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ACTH/CLIP IN CARDIOVASCULAR CENTERS OF THE CAT BRAIN. R. Coveñas, M. de León, J. A. Narváez*, J. A. Aguirre*, S. González-Barón*. Dept. of Cellular Biology, Faculty of Medicine, University of Salamanca and *Dept. of Physiology, Fac. of Medicine, University of Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 67, 1997. O 1

The aim of this work has been the study of the presence of adrenocorticotropin hormone/corticotropin-like intermediate lobe peptide (ACTH/CLIP) in cardiovascular areas of the cat. Control adult cats, as well as animals pretreated with colchicine (intraventricular or intracisternal) were used. The cats were anaesthetized with Ketamine and perfused via the ascending aorta with buffered formaldehide 4 %. The brain sections (40 µm) were processed by indirect immunoperoxidase technique. Fibers containing ACTH/CLIP, but no immunoreactive cell bodies, were found in the nucleus of the solitary tract, the ventrolateral medulla, the locus coeruleus, the marginal nucleus of the brachium conjuntivum and the hypothalamus lateralis. Immunoreactive perikarya were only observed in the nucleus arcuatus. These data suggest: 1) Nucleus arcuatus could be the source of afferents containing ACTH/CLIP projecting to the cardiovascular areas of the brainstem; and 2) that ACTH/CLIP could be involved in central cardiovascular control.

This work has been supported by Spanish DGICYT (PB93-0992).

VENTROLATERAL PAG NEURONS PROJECTING TO RESPIRATORY NUCLEI: *IN VITRO* AND *IN VIVO* CHARACTERIZATION. J. Ribas*, S. P. Gaytán, E. Núñez-Vázquez, and R. Pásaro. *Dept. of Medical Physiology and Biophysics, Sevilla 41009 and Dept. of Animal Physiology and Biology, University of Sevilla, Sevilla 41012 (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 67, 1997. O 2

The Periaqueductal Gray (PAG) is interconnected with numerous brain regions that allows the PAG be influenced by, and to influence sensory, motor and autonomic functions. In this sense, the respiratory function must be also modulated by the PAG. In this report, the PAG link with the Ventral Respiratory Group (VRG) was elucidated by means of the electrophysiological identification of the VRG and injection of a retrograde (Fast Blue, FB) and an anterograde (Fluoro Ruby, FR) fluorochromes. The results showed four different populations of PAG FB-labelled neurons and scarce FR labelling. From all of the populations of PAG neurons, the ventrolateral one was intracellular recorded in slice preparation. The membrane resting potential of these neurons ranged from 60-75 mV and controlled the type of electrophysiological response induced by the depolarizing pulses. The higher membrane potentials were related to tonic firing and the lower to burst firing of action potentials, the latter lacked calcium conductance. Those morphological and physiological features might account for the integrative role of the PAG.

Supported by a CICYT PB94-1443 grant.

O 3 EFFERENT PROJECTIONS OF THE LATERAL RETICULAR NUCLEUS TO THE VENTROLATERAL MEDULLA. R. Pásaro, E. Núñez-Vázquez, S. P. Gaytán, and J. Ribas*. Dept. of Animal Physiology and Biology, Sevilla, 41012 and *Dept. of Medical Physiology and Biophysics, University of Sevilla, Sevilla 41009 (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 68, 1997.

The Lateral Reticular (LRt) nucleus is involved with sensorimotor activity, pain mechanisms and cardiorespiratory neuron activity. Furthermore, the involvement of the LRt in cardiorespiratory events may be also related to its functions in motor control, because the mechanic respiratory movements also depend on pure somatic motricity. However, the relation between the LRt and the ventrolateral medulla (VLM), where the cardiorespiratory neurons are located, is far from clear. The projections of the LRt to the VLM have been elucidated by means of different injections of the anterograde neuronal tracer biotin dextran amine (BDA) within the LRt different parts. The labelled axonal bundles addressed towards the nucleus of the solitary tract, to the spinal trigeminal nucleus, to the VLM, and a few axonal branches followed a ventral course to the surface of the medulla, giving off varicosities, indicating terminal fields. Supported by a CICYT PB94-1443 grant.

O 4 GALANIN INDUCES *c-fos* EXPRESSION IN CARDIOVASCULAR AREAS OF THE BRAINSTEM OF THE RAT. Z. Díaz, J. A. Narváez, R. Coveñas, P. Marcos, J. A. Aguirre, M. de León, M. P. Cordón, K. Fuxe* and S. González-Barón. Dept. of Physiology, Faculty of Medicine, Málaga (Spain) and *Dept. of Neuroscience, Karolinska Institutet, Stockholm (Sweden). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 68, 1997.

Since Galanin has a role on the central cardiovascular regulation, we have studied the expression of *c-fos* in cardiovascular central administration of Galanin. Groups of rats received an effective dose of Galanin (3.0 nmol) intracisternally and they were sacrificed 90 minutes or 6 hours after injections. One group of rats received artificial cerebrospinal fluid (aCSF) alone and another group of rats was sham operated and did not receive any treatment. These two groups were used as controls. The presence of *c-fos* was evaluated by immunofluorescence. In sham-operated animals and in rats which received aCSF no *c-fos* expression was found. From 90 minutes after Galanin injection *c-fos* immunoreactivity was detected in the nucleus of the solitary tract, the nucleus reticularis paragigantocellularis, the nucleus reticularis lateralis and the nucleus reticularis. These results indicate that Galanin increases *c-fos* expression for a longer duration in subsets of nerve cells located in cardiovascular centers of the rat and thus, this *c-fos* expression, could reflect the site of action of Galanin for its cardiovascular action.

This work has been supported by the Spanish DGICYT (PB93-0992).

SOMATIC AND VISCERAL INPUTS ONTO NUCLEUS TRACTUS SOLITARIUS NEURONS (NTS) IN A NEONATAL RAT BRAINSTEM-SPINAL CORD PREPARATION *IN VITRO*. O 5

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We examined neurons in the NTS for responses to a lower cervical dorsal root (DR) and convergent vagal (V) inputs in the neonatal rat. The brainstem and spinal cords, isolated from 2-4 day-old rats, were perfused with artificial CSF at 27 °C equilibrated with 95 % O_2 and 5 % CO_2. Both inputs evoked bursts of ventral root activity. Whole cell patch recordings were obtained from 8 NTS neurons responding to DR stimulation. Mean resting membrane potential and input resistance were -43 ± 2.8 mV (± SD) and 546 ± 217 MΩ, respectively. DR stimulation evoked polysynaptic EPSPs (n = 2), IPSPs (n = 5) or an EPSP/IPSP complex (n = 1). The latency to onset varied from 20 ms to 71 ms between the neurons (mean 40 ± 17 ms). The amplitude of EPSPs at -60 mV was 2.7 ± 0.9 mV and that of IPSPs at -40 mV was 6.5 ± 2.5 mV. In 4/8 neurons, V stimulation evoked polysynaptic IPSPs (n = 3) or monosynaptic EPSPs (n = 1). For each neuron, both inputs evoked either excitation or inhibition. The neonatal NTS neurons were most frequently inhibited by somatic inputs. The postsynaptic responses evoked by both DR and V stimulations imply convergence of functionally different inputs onto NTS neurons. This study suggests that NTS neurons can participate in the interaction between visceral and somatic afferent activity and the creation of adequate physiological response during early postnatal life.

(We acknowledge the support of the British Heart Foundation).

A5 MODULATION OF CARDIORESPIRATORY RESPONSES TO PARABRACHIAL STIMULATION IN THE ANAESTHETISED RAT. O 6

M. P. López de Miguel, P. González-Alegre, J. P. Lara, M. S. Dawid-Milner and S. González-Barón. Dpto. de Fisiología, Facultad de Medicina, Universidad de Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 69, 1997.

We have previously described the cardiorespiratory effects of specific activation of cell bodies located in two pontine structures reciprocally connected: the parabrachial (PB) complex and the catecholaminergic A5 region. A possible modulation of the A5 region in the cardiorespiratory response evoked from the PB complex has been studied.

Experiments were performed in spontaneously breathing anaesthetised rats (Pentobarbitone 60 mg kg^{-1}, i.p.). Phrenic nerve activity, respiratory flow, pleural and blood pressure were recorded. Multibarrel electrodes were positioned stereotaxically in A5 and PB. Glutamate injections (20-30 nl, 100 mM, pH 7.4 ± 0.1) in the PB were made before and after lidocaine injections (20 nmol in 30 nl) in A5. Pontamine Sky Blue injections marked both sites.

PB stimulation evoked two different respiratory responses: and inspiratory facilitatory response, showed by increased respiratory rate ($p < 0.05$, paired sample test) and an expiratory facilitatory response, showed by decreased respiratory rate ($p < 0.05$). Blood pressure and heart rate increased with both respiratory responses ($p < 0.01$ and < 0.05, respectively). After the injection of lidocaine in A5, glutamate injections in PB evoked none of these effects. These results suggest a modulation of the cardiorespiratory response evoked from the PB complex by the A5.

O 7 INCREASED VASOPRESSOR ACTIONS OF NEUROPEPTIDE Y (13-36) IN SHR WKY RATS. POSSIBLE RELATIONSHIP TO INCREASES IN Y_2 RECEPTOR BINDING IN THE nTS. J. A. Aguirre, J. A. Narváez, P. B. Hedlund*, B. Bunnemann* D. Ganten** and K. Fuxe*. Depto. de Fisiología, Facultad de Medicina, Málaga (Spain), *Dept. of Neuroscience, Karolinska Institutet, Stockholm (Sweden) and **Max Delbrück Centrum, Berlin (Germany). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 70, 1997.

