O27

Age-dependent effects on the inter-relationships between dopamine and GABA, noradrenaline, serotonin or taurine

P. Cano, F. Chacón, V. Jiménez, M.P. Alvarez, D.P. Cardinali† and A.I. Esquifino

*Departamentos de Bioquímica y Biología Molecular III and *Biología Celular, Facultad de Medicina, UCM, Madrid, Spain and †Departamento de Fisiología, Facultad de Medicina, UBA, Buenos Aires, Argentina*

The existence of interrelating mechanisms between the neurotransmitters involved in prolactin secretion regulation has been suspected from the data previously published in reference to dopamine (DA) and γ-amino butiric acid (GABA) or to DA and serotonin (5-HT) at the hypothalamic level in basal conditions (Esquifino *et al.* 1999). However, the concentration of each neurotransmitter studied follows a 24 h variation. The aim of this work was to analyse the interactive mechanisms among these neurotransmitters from the chronobiological point of view.

The 24 h variations of DA, noradrenaline (NA), 5-HT, GABA and taurine (Tau) were studied in 2- and 18-month-old male rats. Groups of eight young (2 months) and eight middle-aged (18 months) male rats of the Wistar strain were killed, by decapitation, at six time intervals around the clock, beginning at 09.00 h. Both biogenic amines and amino acid neurotransmitter determinations were performed by specific HPLC techniques. Statistical significance was determined by one-way ANOVA, Cosinor and Scheffe test.

The 24 h variations of DA, NA, 5-HT, GABA and Tau in the median eminence were specific for each neurotransmitter analysed in young rats, although some of these patterns disappeared or changed in middle aged rats. Both DA and NA patterns exhibited a positive polynomial correlation ($P < 0.03$) in the younger group and disappeared in middle-aged rats. Also DA and 5-HT showed a positive lineal correlation ($P < 0.04$) that remained in middle-age rats ($P < 0.02$). As for NA, DA and GABA exhibited a positive polynomial correlation ($P < 0.04$) in young rats and disappeared in middle-aged rats. As for NA and GABA, DA and Tau exhibited a positive polynomial correlation ($P < 0.03$) in young rats and disappeared in middle-aged rats.

The interactions of DA with any other neurotransmitter analysed in this study explain the circadian changes in plasma prolactin level. This correlation disappeared in middle aged rats and partially explains the modification in the circadian secretion of prolactin. All these data suggest that there are age-related changes in the interactive mechanisms among neurotransmitters at the median eminence level that explain, at least in part, the changes in plasma prolactin levels that occurred with age.

Esquifino AI *et al.* (1999). *Chronobiol Int* **16**, 451–460.

All procedures accord with current National guidelines.

O28

Effects of calorie restriction in growing male rats on the 24 h variations of pituitary, adrenal hormones and leptin

F. Chacón, V. Jiménez, M. Perello, P Alvarez, A. Marcos, E. Spinedi and A.I. Esquifino

*Departamentos de Bioquímica y *Biología Celular, Facultad de Medicina, UCM, †Instituto de Nutrición y Bromatología (CSIC), Edificio del Instituto del Frío Madrid, Spain and Unidad de Neuroendocrinología Instituto Multidisciplinar de Biología Celular, La Plata, Argentina*

The effect on the endocrine system of controlled diets with calorie restriction is a matter of controversy. The aim of the present work was to determine the possible effect of calorie restriction on the secretory patterns of plasma adreno-corticotropin (ACTH), corticosterone (B) and leptin as well as the content of B in the adrenal gland.

Growing male Wistar rats were submitted to a 66 % calorie restriction from days 35 to 65 of life. To avoid cannibalism, calorie-restricted animals were isolated. Age-matched intact and isolated rats were used as controls. On day 65 of life, animals were humanely killed by decapitation at six time intervals around the clock beginning at 09.00 h. Specific RIA systems were used to measure plasma levels of ACTH, B and leptin as well as B content in the adrenal gland.

