTERATIONS EXHIBITED BY CIRRHOTIC RATS ARE NORMALIZED BY LOW DOSES OF IGF-I. P3 58

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Liver cirrhosis (LC) evolves to malnutrition state. Sugar malabsorption could be involve in this process. On the other hand, plasma levels of IGF-I are diminished in subjects with IGF-receptors are expressed on basolateral membrane of enterocytes. On the basis of our previous results on this subject, we have focused the aim of this research in further exploring the effect of IGF-I on the *in vitro* and *in vivo* intestinal transport of D-galatose 2 mM in healthy control (C, n=38), Cirrhotic rats (CCl_4 inhalation, CI, n=38), CI rats treated with 2 µg IGF-I/100 g b.w.over 14d (CI+IGF, n=38), and control which received IGF treatment (C+IGF, n=8). ^{14}C-labelled sugar was used as isotopic tracer. Kinetic (in brush-border-membrane-vesicles, BBMV) and morphological studies were included. LC was histologically confirmed. As compared to C rats, both *in vitro* (µmol/mL of intracellular water) and *in vivo* (µmoles/cm jejunum) trials showed that sugar absorption was significantly reduced in LC ($p<0.01$). However, a significant improvement in D-galactose transport was reached in CI+IGF animals.The kinetic constants were altered in CI group (Km=6.342 mM; Vmax=2,447 pmol D-Gal/mg protein.3s), whilst CI+IGF rats (Km=5.497 mM; Vmax=3,664 pmol/mg prot.3s) displayed an absorption pattern statistically equal to that of C animals (Km=5.343 mM; Vmax=3,746 pmol/mg prot.3s). However,IGF-I did not change sugar transport in C rats. Morphological changes in CI rats (marked elongation of jejunal microvilli and villi) were verified by EM and LM, whilst a normal structure was present in CI+IGF animals. Our results show that: 1) D-Galactose jejunal transport are depressed in rats with LC; 2) this malabsorption is associated with morphological alteration; and 3) IGF-I administration is able to restore sugar absorption (both *in vivo* and *in vitro*) and return to normal histological structure.

P3 59 REAPPEARANCE OF FETAL BILE ACIDS DURING LIVER NEOPLASIA. M. F. Domínguez, M. Y. El-Mir, N. Luengo, M. I. Monte, R. I. R. Macías, M. A. Serrano, J. J. G. Marín. School of Pharmacy, University of Salamanca, Salamanca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 184, 1997.

Neoplasia is characterized hy the reappearance of structural and enzymatic proteins typical of fetal life. The aim of this work was to investigate whether liver carcinogenesis-related retrodifferentiation can affect bile acid (BA) metabolism. Urine samples were collected from healthy volunteers (H, n = 20), and 49 patients with cirrhosis (C, n = 30) or liver cancer (12 with metastasis -M- and 7 with hepatocellular carcinoma -HCC-). After extraction BA species were separated, identified and quantified by GC-MS. Major BAs were 5β-cholanoic acids, i.e., A and B rings in perpendicular configuration. However, unsaturated BAs with A and B rings with "flat" configuration, as typically occurs in human fetus, were found only as trace amounts in C group (0.03±0.02 μmol/24 h). These were increased in M (0.57±0.14 μmol/24 h, $p < 0.05$) and in C (0.73±0.17 μmol/24 h, $p < 0.05$) patients, and markedly higher in HCC patients (2.40±0.90 μmol/24 h, $p < 0.05$). Owing to the presence of cholestasis in some of these patients the relationship between fetal BAs and liver neoplasia was further investigated in the rat after induction of liver cancer by the Solt & Farber's protocol. A monoclonal antibody against the placental isoenzyme of the glutathione-S-transferase (GST-P) was obtained and used for immunohistochemistry labelling of neoplasic tissue. Bile sample collection confirmed the absence of cholestasis up to 20 weeks during hepatocarcinogenesis, GC-MS of these samples revealed very low levels of "flat" BAs in healthy adult rat bile (0.02 ± 0.01 mM). These fetal BAs were detected early during carcinogenesis and its bile concentration increased progressively (12 weeks: 0.33 ± 0.08 mM; 16 weeks: 0.56 ± 0.11 mM; 20 weeks: 0.62 ± 0.10 mM; all of them $p < 0.05$ vs control). Moreover, a significant ($p < 0.001$) correlation between bile "flat" BA output and the amount of GST-P positive tissue was found. These results suggest that neoplasic liver parenchyma re-expresses fetal metabolic pathways involved in BA metabolism that are absent in normal adult hepatocytes.