The cardiovascular responses (CR) of NPY-(13-16) together with the distribution of NPY receptor subtypes within the nucleus tractus solitarius (nTS) have been studied in spontaneously hypertensive rats (SHR). NPY-(13-16) was injected intracerebroventricularly in awake rats to evaluate the CR, and NPY receptor subtypes were studied by autoradiography using [^{125}I]peptide YY as a radioligand. In both male SHR and agematched male normotensive Wistar-Kyoto rats (WKY) NPY-(13-16) injections elicited vasopressor effects. In the WKY this effect was dosedependent and became significant at doses from 75 pmol, whereas in the SHR the vasopressor effect had a longer duration than in the WKY rats. Autoradiography showed an increase in Y_2 receptor binding within the nTS in SHR. These results suggest that the inverse potency of NPY (13-36) in SHR might be due to an increased Y_2 binding.

This study has been supported by the Spanish DGCYT (SAL 91-0485 and PB91-0769) and the Swedish MRC (04X-715).

O 8 AT_2 RECEPTOR MEDIATE VASODEPRESSOR RESPONSE TO FOOTSHOCKS. A. Israel, M. Cierco, and C. I. Gutiérrez. School of Pharmacy. Department of Biological Sciences. Universidad Central de Venezuela, Caracas (Venezuela). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 70, 1997.

Angiotensin II facilitates sympathetic activity. In rats pretreated with losartan (LOS), footshocks resulted in a vasodepressor response. This action could be mediated through the AT_2 receptor. We assessed the effects of ICV or IP administration of LOS and/or PD 123319 (PD) on the cardiovascular response to footshocks (FS). Male S-D rats, 160-200 g, were treated with LOS (10 mg/kg, i.p.), an AT_1 antagonist, PD (20 mg/kg, i.p.), an AT_2 antagonist or LOS+PD. Another group of rats, with an ICV cannula, were injected ICV with: LOS (100 µg/5 µl), PD (3 µg/5 µl) or LOS+PD. Half an hour after treatment rats were subjected to mild inescapable FS delivered by Grass stimulator (2Hz/100 V/10 ms/5 min). Mean arterial pressure (MAP) and heart rate (HR) were recorded daily, and immediately after FS using a tail-cuff pletismograph (LETICA). FS increased PAM and HR (23 ± 1.8 mmHg 63.2 ± 13 bpm). Peripheral or central administration of LOS resulted in a hypotensive response to footshocks, while HR response was not altered. PD-ICV reduced, whereas PD-IP did not alter, the hemodynamic responses to FS. Simultaneous blockade of AT_1 and AT_2 receptors (ICV or IP) eliminated the vasodepressor response observed after FS in rats pretreated with LOS alone. Our results suggest that both, central and peripheral AT_2 receptors are involved in the hypotensive response to FS during AT_1 receptor blockade.

TWO MODELS OF HYPERTENSION WITH DEFICIENT BRAIN NO SYNTHASE. O 9

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We have reported that nitric oxide (NO) administered intracerebroventricularly (i.c.v.) has a depressor effect, and that the central production of NO in genetically hypertensive rats (SHRSP) is deficient (*Am.J.Hypertens.*, 9, 237, 1996). In the current study we determined whether deficient central production of NO contributes to the hypertension resulting from chronic NOS blockade. Normotensive WKY rats (115 ± 4.7 mmHg) were treated with LNNA (1.2 mg/kg/day) in drinking water for three weeks (178 ± 7.4 mmHg). Studies were conducted on normotensive control (WKY), on genetic hypertensive (SHRSP) and on NOS blocked (LNNA treated) rats. Blood pressure responses to i.c.v. administration of DEA/NO (an NO donor), of calcium (an NOS stimulant) and of L-NAME (an NOS blocker) are presented:

	Changes in MAP (mmHg)		
	WKY (4)	SHRSP (5)	LNNA (6) TREATED
DEA/NO (100 nmmol)	−39.5 ± 2.6	−66.1 ± 3.9	−54.2 ± 3.9
$CaCl_2$ (100 nmol)	−18.5 ± 1.1	−12.6 ± 1.2	−10.8 ± 1.9
L-NAME (1000 nmol)	+34.25 ± 2.6	+15.0 ± 2.7	+19.3 ± 1.8

The abnormalities of the blood pressure responses to manipulations of central NO in the LNNA hypertensive rats are entirely parallel to those in SHRSP. Both are more sensitive than normal to the depressor effects of NO. The depressed blood pressure responses of LNNA-treated rats to central manipulations of NOS indicate that a deficiency of central NOS activity may contribute to the hypertension resulting from LNNA-treatment. Finally, since LNNA hypertension results from NOS blockade, these observations support our earlier conclusion that a deficiency in central NOS contributes to the arterial pressure elevation in SHRSP.

IN VIVO TRYPTOPHAN HYDROXYLASE ACTIVITY SUPPORTS THE PRESENCE OF A SEROTONERGIC INNERVATION FROM CENTRAL ORIGIN IN RAT CEREBRAL ARTERIES. O 10

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The *in vivo* presence of tryptophan hydroxylase activity in rat major cerebral arteries as well as the possible origin of the structure containing it were explored. Enzyme activity was appraised from 5-hydroxytryptophan (5-TP) accumulation after aromatic L-amino acid decarboxylase inhibition. Decarboxylase blockade evoked a significant increase in 5-HTP levels in rat cerebral arteries, striatum, hippocampus, hypothalamus, and plasma but had no effect on aorta. p-Chlorophenylalanine reduced 5-HTP accumulation in cerebral vessels and brain nuclei whereas α-methyl-tyrosine did not modify it except in hypothalamus where it was enhanced. α-Methyl-tyrosine significantly reduced noradrenaline levels in cerebral arteries and L-dopa accumulation after inhibition of the decarboxylase in striatum. Dorsal raphe nucleus lesion significantly diminished 5-HTP formation in cerebral arteries, striatum, and hypothalamus without affecting it in hippocampus. Lesion of median raphe nucleus reduced 5-HTP accumulation in hippocampus and hypothalamus but not in cerebral blood vessels and in striatum. Superior cervical gangliectomy decreased noradrenaline levels in cerebral blood vessels without affecting 5-HTP accumulation. These results indicate the presence of a functionally active tryptophan hydroxylase in rat cerebral arteries associated with fibers originating from dorsal raphe nucleus.

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O 11 EFFECT OF CHANGES IN O_2 AFFINITY OF HEMOGLOBIN ON MAXIMAL O_2 UPTAKE OF CHRONIC HYPOXIC RATS. N. C González, W. McCanse, T. Urano and R. L. Clancy. Dept. Physiology, Univ. Kansas Medical Center, Kansas City KS (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 72, 1997.

O_2 affnity of hemoglobin (Hb) of chronic hypoxic rats (CHx; 3 weeks at PB 370 Torr; PI_{O2} ~70 Torr) was increased by administration of sodium cyanate (NaCNO) in the drinking water. Controls (Ctl) were non-treated CHx rats. NaCNO decreased the P_{O2} for 50 % saturation of Hb (P50: 38.3 ± 1.8 Torr vs 26.5 ± 1.4 Torr). At 3 weeks of hypoxia, the rats exercised in a treadmill at PI_{O2} 70 Torr (Hx) or 140 Torr (Nx). Maximal O_2 uptake ($\dot{V}_{O2max}$) was determined using an incremental exercise protocol. Maximal cardiac output ($\dot{Q}_{max}$) was obtained from arterial (Ca_{O2}) and pulmonary arterial ($C\bar{v}_{O2}$) blood oxygen content and $\dot{V}_{O2}$. In Hx, both Ca_{O2} and the convective rate of O_2 transport ($\dot{T}_{O2max} = \dot{Q}max * Ca_{O2}$) were - 60 % higher in NaCNO rats; however, since O_2 extraction and $a\text{-}\bar{v}C_{O2}$ were significantly lower, $\dot{V}_{O2max}$ in NaCNO was unchanged from Ctl. In Nx, Ca_{O2} and $\dot{T}_{O2max}$ were essentially the same in NaCNO and Ctl, but O_2 extraction and $a\bar{v}C_{O2}$ were lower in NaCNO, resulting in a decrease in $\dot{V}_{O2max}$ of ~10 % below Ctl. These data show that $\dot{V}_{O2max}$ can be dissociated from $\dot{T}_{O2max}$ in intact animals, indicating that convective O_2 delivery is not the only determinant $\dot{V}_{O2max}$. As P50 decreases, the P_{O2} required to sufficiently lower O_2 saturation of Hb in tissue capillaries may be too low to maintain an effective O_2 diffusion gradient between capillaries and tissues.

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O 12 MATHEMATICAL MODEL OF THE GAS TRANSPORT SYSTEM. L. Roa, T. Gómez-Cía and J. Ortega-Martínez. Grupo de Ingeniería Biomédica, E. S. Ingenieros, U. S. Av. Reina Mercedes, 41012 Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 72, 1997.

A mathematical model of the gas transport system is presented. The use of causal diagrams, which relate the variables and the relations considered in the model, allows an easy interaction between the members of our interdisciplinary team. The construction of the model is based on the dynamic system theory. We present the results obtained by simulation of the influence of blood flow changes in the transport of gases under different metabolic circumstances. These results agree with experimental and clinical data and validated the model. In our opinion, mathematical modelling of physiological systems could be a good research and teaching tool in physiological sciences.