Plasma ACTH levels in intact control rats peaked at 17.00–21.00 h. In isolated controls plasma ACTH levels peaked at 17.00 h. The mean 24 h values of the hormone remained similar in both groups. However, in calorie restricted rats the mean values of the hormone were reduced. The pattern of ACTH showed lower amplitude in calorie-restricted rats, although the pattern was similar to that described in intact controls. Plasma B levels showed a plateau between 17.00 and 05.00 h. The mean levels of the hormone were increased and the pattern disappeared in both isolated control and calorie-restricted rats, as compared to intact controls. These changes were associated with a marked decrease in adrenal B content in both isolated groups and was higher in intact controls. Plasma levels of leptin peaked at 05.00 h in intact controls. In isolated controls a phase advance in leptin peak to 21.00–01.00 h. was observed. In calorie-restricted rats this pattern disappeared as compared to either isolated or intact controls. Mean values of leptin were markedly increased in isolated controls and in calorie restricted rats it was markedly decreased as compared to intact controls.

All these data suggest that calorie restriction disrupts the regulatory mechanism that modulates carbohydrate and lipid metabolisms.

All procedures accord with current National guidelines.

O29

Effects of melatonin on age-induced liver injury

C. Castillo, E. Vara*, V. Salazar, A. Martínez, M.A. Ramírez, A. Holguín, C. Ariznavarreta and J.A.F. Tresguerres

*Departments of Physiology and *Biochemistry, School of Medicine, Complutense University, Madrid, Spain*

We have previously demonstrated that ageing decreases the synthesis of phosphatidylcholine in isolated Wistar rats hepatocytes. Accumulating evidence indicates that free radicals (FR) play a major role in tissue and cell damage associated with

ageing. FR generated within cells include the superoxide anion radical, the hydroxyl radical and nitric oxide (NO). Because of their high reactivity, these radicals can be devastatingly toxic to other molecules and cause cellular dysfunction and sometimes death of cells. Melatonin is an important antioxidant molecule. Besides its ability to scavenge highly reactive radicals, melatonin's antioxidant activity is augmented by its ability to stimulate enzymes related to antioxidative defence systems. This study was designed to investigate a possible protective effect of melatonin against age-induced hepatocyte injury.

Hepatocytes were isolated from young (2 months old, $n = 8$) and old (24 months old) male Wistar rats. Old rats were randomly separated into three groups: non-treated rats ($n = 8$), rats treated with melatonin (1 mg kg^{-1} day^{-1}) for 2.5 months (mel 2.5; $n = 8$), and rats treated with melatonin for 5 months (mel 5; $n = 8$). After humanely killing the animals by decapitation, cells were cultured in RPMI 1640 medium (supplemented with serum, glutamine, antibiotics and insulin) for 24 h. Then, media and cells were collected separately and CO and NO release to the medium, and cGMP, phosphatidylcholine (PC), and lipid peroxide (LPO) content of the cells were measured. Results are presented as means ± S.E.M. Mean comparison was done by Friedman's analysis of variance followed by Wilcoxon's two-tailed test for paired data; a confidence level of 95 % ($P < 0.05$) was considered significant. Experimental procedures employed are in accordance with the National rules of Spain (RD 223/1986).

Age increased LPO (5.6 ± 0.03 *vs.* 0.91 ± 0.05 fmol (μg protein)$^{-1}$, $P < 0.01$). This increase was attenuated when animals were treated with melatonin (2.6 ± 0.03 and 2.19 ± 0.01 for mel 2.5 and mel 5, respectively). NO (1.99 ± 0.02 *vs.* 1.24 ± 0.005 nmol (μg protein)$^{-1}$, $P < 0.05$), and CO (5.33 ± 0.1 *vs.* 1.93 ± 0.09 pmol (μg protein)$^{-1}$, $P < 0.05$) release to the medium and cGMP (248 ± 2.1 *vs.* 46.6 ± 4.1 fmol (μg protein)$^{-1}$, $P < 0.01$) content of the cells were increased in 24-month-old rats, and again this effect was diminished by melatonin treatment (1.05 ± 0.004, mel 2.5 and 1.04 ± 0.003, mel 5; 1.96 ± 0.1, mel 2.5 and 1.86 ± 0.2, mel 5; and 70.5 ± 3.7, mel 2.5 and 67.3 ± 2, mel 5 for NO, CO and cGMP, respectively). Additionally, melatonin was able to attenuate the decrease in PC synthesis induced by ageing.

In summary, these results suggest a possible protective effect for melatonin in age-induced liver injury. This effect could be mediated by reduction of FR generation.

This work was supported by a grant of C.A.M. 8.5/0062/2001.

All procedures accord with current National and local guidelines.