VLDL SECRETION AND CHOLESTERYL ESTERS TURNOVER IN ISOLATED LIVER PARENCHYMAL CELLS ARE ALTERED BY LOVASTATIN AND SIMVASTATIN. O 13

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Lovastatin and simvastatin act at the hepatic level as very effective HMG-CoA reductase inhibitors and are currently used for the treatment of hypercholesterolemia. The blockage of cholesterol synthesis triggers an overexpression of LDL receptors, which enhances the clearance of LDL. Besides cholesterogenesis and lipoprotein uptake, cholesterol homeostasis in the liver involves esterification of cholesterol for storage and further mobilisation; formation of bile acids and lipoprotein secretion. As a consequence of the hepatic cholesterol depletion, statins might alter the free and esterified sterol pools within the hepatocyte as well as those secreted in VLDL. The present study examined the short-time effect of lovastatin and simvastatin i) in the amount of lipids associated with VLDL and ii) on cholesteryl esters synthesis and hydrolysis by assaying the activity of acyl-CoA:cholesterol acyltransferase and lysosomal, cytosolic and microsomal cholesterol ester hydrolases in rat hepatocytes. Lovastatin and simvastatin gave slight and transient modifications in the specific activities of the enzymes controlling the cholesteryl ester cycle. In addition, the situation of reduced capacity to synthesize cholesterol in the hepatocytes led to a reduced secretion of lipoprotein lipids. VLDL not only had marked reductions in their cholesterol and cholesteryl esters content, but also exhibited differences in a number of structural features.

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SALIVA LACTATE CORRELATES WITH BLOOD LACTATE AND CAN BE USED AS A PROCEDURE FOR MONITORING THE METABOLIC RESPONSE TO DIFFERENT WORKLOADS. O 14

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Objetives: To test our hypothesis that saliva lactate concentrations may reflect those present in blood and that saliva lactate can be used as a very convenient and useful parameter in the study of anaerobic metabolism. Material and Methods: Parallel determinations of lactate in saliva and in capillary blood samples, obtained at 3 minute intervals, from 9 sportsmen of international category during the performance of a maximal graded exercise test, on a specific ergometer. Lactate determinations were done by means of an electroenzymatic method using 25 µl samples in both types of fluids. Results: The degree of correlation between the saliva and the blood lactate values along the different steps of test is excellent ($r = 0.96$; range = 0.997-0.954). The absolute lactate values have been found to be lower in the saliva than in the blood samples but following a similar evolution pattern when increasing the work load (or output). It is worth noting that the "inflexion" points in the lactate curves constructed from the values obtained in the blood samples are identical to those obtained from saliva samples suggesting that this fluid can be used as an alternative to blood sampling in estimating the lactate (LT) or anaerobic threshold (AT). Conclusion: The determination of lactate in saliva can be used as an alternative to its determination in blood overcoming most of the drawbacks of the procedures being used at present, the collection of the samples requiring no special expertise.

O 15 COMPARATIVE EFFECT OF DIETARY RESTRICTION (DR) INITIATED IN ADULTHOOD VS AFTER WEANING ON BILE FORMATION AND GLUTATHIONE SECRETION IN OLD RATS. B. Tuchweber, G. Bouchard, G. Ferland, S. Chevalier and I. M. Yousef*. Depts. of Nutrition and of *Pharmacology, Université de Montréal (Canada). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 74, 1997.

Hepatic and biliary functions are known to be adversely affected by aging, resulting in greater toxicological risks. We previously demonstrated that life-long DR implanted soon after weaning (DRW) prevents the decrease in bile formation in aging rats, mostly through an increase in the biliary content of the osmotic factor glutathione (GSH). However, since DRW is known to interfere with growth and maturation, initiation of the DR at an adult age (DRA) would eliminate this factor. Thus, we investigated in female Sprague-Dawley rats, the effect of a 40 % DR initiated at 8 months of age on bile formation in aged rats (20 months). DRA resulted in a bile flow 88 % greater than that of *ad libitum*-fed rats of the same age. This increase was of the same magnitude as that observed in DRW. Biliary secretion of GSH, like in DRW was also significantly improved by DRA. This stimulation was associated with increased hepatic GSH concentration in both DR groups but was more marked in the DRW-rats. Interestingly, the secretory rate of bile salts, which was unchanged after DRW in our conditions, was also significantly increased by DRA. Thus, DRA is as beneficial as life-long DRW to maintain bile formation in old rats.

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O 16 FOOD INTAKE DATA ACQUISITION SYSTEM FOR THE WATER BUFFALO (*Bubalus bubalis*). S. Y. Zhang, Z. K. Zhu, F. L. Wu, X. Z. Mao, F. F. Bermúdez, J. González-Gallego and J. P. Barrio. Depto. Fisiol. Pharmacol. & Toxicol., Universidad de León, 24071 León (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 74, 1997.

An automatic food intake data acquisition system has been developed in the Agricultural University of Nanjing (Jiangsu, People's Republic of China) for studying the feeding behavior of up to eight water buffaloes (*Bubalus bubalis*) within the framework of an INCO project from the European Union involving researchers from China, Germany, Spain and United Kingdom to develop new therapeutic and diagnostic approaches to fasciolosis in China. The system provides a voltage proportional to the weight of the food containers, to be analyzed by a personal computer after appropriate amplification and analogue-to-digital conversion. Observations were made from June 25th to August 7th 1996 with an indoor average temperature of 28 °C (max 34, min 25 °C). The animals were offered herbage hay and water *ad libitum*, and they were allowed to exercise (1 hour) prior to the daily recording period. The total weight of food offered at 9:00 every day was recorded as well as the animal feeding activity throughout the day. The average food intake per animal and day was 9.59 ± 0.83 kg hay.

C/EBP TRANSCRIPTION FACTORS ARE INVOLVED IN THE REGULATION OF BROWN FAT THERMOGENESIS: FROM MOLECULAR BIOLOGY TO PHYSIOLOGY. O

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Brown adipose tissue (BAT) is the main site for non-shivering thermogenesis in mammals in response to environmental temperature or to diet. This thermogenic activity depends on the presence in the inner membrane of BAT mitochondria of the uncoupling protein (UCP), that is uniquely expressed in this tissue. We have studied the involvement of the C/EBP family of transcription factors in the acquisition of BAT thermogenic capacity. Northern and Western blot analysis of the C/EBPα and C/EBPβ expression in BAT showed a developmentally and cold-induced regulation of their expression in closely correlation with BAT *in vivo* differentiation and activation. Furthermore, C/EBPs were identified as transcriptional activators of the *ucp* gene by co-transfection experiments into primary brown adipocytes differentiated in culture. Two main C/EBP binding sites in the *ucp* gene promoter were mapped by footprinting and gel-shift and by functional assays. The *in vivo* significance of these results was demonstrated by the impaired thermoregulation observed in transgenic mice carrying either a targeted disruption in the *c/ebpα* or in the *c/ebpβ* genes.

MECHANISMS OF HEAT STRESS INDUCED MYOPATHY IN THE DOMESTIC FOWL. O 18

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Acute exposure of the domestic fowl to elevated thermal loads results in increased plasma activity of the skeletal muscle isoform of the intracellular enzyme creatine kinase. The mechanism of this stress induced myopathy has not been elucidated. An isolated *in vitro* skeletal muscle preparation from the fowl has been employed to characterise the effects of disturbances in sarcoplasmic sodium and calcium homeostasis, which may be altered during heat stress, upon sarcolemmal integrity assessed by efflux of the creatine kinase (CK). ^{45}Ca influx into the tissue was stimulated maximally ($p < 0.001$) by incubation with a sodium ionophore (100 μM monensin) concomitant with a 20 x increase in CK efflux ($p < 0.001$). Sodium influx and sarcolemmal sodium-calcium exchange may mediate this effect but monensin treatment induced CK efflux (12 fold, $p < 0.001$) even in the absence of extracellular calcium. Release of calcium from intracellular stores was thus implicated and inhibition of the ryanodine sensitive calcium channel of the sarcoplasmic reticulum (SR) with dantrolene significantly reduced CK efflux (52 %, $p < 0.01$) from monensin treated muscle. In parallel *in vivo* studies birds treated with dantrolene and subjected to 2 hours of heat stress exhibited a 10 fold decrease in CK efflux compared to vehicle treated controls. It is proposed that acute heat stress stimulates calcium release from the SR via the ryanodine receptor which induces altered sarcolemmal permeability thus mediating subsequent myopathy.

O 19 MEASUREMENT OF THE METABOLIC ACTIVITY IN LYMPHOCYTES BY THE AMINO ACID TRANSPORT EVALUATION. M. Morell, M. Perán, F. Cardona, J. L. Gil, A. Manteca and S. Perán. Dpto. de Bioquímica. Facultad de Medicina. Malaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 76, 1997.

One of the more interesting effects of the mitogenic agents on cellular metabolism are the early changes in amino acid flux through plasmatic membrane. These changes were used to investigate the first manifestation of malign cells tranformation and to evaluate the cellular activity in lymphocytes from patients with leukaemia and from a population of high incidence of leukaemic process.

The initial L-Gln and L-Leu uptake by human lymphocytes were studied by applying a single-passage, paired-tracer dilution technique to perfused population of isolated cells. Lymphocytes were isolated from a blood 40 ml sample and perfused in a dialysis column at 1 ml/min flow with Hanks' balanced salt solution that contained the triated amino acid and an extra cellular reference molecule D-[^{14}C]mannitol.

Both L-Gln and L-Leu uptakes in resting lymphocytes were saturable and temperature dependent with an apparent Km of 163 μM and 9 μM respectively. Inhibition of glutamine transport was observed for N system competitor L-His and L-Asn. In cells exposed to Conca A, amino acid transport rates and [3H]thymidine uptake were lineary augmented with the incubation time. In leukaemic lymphocytes the amino acid uptakes were significantly elevated in concordance with the raising of [3H]thymidine uptake. Cells from people with Down syndrome showed individual transport features with different rates of amino acids transport and different responses to Conca A.

O 20 FILOGENY OF INSULIN AND IGF-I RECEPTORS. I. Navarro, M. A. Maestro, N. Baños, C. Castejón, E. Méndez, J. V. Planas and J. Gutiérrez. Departament de Fisiologia, Facultat de Biologia, Universitat de Barcelona, Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 76, 1997.

Insulin and IGF-I are members of the same family of polipeptides, involved in the regulation of metabolism and growth. The structure of both molecules is very similar, reflecting a common origin (Chan *et al.*, 1993). Using the affinity chromatography method (Gutiérrez *et al.*, 1991) the receptors have been studied in muscle and other tissues in various species of vertebrates and invertebrates, and the number of receptors, affinity, specificity and tyrosine kinase activity have been characterised. Interestingly, we have observed that in skeletal muscle or heart of all ectothermic vertebrates studied, receptors for IGF-I (28-535 fmol/g of tissue) are more abundant than those for insulin (8-394 fmol/g). Moreover, IGF-I receptors present higher affinity and specificity than insulin receptors, resulting in higher binding of IGF-I. This is not in agreement with the data found in birds and mammals, where the number of insulin receptors (298, 282 fmol/g, respectively) is higher than the number of IGF-I receptors (135, 66 fmol/g, respectively). In muscle of molluscs, bivalves or gasteropods we detected the presence of receptors for IGF-I (4-8 fmol/g), but not for insulin. In studies during the embryonic development of trout, IGF-I receptors were found to appear earlier and in higher numbers (10-fold) than insulin receptors. All these data point towards the relative importance of IGF-I and insulin receptors during vertebrate evolution.

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EFFECTS OF GHRH AND SOMATOSTATIN (SS) ON GH RELEASE AND GROWTH IN LAMBS. O 21

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 77, 1997.

In order to find the pattern of GHRH and SS administration being able to stimulate growth rate and GH release, male lambs were treated for three weeks as follows: A) 3 daily GHRH (250 µg) s.c. injections; B) alternating 2 daily injections of GHRH (250 µg) and 2 of natural SS (250 µg); C) continuous s.c. infusion of GHRH (1200 µg/day) using a pellet; D) the same as C plus one injection of octreotide (20 µg/day); E) continuous infusion of GHRH (900 µg/day) with a mini-osmotic pump; F) the same as E, plus one injection of octreotide (20 µg/day); G) controls. Animals were weekly weighed. Basally and every 7 days, GH response to an i.v. GHRH challenge was evaluated. At the end animals were sacrificed and pituitary GH content measured by RIA. No significant differences were found on percentual weight increments. Pituitary GH content was lower in groups A and B than in G, whereas basal plasma values were higher and showed only a low GH response to GHRH. A high GH response to GHRH similar to G was observed in the rest of groups, without changes in pituitary GH content. GH release was only modified by pulsatile but not by continuous GHRH administration. None of the treatments altered growth pattern.

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REGULATION OF ATRIAL NATRIURETIC PEPTIDE (ANP) INDUCED GUANYLYL CYCLASE (GC) ACTIVITY. O 22

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ANP is a peptidic hormone involved in fluid and electrolyte balance. ANP exert its physiological actions via membrane receptors (R), coupled to GC. ANP stimulates cGMP production in the subfornical organ (SFO) and choroid plexus (CP). The ANP binding and possibly, the GC activity, are susceptible of regulation/modulation by different factors. We evaluated the effect on ANP-induced cGMP production of the diuretic amiloride (A), which has been reported to increase the ANP binding to its R, and the action of ATP in the presence of the cofactor Mn^{++}. Male, S-D rats, 220 g, were killed by decapitation and tissues extracted and homogenized. cGMP formation was measured by radioimmunoassay. A increased the GC activity in SFO and CP. ATP inhibited, basal and ANP-induced cGMP production in both structures. In addition, in the inferior olive (IO), a structure known to express AT2-R, we assessed the possibility of a heterologous regulation between ANP and angiotensin II (ANG II), via ANG-AT2-R and through the action of phosphotyrosine phosphatases (PTPases). In IO ANP increased cGMP production. ANG II, PD123319 and CGP421 12A, significantly inhibited basal and ANP stimulable GC activity. This effect was reverted by the PTPases inhibitor, sodium orthovanadate. Our results indicate that ANP induced cGMP production is regulated by factors like A, ATP and AT2-R stimulation.

O 23 DISTRIBUTION OF CONSTITUTIVE NITRIC OXIDE SYNTHASES IN THE KIDNEY IN SPONTANEOUS HYPERTENSION. F. J. Salazar*, E. Nava*, J. M. de Velasco, R. Martínez-Murillo, A. P. Fernández, J. Serrano and J. Rodrigo. *Dpto Fisiología, Facultad de Medicina, Murcia and Instituto Cajal, Madrid (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 78, 1997.

We previously reported that the activity of constitutive NO synthase (NOS) is upregulated in the renal medulla of spontaneously hypertensive rats (SHR) suggesting that the hypertensive kidney produces more NO than the normotensive. We now aimed to know which form(s) of NOS is (are) responsible for this higher activity.

We have analyzed the distribution of immunoreactivity (IR) of endothelial NOS (eNOS) and neuronal NOS (nNOS) by means of specific antibodies in kidneys from SHR as compared to normotensive Wistar Kyoto (WKY) rats. eNOS: IR was equally and homogeneously distributed among the cortical and medullary vessels of kidneys from both WKY and SHR. nNOS: IR was detected in a) macula densa; b) Bowman's capsule; c) inner medullary collecting ducts; and d) perivascular nerve fibres. Kidneys from SHR displayed a remarkably higher nNOS IR in the macula densa, the Bowman's capsule and inner medullary collecting ducts. Nerve fibres of SHR showed less IR to nNOS.

We conclude that the higher activity of NOS in the SHR kidney occurs at the expense of a higher nNOS expression in the collecting duct and the macula densa. These results support the concept of a higher renal production of NO in hypertension.

O 24 CHANGES IN NITRIC OXIDE RELEASE *IN VIVO* IN RESPONSE TO VASOACTIVE SUBSTANCES. E. Nava, F. J. Salazar, F. Rodríguez, J. D. González and C. Moreno. Dpto. de Fisiología, Facultad de Medicina, Murcia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 78, 1997.

Changes in the release of NO *in vivo* were studied in rats following the administration of endothelium-dependent (EDVD) and endothelium-independent vasodilators (EIVD). NO production was assessed by measuring variations of nitrate in plasma by capillary ion analysis.

EDVD: bradykinin (2 and 10 $\mu g\ kg^{-1}\ min^{-1}$, iv) or substance P (0.3-3 $\mu g\ kg^{-1}\ min^{-1}$, iv) decreased blood pressure and increased plasma nitrate concentration.

EIVD: prostacyclin (0.6 $\mu g\ kg^{-1}\ min^{-1}$, i.v.) or adenosine (3 $mg\ kg^{-1}\ min^{-1}$, i.v.) diminished blood pressure and decreased plasma nitrate.

Control experiments: Sodium nitrate (200 $\mu g\ kg^{-1}$, iv) or authentic NO (400 $\mu g\ kg^{-1}$, iv) elevated plasma nitrate in a similar magnitude as EDVD. N^G-nitro-L-arginine methyl ester (L-NAME, 10 $mg\ kg^{-1}\ min^{-1}$, i.v.) decreased plasma nitrate in a similar magnitude as EIVD.

This study demonstrates that: 1) Changes in plasma nitrate can be detected *in vivo* after stimulation of NO synthase with EDVD or inhibition with L-NAME. 2) A diminished concentration of plasmatic nitrate is associated to the hypotension induced by EIVD, suggesting that the L-arginine, NO pathway is capable of down-regulation in response to a fall in blood pressure.

MECHANISMS INVOLVED IN THE RENAL DAMAGE INDUCED BY CHRONIC INHIBITION OF NITRIC OXIDE SYNTHESIS. O 25

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Chronic oral administration of nitric oxide (NO) synthesis inhibitors, such as N^G-nitro-L-arginine methyl ester (LNAME) induces a sustained elevation of blood pressure (BP) and renal damage in rats. In addition, arachidonic acid derivates, such as TXA_2, are involved in the renal damage in different pathologies. To investigate the mechanisms underlying the renal damage in LNAME hypertensive rats, we studied the effects of prolonged treatments with the ACE inhibitor, quinapril (10 mg/kg/day), the calcium channel blocker, diltiazem (100 mg/kg/day) and the cyclooxigenase inhibitor, indomethacin (1 mg/kg/day) on LNAME treated rats 40 mg/kg/day). Drugs were given in drinking water for 8 weeks. LNAME progressively increased BP levels (from 105 ± 5 to 182 ± 8 mmHg; $p < 0.05$) without changing plasma creatinine levels or sodium excretion during the study. Quinapril and diltiazem treatments reduced the increase in BP levels induced by LNAME by 74 % and 31 %, respectively. By contrast indomethacin did not modify it. LNAME administration increased urine protein excretion ($p < 0.05$). All treatments prevented the development of proteinuria induced by LNAME. None of the treatments were able to modify BP and renal function by themselves. Conclusions: 1) The prevention of proteinuria seems to be partially independent on BP reduction; 2) angiotensin II seems to be an important factor involved in LNAME-induced hypertension and renal damage; 3) an arachidonic acid derivative seems to be involved in the proteinuria induced by LNAME.