O30

α-Melanocyte-stimulating hormone induces Fos expression in oxytocin neurones but decreases systemic oxytocin secretion in the rat

Celine Caquineau, Nancy Sabatier, Philip Bull, Alison Douglas and Gareth Leng

Department of Biomedical Sciences, University of Edinburgh, Edinburgh, UK

α-Melanocyte-stimulating hormone (αMSH) is produced in the brain mainly in the neurones of the arcuate nucleus. αMSH acts centrally in behaviours such as feeding, stretching–yawning reflex and sexual behaviour via MC3 and MC4 receptors. These central actions are remarkably similar to those mediated by central oxytocin. Projections between the arcuate nucleus and the supraoptic nucleus (SON) have been described and MC4 receptor mRNA is expressed in the SON (Mountjoy *et al.* 1994). Consequently, we investigated the modulatory effect of αMSH on oxytocin neurone activity and oxytocin secretion.

Firstly, urethane-anaesthetised Sprague-Dawley rats were fitted with femoral artery and I.C.V. cannulae. Blood samples were collected every 5 min after I.C.V. injection of MC4 agonist (400 ng/2 μl) or vehicle. In a second experiment, rats were implanted with jugular vein and I.C.V. cannulae under brief halothane-anaesthesia. After two days, blood samples were taken 10 min before, and 10, 15 and 90 min after I.C.V. injection of αMSH(1 μg/5 μl) or vehicle. Since cholecystokinin (CCK) increases oxytocin secretion and Fos expression in oxytocin neurones, other rats were given I.V. injection of CCK (20 μg kg^{-1}) or vehicle as controls. Rats were given an overdose of pentobarbitone and perfused transcardially 90 min after drug injection. Free-floating brain sections were processed for Fos immunocytochemistry and for Fos/oxytocin double immunocytochemistry. Oxytocin was measured in unextracted plasma samples by specific RIA.

In anaesthetised rats, MC4 agonist decreased plasma oxytocin concentration ($n = 7$), while vehicle had no effect ($n = 5$; One-way RM ANOVA $P < 0.05$). In conscious rats, αMSH ($n = 7$) had no significant effect on oxytocin secretion compared to vehicle ($n = 6$), whereas rats injected I.V. with CCK showed a 180 % increase in oxytocin secretion ($n = 7$; $P < 0.05$). In the same rats, αMSH increased Fos expression in the SON (mean ± S.E.M.; 110 ± 19 Fos-positive cells per section; $n = 7$) significantly more than either vehicle (18 ± 6; $n = 6$; Mann-Whitney test $P < 0.05$) or CCK (55 ± 11 Fos-positive cells per section; $n = 8$; $P < 0.05$). Double immunocytochemistry confirmed the increased Fos expression in oxytocin neurones after αMSH (52 ± 6% Fos-positive oxytocin cells/section) compared to CCK (37 ± 5% Fos-positive oxytocin cells/ section; $P < 0.05$).

Thus central administration of αMSH induces strong Fos expression in oxytocin neurones but decreases oxytocin secretion from the pituitary gland. This illustrates that Fos induction does not necessarily reflect excitation of the neurones. These studies confirm the modulatory effect of αMSH on oxytocin neurones via MC4 receptors.

Mountjoy KG *et al.* (1994). *Mol Endocrinol* **8**, 1298–1308.

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All procedures accord with current UK legislation.

O31

α-Melanocyte-stimulating hormone mobilises Ca^{2+} from intracellular stores in rat supraoptic oxytocin neurones and induces dendritic release of oxytocin

Nancy Sabatier*, Celine Caquineau*, Govindan Dayanithi† and Gareth Leng*

**Department of Biomedical Sciences, the University of Edinburgh, Edinburgh, UK and †INSERM 432, University of Montpellier II, Montpellier, France*

O32

Hypothalamo–pituitary–adrenal responses to social defeat are reduced in pregnant rats

P.J. Brunton*, S.L. Meddle*, S. Krömer†, I.D. Neumann†‡ and J.A. Russell*

**Division of Biomedical & Clinical Laboratory Sciences, Hugh Robson Building, George Square, University of Edinburgh, Edinburgh EH8 9XD, UK, †Max Planck Institute for Psychiatry, Kraepelinstrafle 2, 80804 Munich, Germany and ‡University of Regensburg, Universitatsstrafle 31, 93043 Regensburg, Germany*

The responsiveness of the hypothalamo–pituitary–adrenal (HPA) axis to a range of 'emotional/psychological' stressors is reduced in late pregnancy in the rat. Here we employed the social defeat model of 'maternal defence' (previously validated as a relevant emotional stressor for female rats (Neumann *et al.* 2001) to test whether pregnant rats demonstrate attenuated HPA axis responses to an acute social stressor relevant to females. The test relies upon aggression displayed by a lactating rat directed against an intruder, male or female, that approaches the nest.