ENDOTHELIAL DYSFUNCTION IN DYSLIPEMIC RABBITS: EFFECT OF ATORVASTATIN TREATMENT. O 26

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The endothelial dysfunction induced by dyslipidemia is characterized by a diminished relaxation in response to nitric oxide (NO)-dependent vasodilators. However, it is not well established whether the endothelium-derived contracting factors (EDCFs) can be also involved. Therefore, we have investigated the role of EDCFs in the altered endothelial function in aortic rings from rabbits fed a diet containing 0.5 % cholesterol + 14 % coconut oil for 9 weeks. The effects of the HMG-CoA reductase inhibitor, atorvastatin (2.5 mg/kg/day) were also studied. The administration of the experimental diet induced a significant increase in plasma levels of both cholesterol (1910 ± 111 vs 55 ± 4.7 mg/dl) and triglyceride (410 ± 43 vs 112 ± 22 mg/dl) as compared with the control group, respectively. Atorvastatin treatment significantly reduced both concentrations. The vasorelaxation induced by acetylcholine (Ach) was reduced ($p < 0.05$) in dyslipemic rabbits compared to controls. This minor response was totally prevented by the administration of atorvastatin. The participation of an EDCF was studied through the concentration-related constrictor response to Ach in presence of an NO synthase inhibitor. This response was higher ($p < 0.05$) in dyslipemic rabbits than in controls, and was prevented by atorvastatin treatment. In summary, the endothelial dysfunction presented in rabbits with dyslipemia seems to be due not only to a lower NO availability, but also to an elevated production of an EDCF. Likewise, the administration of atorvastatin totally prevents these effects.

O 27 MYOBLAST TRANSFER THERAPY (MTT) PHASE II CLINICAL TRIALS. P. K. Law, T. G. Goodwin, Q. Fang, T. Quinley, G. Vastagh, T. Hall, T. Jackson, M. B. Deering, V. Duggirala, C. Larkin, J. A. Florendo, L. M. Li, T. J. Yoo, N. Chase, M. D. Neel, T. Krahn and R. L. Holcomb. Cell Therapy Research Foundation, 1770 Moriah Woods Blvd, Suite 18, Memphis, TN (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 80, 1997.

MTT is completing Phase II clinical trials on Duchenne muscular dystrophy (DMD). The whole body trial (WBT) consists of injecting 25 billion myoblasts in two MTT procedures separated by 3 to 9 mo. Each procedure delivers up to 200 injection or 12.5 billion myoblasts to either 28 muscles in the upper body (UBT) or to 36 muscles in the lower body (LBT). A randomized double-blind portion of the study is conducted on the biceps brachii or quadriceps. Subjects take oral cyclosporine for 3 mo after each MTT. One infantile facioscapulohumeral dystrophy and 40 DMD boys aged 6 to 16 have received WBT in the past 36 mo with no adverse reaction. Nine months after MTT immunocytochemical evidence of dystrophin has been demonstrated in 18 of the 20 subjects biopsied. Forced vital capacity increases by 33.3 % and maximum voluntary ventilation increases by 28 % at 12 months after UBT. Plantar flexion increases by 52 % in force in 9 mo in the ambulatory subjects. Behavioral improvements in running, balancing, climbing stairs and playing ball are noted. Six years after MTT the world's first MTT patient continues to show dystrophin in the myoblast-injected foot muscle. Dystrophin is absent in the sham-injected muscle. The latest development involves a one time injection of 50 billion myoblasts into 82 muscles with 179 punctures approved for subjects with DMD, Becker MD and Limb-girdle MD. Ten subjects in this trial experience no adverse reaction.

O 28 A QUANTIC FORMATION MECHANISM FOR REFLEX AND MOTOR LEARNED RESPONSES. A. Gruart, J. A. Domingo and J. M. Delgado-García. Laboratorio de Neurociencia, Facultad de Biología, 41012-Sevilla (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 80, 1997.

Eyelid movements represent an excellent experimental model for the study of spontaneous blinks and conditioned eyelid responses (CRs). Animals were implanted bilaterally with search coils in the upper lid and with EMG electrodes in the orbicularis oculi muscle. Reflex responses were evoked with air puffs, flashes and tones. Five classical conditioning paradigms were used to analyze the frequency-domain properties of CRs.

It was found that the CR appeared as formed by a succession of small waves at a dominant frequency of ≈20 Hz. The amplitude (and number) of the constituting waves depended on the characteristics of the CS and on the time interval until US presentation. The CR seemed to be formed from a minimum (quantum) lid displacement of 2-6 deg, and ≈50 ms, that increased in number and/or amplitude, along conditioning sessions, until a complete CR was reached. It is suggested that a ≈20 Hz oscillator underlies the generation of reflex and conditioned eyelid responses. The oscillator is susceptible of being neurally modulated in order to modify the velocity of a given quantum of movement, and the total duration of the lid response. Learned eyelid movements are probably the result of a successively-longer release of the oscillator as a function of the temporary-spatial needs of the motor response.

ENHANCEMENT OF CONTRACTILE PROPERTIES OF REGENERATING MUSCLE RECEIVING EXOGENOUS MYOBLASTS. O 29
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Myoblast, injected into skeletal muscle, may fuse with established myofibers or may fuse with each other to form new myofibers. Little is known about the ability of injected myoblasts to enhance a muscle's functional capacity. Donor myoblasts were derived from a cell line (MM14) that was transfected with a construct consisting of a nlsβ-gal under the control of the MLC3F promoter/enhancer. The extensor digitorum longus muscles (EDL) of 8 wk SCID mice were orthotopically transplanted to induce regeneration and enhance incorporation of injected cells. 2 days later, some transplanted muscles were injected with $7x10^5$ donor myoblasts (EXP). A second group received injections of vehicle only (no myoblasts-SHAM). A third group received no injection (TRANS). Other mice received neither transplant nor injection (UN). 12 wk later, blue myonuclei were still present in the EXP muscle, as determined by whole amount X-gal staining. *In vitro* isometric tension analysis by direct stimulation (100 Hz, 500 ms) determined that EXP generated a twitch tension of 3X that of SHAM and 1.5 X that of TRANS ($p < 0.001$). EXP muscles exhibited similar increases in tetanic tension as compared to SHAM and TRANS. The EXP achieved 46 % and 62 % of twitch and tetanic tensions, respectively, of UN EDL. Force/unit area of all groups were similar. Injected myoblasts clearly enhance the contractile properties of regenerating muscles.

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BRAINSTEM MOTONEURONS RESPONSES DURING A LEARNED MOTOR TASK. O 30
J. A. Trigo, A. Gruart and J. M. Delgado-García. Laboratorio de Neurociencia, Facultad de Biología, Universidad de Sevilla, Avda Reina Mercedes 6, Sevilla-41012 (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 81, 1997.

The nictitating membrane/eyelid response has been used as a suitable paradigm to study a learned motor response, a model used to try to get some insights into the general process of learning. In the cat, the control of the upper eyelid downward movement is carried out by the joint activity of the accessory abducens nucleus and the dorsolateral subdivision of the facial nucleus. These groups of motoneurons were recorded during reflex and conditioned eyelid responses. Main results indicate that the closure of the eyelid during both responses is the result of the contribution with different latency and gain of each group of motoneurons. *Phasic* facial motoneurons seemed to be synchronized when a CS involving a trigeminal pathway was used and thus they must be the responsible of the 20 Hz oscillation observed in actual lid displacements (Domingo *et al.*, *Soc. Neursci. Meeting*, Washington, 1996). Such synchronization was not present if an auditory CS was used. *Tonic* facial motoneurons were active during both types of conditioning paradigms but not during the initial conditioning sessions when a short, weak air puff was used as a conditioned stimulus.

O 31 CROTONITRILE-INDUCED VESTIBULAR HAIR CELL LOSS: BEHAVIORAL AND MORPHOLOGICAL CHARACTERIZATION IN THE RAT. J. Llorens, A. Aguiló and E. Rodríguez-Farré*. U. de Fisiologia Bellvitge, Universitat de Barcelona, Hospitalet de Llobregat and *Dept. de Farmacologia i Toxicologia, IIBB, CSIC, Barcelona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 82, 1997.

Exposure to the dinitrile 3,3'-iminodipropionitrile (IDPN) induces a syndrome of abnormalities in behavior associated with degeneration of the vestibular sensory hair cells (Llorens *et al.* Toxicol. Appl. Pharmacol., 123:199, 1993). In this study, the vestibular toxicity of a mononitrile, crotonitrile, was characterized in adult male Long-Evans rats. Crotonitrile (0, 100, 125 or 150 mg/kg/day x 3 days, i.p., in corn oil, n = 8/group) caused dose-dependent changes in behavior congruent with loss of vestibular function. These included high rating scores in a test battery assessing loss of vestibular function (125 and 150 mg/kg), and increased locomotor activity (150 mg/kg). Scanning electron microscopy observation of vestibular sensory epithelia at 3 weeks post-exposure (n = 3/group) indicated a dose-dependent loss of hair cells, in good agreement with the behavioral data. The present data indicate that vestibular toxicity is a property of IDPN shared by mononitriles lacking the imino group that characterizes the IDPN molecule. The nitriles appear as a new family of ototoxic compounds with potential use as tools in vestibular research.

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O 32 ACTIVE CHLORIDE ABSORPTION THROUGH MITOCHONDRIA-RICH CELLS OF TOAD EPIDERMIS. N. J. Willumsen, L. J. Jensen and E. H. Larsen. August Krogh Institute, University of Copenhagen (Denmark). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 82, 1997.

The epidermis is a primary osmoregulatory organ in amphibians. Thus, in a freshwater environment uptake of Na^+ and Cl^- takes place through the common principal cells and the much less abundant mitochondria-rich (MR) cells, respectively. In the absence of external Cl^-, Ussing chamber experiments revealed a marked acidification of the external bathing solution, dependent on serosal CO_2 supply, whereas little or no acidification was observed when Cl^- or other halides were present. When active Na^+ uptake was blocked by amiloride, changes in short-circuit current, induced by varying serosal CO_2 partial pressure, matched changes in the proton flux. The observations suggest that the apical MR cell membrane contains active electrogenic proton pumps which provide energy for uptake of Cl^- (and other halides) in exchange for cellular bicarbonate against steep concentration gradients. Being concentrated above its equilibrium concentration, Cl^- leaves the MR cell across the basolateral membrane through anion channels. We have in cell-attached and excised inside-out patch-clamp studies identified 3 types of resolvable Ohmic anion channels (10, 30 and 150 pS, respectively) in the basolateral MR cell membrane. Furthermore, we have observed a very small channel that escapes resolution of single-channel events. The channel type responsible for cutaneous Cl^- uptake remains yet to be positively identified.

NEURONAL RESPONSES TO TARGET LOSS AND AXOTOMY ANALYZED IN THE CAT OCULOMOTOR SYSTEM. O 33

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 83, 1997.

The present study has been carried out to evaluate the influence that target cells exert on the physiology of their afferent neurons in the adult mammal. The experimental approach involved the use of two different lesioning procedures, both producing target disconnection: i) selective cytotoxic target ablation, and ii) axotomy. The anatomical substrate of the study was the projection of abducens internuclear neurons (Abd Ints) onto the medial rectus motoneurons of the oculomotor nucleus. Target motoneurons were killed by injection of toxic ricin into the medial rectus muscle; this procedure leaves intact Abd Ints deprived of their target. The second procedure was the transection of the medial longitudinal fascicle, where Abd Ints axons travel. In both cases, Abd Ints survive the insult. However, some differences were found regarding the changes observed in their discharge pattern, which was analyzed under alert conditions and in correlation with eye movements. Target loss produced transitory firing alterations since recovery occurred by one month probably due to the availability of novel neuronal targets within the oculomotor nucleus. Axotomized Abd Ints showed failure of axonal regeneration and this could be related to their altered low-rate discharge maintained in the long-term.

CONVENTIONAL AND NONLINEAR ANALYSIS OF THE ECG. O 34

G. Ezpeleta, C. Varela, P. Berraondo and E. J. Díaz-Calavia. Biofísica. Facultad de Medicina. Universidad de Navarra. 31080 Pamplona (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 83, 1997.

The ECG is analysed following the methods employed at present in clinical medicine and the methods of the nonlinear dynamics. The latter give more information, and sooner, than the former.

Methods: A five minutes register of the ECG was obtained from 20 healthy subjects in which there were four retrospective studies of valvular mitral prolapse (VMP) with healthy ECG.

Conventional method of analysis: 1) Diagnosis of the ECG by an experimented cardiologist; 2) FFT; and 3) Autocorrelation function in order to study the periodicity of the signal.

Nonlinear method of analysis: Calculate: 1) Correlation dimension following the FASA algorithm, (obtained from the one proposed by Grassberger-Proccacia); 2) Kolmogorov entropy (indicates how chaotic the system is); 3) Maximum Lyapunov exponent (calculated following Wolf); and 4) Reconstruction of the possible attractor by Takens's method.

Results: 1) The conventional method finds normal ECG in all the patients; 2) the nonlinear method finds significative difference in 4 of the 20 subjects, due to VMP; 3) there are no significative differences in the FFT and in the autocorrelation function of the 20 patients.

Conclusions: There is significative difference between both methods of analysis. The nonlinear method is more sensible than present day methods of morphologic ECG diagnosis.

References: Jalife (1991): *J. Ann. NY Acad. Sci.*, 591.

O 35 HYDROCEPHALUS AND SUBCOMMISSURAL ORGAN. P. Fernández-Llebrez, J. M. Grondona, M. Pérez-Martín, M. Cifuentes, J. Pérez, A. Jiménez* and J. M. Pérez-Figares. Depts. de Biología Animal y *Biología Celular, Facultad de Ciencias, Universidad de Málaga, Málaga (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 84, 1997.

Obstructive hydrocephalus by stenosis of the cerebral aqueduct is related to malformations or disfunctions of the ependyma lining the brain ventricles. The subcommissural organ (SCO) is an ependymal gland specialized in the release of large glycoproteins into the cerobrospinal fluid that polymerize in a Reissner's fiber (RF). Some authors have suggested a causal relationship between SCO and hydrocephalus. By using specific antisera against SCO secretion and lectins, we have investigated the SCO in three animal models of hydrocephalus: 1) congenital hydrocephalus in newborn *hyh* mice and 2) *hy3* mice; 3) experimental acquired hydrocephalus in adult rats by injection of neuraminidase.

In all three cases the ependyma was denudated and the SCO was present and active, but glycoproteins do not polymerize forming a RF. Detailed immunocytochemical, lectin histochemical and morphological study of the SCO in hydrocephalus showed some interesting alterations although it is not clear whether they are a cause or a consequence of hydrocephalus.

Supported by DGICYT PB93 0979 Madrid, and FIS 95 1591 (Spain).

O 36 ROLE OF OXIDATIVE STRESS IN EXPERIMENTAL NEUROPATHIES. S. Weber. F. J. Romero and F. Bosch-Morell. U. Toxicol. Neurotoxicol. Exper., Depto. Fisiol., Fac. Medicine y Odontología, Universidad de Valencia. 46010-Valencia (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 84, 1997.

Decreased antioxidant availability has been observed in different neurological disorders in the central nervous system, e.g. Parkinson's disease, Alzheimer's disease, epilepsy, amyotrophic lateral sclerosis, cerebral ischaemia, etc. Acute phenytoin intoxication has been associated with a decrease in Na,K-ATPase in several tissues but not in peripheral nerve. Since this enzyme is a substrate of PKC, we have determined the Na,K-ATPase activity in sciatic nerves of control and phenytoin-treated rats. This activity is decreased in phenytoin intoxication and this decrease is associated with electrophysiological changes and a decrease in GSH content of these nerves. This effect could be prevented by the administration of the PKC inhibitor H7. Acute ethanol intoxication induces electrophysiological changes associated with GSH content decreases, whereas in the chronic model, the function is less affected but the GSH decreases appear. In these animals S-adenosyl-methionine was able to recover GSH levels and reduce malondialdehyde (MDA) accumulation in sciatic nerves. Finally, administration of methylmercury reduces GSH content in sciatic nerves but not in brains of treated rats. This change is transient and recovers on day 3 after treatment. The common features of these experimental models are discussed.

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VOLTAGE- AND CALCIUM-DEPENDENT OUTWARD MEMBRANE CURRENTS OF NURSE LIKE STROMAL CELLS ISOLATED FROM FISH THYMUS. O 37

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 85, 1997.

Enzymatic and mechanical dissociation of fish (carp) thymus was used to isolate cells identifiable by morphological criteria as nurse-like epithelial similar to mammalian cells of this type. Whole cell voltage clamp recordings revealed evidence for at least three components to the macroscopic voltage-activated outward current. We have termed these Ia, Ib and Ic, because of their resemblance to known current types: Firstly, (Ia) a fast, transient outward potassium current (likened to IA); secondly, (Ib) a noisy, high conductance, Ca^{2+}-dependent, potassium current (comparable to the 'maxi' conductance type, IBK); and finally, (Ic) a high voltage activated current, sensitive to anion transport blockers, and displaying a reversal potential close to the chloride equilibrium potential. Comparisons are drawn with the existing knowledge of currents from rodent TNCs and human thymic epithelia. Expression of TNCs in fish, and of voltage-activated and calcium-dependent outward currents in these cells appears to be a common finding at widely separate phylogenetic time points, indicate a degree of conservation in the functional plan of the vertebrate thymus and the ionic mechanisms of nurse cells.

EFFECTS OF NA AND ATP ON THE VOLTAGE GATED K^+ CHANNELS IN WHITE ADIPOCYTES. O 38

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We have described the characteristics of macroscopic membrane currents of white adipocytes using the whole-cell variant of the patch-clamp technique. These cells were obtained by differentiating preadipocytes from rat epididymal tissue. All mature cells recorded had outward currents voltage dependent. The ion channels underlying the macroscopic current were selective for K^+. By their pharmacological characteristics we propose that in white adipocytes could exist different K^+ channels with properties almost identical to those described in brown fat cells. In these cells, the noradrenaline (NA) evokes an electrical and metabolic responses and it is well established that K^+ channels play a significative rolle in the electrical response to this hormone; although up to date it is not known if they are involved in the metabolic response. Similarly, in white adipose cells multiple K^+ channels could coexist with distinct physiological roles. Preliminary experiments show that external addition of NA (40 μM) and ATP (100 μM) decrease reversibly the amplitude of macroscopic currents in white adipocytes. We concluded that in these cells voltage gated K^+ channels could participate in the hormonal regulation of their metabolic activity.

O 39 INTRACELLULAR CALCIUM CHELATION DOES NOT PREVENT THE FUNCTIONAL INCORPORATION OF RECONSTITUTED PROTEINS INTO THE *XENOPUS* OOCYTE MEMBRANE. I. Ivorra, B. Gal, A. Fernández and A. Morales. Instituto de Neurociencias. Universidad de Alicante. 03080 Alicante (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 86, 1997.

We have previously shown that nicotinic acetylcholine receptors (AChRs) reconstituted in lipid vesicles and microinjected in *Xenopus* oocytes are functionally incorporated into the oocyte membrane (Morales *et al.*, *Proc. Natl. Acad. Sci USA.* 92:8468-8472, 1995). Now we have studied if calcium ions play an important role in the incorporation of foreign proteins. For this purpose asolectin lipid vesicles containing AChR and ClC-0 proteins from *Torpedo marmorata* electroplaques were injected in oocytes kept in Barth's solution or in a Barth's solution to which 50-200 µM BAPTA-AM was added before injection. Acetylcholine and voltage dependent chloride currents elicited in oocytes from control or BAPTA groups were of similar amplitude. Only BAPTA group cells which showed a clear chelation of intracellular calcium (oocytes unresponsive to serum (1:100 dilution) and in which the T_{in}, current could not be evoked) were included. Similar results were found when preloading the cells with EGTA (≈5 nmol). It is concluded that incorporation of foreign proteins into the oocyte membrane is not dependent on intracellular calcium.

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O 40 MODULATION OF SWELLING-ACTIVATED Cl^- CHANNELS BY Cl^-. F. V. Sepúlveda, A. Stutzin, A. L. Eguiguren and L. P. Cid. *Facultad de Medicina, Universidad de Chile y Centro de Estudios Científicos de Santiago (Chile). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 86, 1997.

The efflux of Cl^- and intracellularly-accumulated organic osmolytes plays a central role in regulatory volume adjustments in many cells. It has been postulated that a single swelling-activated anion channel mediates transmembrane movement of both solutes. Our present patch-clamp studies in epithelial HeLa cells show that the activity of these channels decreases when extracellular Cl^- is replaced by a relatively impermeant anion. The effect of Cl^- removal does not appear to be due to depletion of intracellular anion and it is postulated to relate to a direct effect on the gating akin to what has been termed "gating by the permeant ion". If the same channels were to provide the exit pathway for intracellular organic osmolytes released during regulatory volume decrease, removal of extracellular Cl^- should also decrease their flux. This was tested by measuring the swelling-induced release of taurine, a sulphonic amino acid accumulated up to 70 mM in many mammalian cells. Taurine permeability is increased up to 20-fold upon osmotic swelling. The increase in permeability is enhanced 2-3-fold by replacement of Cl^- in the extracellular medium, an effect that is not dependent upon changes in membrane potential. It is concluded that separate entities with opposing sensitivities to extracellular Cl^- mediate swelling-activated Cl^- and taurine efflux in HeLa cells.

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THE STRUCTURAL BASIS OF RECOGNITION AND ACTIVATION MECHANISMS IN G-PROTEIN COUPLED RECEPTORS AT ATOMIC DETAIL. O 41

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Our Laboratory addresses structural, dynamic and electronic determinants of biological processes underlying physiological functions through the application of methods in theoretical and computational biophysics. Theoretical determinations of molecular structure and properties, and computational simulations of molecular mechanisms are designed to complement experimentation by providing mechanistic insights in the exploration of cellular processes and functions. A central theme is the understanding of mechanisms triggered by molecular recognition and leading to signal transduction. We present results from studies of structural specificity and dynamics in molecular mechanisms of cellular signaling through ligand recognition and activity of G-protein coupled receptors of the neurotransmitter serotonin (5-HT); the gonadotropin releasing hormone (GnRH); and μ,κ,δ-opioid receptors. The findings provide a direct structural context for mechanistic concepts including: • distinct conformational states of receptors and their relation to activation and effector coupling; • the role of ligands in stabilizing or inducing "receptor states"; • pharmacological efficacy and its relation to ligand-induced receptor "states"; • constitutively active receptors; • inverse agonism; • ligand-dependent selectivity in coupling to different effectors; • receptor-determined ligand efficacy.

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IMMUNOLOCALIZATION OF THE Na^+/GLUCOSE COTRANSPORTER (SGLT1) IN PATIENTS WITH GLUCOSE-GALACTOSE MALABSORPTION. O 42

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Glucose-galactose malabsorption (GGM) is a genetic disease caused by a defect in the intestinal Na^+/glucose cotransporter gene, that is characterized by a neonatal onset of severe diarrhea. D28N, C292Y and Q457R are three of the 23 missense mutations identified so far associated with the disease. Using the *Xenopus* oocyte expression system and a combination of biochemical and electrophysiological techniques, we have studied the cause of the defect in these three mutants. D28N and C292Y mutations disrupted the trafficking of the protein between the ER and the plasma membrane whereas the defect in Q457R SGLT1 was functional: reduction of the affinity for sugar and blockage of the sugar release to the inside of the cell immunohistochemical studies of biopsies from patients localized D28N and C292Y proteins in the cytoplasm of the enterocyte and Q457R in the brush border membrane. These studies suggest that the defects in SGLT1 causing GGM in these patients are similar to those found using the heterologous expression system.

O 43 NITRIC OXIDE STIMULATION OF TYROSINE PHOSPHORYLATION ON RAT PANCREATIC ACINAR CELLS. M. García-Benito, J. J. Acosta, J. I. San Román, J. J. Calvo, M. A. López and L. J. García. Depto. Fisiología y Farmacología, Universidad de Salamanca, 37008 Salamanca (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 88, 1997.

Nitric oxide (NO) exerts several effects in mammalian systems and a role for NO has been suggested in the exocrine pancreas as an intracellular messenger. The present study was designed to examine the effects of NO, using sodium nitroprusside (SNP) as NO donor, on tyrosine phosphorylation levels in rat pancreatic acinar cells. Cell lysates were analyzed by anti-phosphotyrosine Western blots of anti-phosphotyrosine immunoprecipitates. SNP 10 μM increased tyrosine phosphorylation of 130, 105 and 75 KD proteins to different extents. A maximal increase in tyrosine phosphorylation of these proteins was detected within 30 min with a 2.8-fold increase in p130, 4.2-fold for p105 and 2-fold for p75. Pretreatment for 1 h with methylene blue (0.1 mM), and inhibitor of soluble guanylate cyclase, caused a significant decrease in the phosphorylation of these proteins. Depletion of the intracellular calcium pool by pretreatment with the tumor promoter thapsigargin (10 μM) induced a slightly increase in the SNP stimulation on tyrosine phosphorylation. However, pretreatment with thapsigargin in a calcium free medium, with EGTA 5 mM, caused a significant decrease in the tyrosine phosphorylation of these proteins by SNP. Our results demonstrate that in rat pancreatic acini the NO donor SNP causes tyrosine phosphorylation of multiple proteins and that this increases could be modulated by a guanylate cyclase activation and by the calcium influx.

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O 44 MUSCARINIC RECEPTORS OCCUPATION CAUSES TYROSINE PHOSPHORYLATION OF $p125^{FAK}$, PAXILLIN AND $p130^{Cas}$ IN PANCREATIC ACINAR CELLS. J. A. Rosado, G. M. Salido and L. J. García. Departamento de Fisiología, Universidad de Extremadura, 10071 Cáceres (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 88, 1997.

Previous studies have demonstrated that muscarinic receptors occupation in Swiss 3T3 cells can cause tyrosine phosphorylation (Tyr-P) of $p125^{FAK}$. However, it is not known which cellular pathways mediate this effect. The purpose of this study was to determine whether muscarinic receptors occupation in rat pancreatic acini play a role in Tyr-P of $p125^{FAK}$ and two of its substrates, paxillin and $p130^{Cas}$. Tyr-P of FAK, paxillin and Cas reached a maximum of 8.42, 3.03 and 5.26-fold stimulation respectively 5 min after addition of the muscarinic agonist, carbachol, with a half maximal effect of 3 μM. Depletion of the intracellular Ca^{2+} pools by treatment with thapsigargin in a calcium free medium or treatment with GF109203X, an intibitor of PKC, had not a significant effect on Tyr-P of these proteins. However, the combined effect of these inhibitors causes a significant reduction, about 50 %, in Tyr-P of these proteins. Cytochalasine D, an agent which disrupts the network of actin microfilaments, completely inhibited carbachol-induced Tyr-P of these proteins, whereas colchicine, which disrupts microtubules, had no effect. These results demonstrate that carbachol causes rapid Tyr-P of FAK, paxillin and Cas in rat pancreatic acini by both Ca^{2+} and PKC-dependent and -independent pathways. Furthermore, the integrity of actin cytoskeleton is essential for this muscarinic receptors occupation effect.

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BASIC FIBROBLAST GROWTH FACTOR (bFGF) INHIBIT CCK8-INDUCED AMYLASE RELEASE IN ISOLATED RAT PANCREATIC ACINI. O 45

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J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 89, 1997.

Basic fibroblast growth factor (bFGF) is a mitogenic polypeptide that belongs to a family of heparin binding proteins that modulate numerous cellular processes. The action of bFGF is initiated by its binding to a specific cell-surface tyrosine kinase receptor. On the other hand, the physiological responses of pancreatic acinar cells to several growth factors remain controversial. bFGF can stimulate amylase release as carbachol does, whereas epidermal growth factor (EGF) has no secretory potency but it inhibits CCK- and bFGF-induce amylase secretion. In the present study, rat pancreatic acini were used to investigate the effect of bFGF on amylase release in response to CCK. The results show that 50 pM bFGF, which had no effect on the basal amylase secretion, shifted the dose-response curve for CCK8 (1 pM-10 nM)-stimulated amylase release. At the maximal CCK8 concentration of 320 pM, bFGF inhibited amylase release from 22.6 ± 0.9 to 18.3 ± 0.7 % of total above basal. The IC50 of bFGF (0.5 pM-500 pM) to inhibit 320 pM CCK8-stimulated amylase release was 7.5 pM. In contrast, EGF had no significant influence either basal or CCK8-evoked amylase secretion. The inhibitory effect of bFGF on CCK8-induced amylase release was completely abolished after pancreatic acini were preincubated with the tyrosine kinase inhibitor tyrphostin 25 (100 μM). In conclusion, the data of the present study show that bFGF inhibits CCK8-evoked enzyme secretion via activation of protein tyrosine kinases.

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ENDOGENOUS INHIBITOR OF THE NA-K-Cl COTRANSPORT SYSTEM IN INBRED DAHL RATS. O 46

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Dahl salt-sensitive rats seem characterized by an ubiquitary increase in Na-K-Cl cotransport activity. Here, an endogenous inhibitor of the Na-K-Cl cotransport system (CIF), was investigated in inbred Dahl salt-sensitive (DS) and salt-resistant (DR) rats. The animals were orally loaded for 10 days with 2 % NaCl. Plasma from salt-loaded DS rats inhibited cotransport with IC_{50} = 6.4 ± 0.6 % (% plasma concentration, v/v) vs 24.2 ± 2.2 % in DR rats ($p < 0.001$). In urines, IC_{50} for cotransport inhibition was constantly lower in DS before and all during the whole salt-loading period (after 10 days of salt-loading IC_{50} was 2.59 ± 0.11 % and 6.00 ± 0.24 % in DS and DR rats respectively, $p < 0.001$). After 3 days of salt loading, higher salt appetite in DS rats magnified the differences in urinary CIF excretion. In erythrocytes from DS rats, increased cotransport activity was strongly correlated with urinary CIF excretion ($r = 0.967$). In conclusion, Dahl salt-sensitive rats present increased plasmatic and urinary CIF levels. This can be a compensatory phenomenon to reduce cotransport hyperactivity and increased NaCl reabsorption at the thick ascending limb of Henle's loop.

O 47 WATER CHANNELS IN FROG CORNEAL EPITHELIUM. O. A. Candia. Dept. Ophthalmology. Mt. Sinai School of Medicine. New York, NY (USA). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 90, 1997.

Cl-activated water channels are present at the apical membrane of the frog corneal epithelium. The contribution of the paracellular pathway to transepithelial water flow is minimal, indicating the need of basolateral water channels for transepithelial water flow. When the apical membrane is permeabilized with amphotericin B rendering this barrier non-limiting, 0.5 mM $HgCl_2$ reduces the unidirectional ^3H_2O flux from 14.8 ± 1.0 to 12.2 ± 0.8 µl/min cm^2 and the osmotically induced net fluid flow from 0.96 to 0.30 µl/min cm^2. K channel blockers (quinidine, lidocaine) reduced ^3H_2O flux by 12 ± 0.9 % ($p < 0.01$) independently of the permeability of the apical membrane, suggesting that a fraction of the basolateral water flow occurs via K channels. Blockers of basolateral K channels produce a parallel reduction of K current and water fluxes. Although water flow at the apical barrier is mainly across specific water channels, its basolateral crossing can be via water and K channels. Thus, K channel regulation can affect transepithelial water flow.

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O 48 DIFFERENT TYPES OF EXERCISE-TRAINING INDUCE MODIFICATIONS OF OXIDATIVE STRESS IN LIVER AND SKELETAL MUSCLE AGED RATS. A. Navarro-Arévalo, M. J. Sánchez-del Pino. Departament of Biochemistry, Faculty of Medicine, University of Cadiz (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 90, 1997.

Antioxidant enzymes play an important role in defending the cells against free radicals mediated oxidative damage. Oxygen free radicals are involved in aging and exercise. These experiments tested the hypothesis that the type, duration and intensity of exercise affect biomarkers of free radical and antioxidant activity. In this study, the activity of superoxide dismutase (SOD), as antioxidant, and thiobarbituric acid-reactive substances (TBARS), as lipid peroxidation marker, were investigated in liver and skeletal muscle soleus of 38 old rats (24-27 mo.). The animals were exercised on a treadmill, and they were divided into four groups: rest (n = 10), exhausted (n = 15), short-training (n = 6), and long-training rats (n = 7). Our results indicated that the peroxidation level is increased in liver long-training rats about all, as well as soleus. The SOD activity was higher in rest, and decreased with exercise in liver. But in muscle, the long-training rats had greater SOD activity. These findings must confirm the hypothesis that endurance training increases both oxidative and antioxidant enzymes activities in old animals. In summary, exercise training can cause adaptative responses in skeletal muscle antioxidant enzymes.

FLUPIRTINE, A NON-OPIATE ANALGESIC, PROTECTS AGAINST β–AMYLOID-INDUCED APOPTOSIS IN PRIMARY CULTURES OF NEURONS. O 49

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β-Amyloid peptide (AB) is the major component of the senile plaques and neurofibrillary tangles of Alzheimer's disease (AD) patients. This peptide is able to elicit apoptosis in neurons. Flupirtine, a triaminopyridine used as non-opiate analgesic, has shown protective effects in other experimental models such as retinal or cerebral ischemia. Here we have studied some related properties of this novel drug. Flupirtine is able to inhibit spontaneous lipid peroxidation in rat embryo homogenates (a tissue very sensitive to oxygen). AB not only triggers apoptotic cell death but also, and this is a novel finding, glutathione (GSH) depletion in primary cultures of neurons. This depletion and apoptosis both could be inhibited by flupirtine. These findings set the bases for a clinical trial of this drug, already proven non toxic, in AD patients.

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4-HYDROXYNONENAL, A LIPID PEROXIDATION PRODUCT, INFLUENCES VASCULAR TONE IN HUMAN MESENTERIC ARTERIES. O 50

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4-Hydroxynonenal (HNE) is one of the major and most biologically active product of nonenzymatic lipid peroxidation (LPO). This and other related compounds have been shown to exert important effects on cellular and *in vitro* systems, among others, relaxation of human cerebral arteries (*J. Cereb. Blood Flow Metab.* ^{14}: 693-696. 1994). HNE is present in human plasma at concentrations ranging 0.3-0.6 μM, whereas at the inflammatory site it may reach the mM range. Moreover, in several systemic diseases serum concentrations of LPO products (the most abundant one, and used as a marker of this process, is malondialdehyde) are increased. LPO products concentration in serum of alcoholic, cirrhotic, and hepatitis C patients have been assayed and found to be significantly increased. HNE relaxes in a concentration-dependent manner the precontracted (norepinephrine 10^{-6} M) artery rings in the organ bath. This effect was strictly endothelium-dependent. Addition of L-NAME to the organ bath partially prevents this HNE-induced relaxation in artery rings with endothelium.

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O 51 COUPLING CONDUCTANCES AND OPTIMAL INPUT RESISTANCE IN MOUSE B-CELLS. E. Andreu, R. Pomares, B. Soria and J. V. Sánchez-Andrés. Departamento de Fisiología, Universidad de Alicante, Apartado de Correos 374, Alicante 03080 (Spain). J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (1), 92, 1997.

Pancreatic beta cells are grouped in islets and coupled through gap-junctions. When couplet in the islet these cells show a typical bursting pattern. Coupling in this system is a necessary requirement as isolated cells are unable to oscillate. The intrinsic properties of single cells are unable by themselves to explain the oscillatory behaviour. Characterization of coupling conductances at different glucose levels and during different electrical patterns has been performed in order to elucidate the role of coupling in this system. Different coupling levels lead cells to different input resistances. When coupling is near to zero, cells on the islet have a behaviour similar to isolated cells, as coupling increases, the dynamical properties of the coupled cells vary until they arrive to a coupled state where they behave as a syncytium and the whole system oscillates between two unstable states. Thus, the contribution of coupling conductance allowing a feedback pathway between cells, makes the global input impedance to be restricted into a narrow window. Outside these impedance values, cells can show random spiking or be silent, in dependence of the membrane conductance heterogeneity shown by these cells. The activation of coupling at physiological glucose ranges leads both spiking and silent cells to the same input resistance state and allows the system to show a synchronic behaviour.