On the morning of the experiment pregnant (day 21) or virgin Sprague-Dawley rats were transferred from their home cages into the cage of a lactating resident and exposed to 'maternal defence' for 30 min, and then killed immediately by decapitation (in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986). Control rats were undisturbed before killing. Trunk blood was collected for determination of plasma ACTH and corticosterone concentrations by radioimmunoassay. Brains were removed and processed by *in situ* hybridisation (ISH) for the immediate early gene: nerve growth factor induced gene-B (NGFI-B) mRNA expression in the parvocellular region of the paraventricular nucleus (pPVN).

Aggressive behaviour displayed by the lactating resident (number of attacks and latency to attack) was not significantly affected by the reproductive status of the intruder rat (Student's unpaired *t* test). Exposure to maternal defence induced a significant increase in plasma ACTH concentration in the virgin group (2.3-fold increase, 2-way ANOVA; $P < 0.01$, $n = 7$), but not in the pregnant group (1.4-fold increase, 2-way ANOVA; n.s., $n = 6$). Similarly, the maternal defence test induced a significant increase in corticosterone secretion only in the virgin group (36.0 % increase, 2-way ANOVA; $P < 0.01$) and had no effect in the pregnant rats (1.7 % increase, n.s.). ISH revealed a significant increase in expression of NGFI-B mRNA in the pPVN of virgin rats in response to social defeat but not in the pregnant rats (2.3-fold *vs.* 1.5-fold increase, respectively; $P < 0.01$; 2-way ANOVA).

Thus the responsiveness of the HPA axis to social defeat is reduced in late pregnant rats consistent with our previous studies using other emotional stressors. This is a consequence of reduced activation of the pPVN corticotropin-releasing hormone and/or vasopressin neurones in the pregnant rats.

Neumann ID *et al.* (2001). *Eur J Neurosci* **13**, 1016–1024.

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All procedures accord with current UK legislation.

O33

Renal responsiveness to the vasopressin analogue DDAVP during the suppressed menstrual cycle

Jane Ward, Niall Boyce, Melanie Rosser and Mary L. Forsling†

Division of Physiology and †Neuroendocrine Laboratories, GKT School of Biomedical Sciences, Guy's Campus, London SE1 1UL, UK

The renal response to vasopressin depends on reproductive status in women (Boyce *et al.* 2001) as well as in the rat (Forsling *et al.* 1996). To investigate the possible role of ovarian steroids, the renal response to DDAVP (Ferring AB, Sweden), an agonist at the renal vasopressin receptor (V2), was monitored in women taking a combined contraceptive pill containing 30 mg ethinylestradiol and 150 mg levonorgesterol (Microgynon-30, Schering Health Care).

The study was carried out on 6 women age 19–28 years with local ethics committee approval and informed subject consent. A water load of 750 ml per 70 kg body weight was given between 08.00 and 09.00 h, followed by 0.2 mg oral DDAVP 2 h later. Hydration was maintained by giving the subjects water to drink equivalent to each urine sample passed; blood and urine samples were taken for 6 h for analysis. Subjects were studied on the last pill-free day, taken as day 7, and day 21.

There were no differences in creatinine clearance on the two days and no changes were observed following DDAVP administration. Control urine flow, osmolality and electrolyte concentrations were similar on the two days, but after DDAVP administration urine flow fell to the lower value of 0.69 ± 0.05 ml min^{-1} on day 21 compared to 0.87 ± 0.07 ml min^{-1} on day 7 ($\pm$ S.E.M., $P < 0.001$, Student's paired *t* test), while the urinary osmolality was higher being 785 ± 22 as compared to 721 ± 16 mosmol kg^{-1} ($P < 0.001$) Excretion of sodium and potassium was also significantly greater following DDAVP on day 21 ($P < 0.005$). Plasma osmolality fell on DDAVP administration, the greater drop to 279 ± 1 mosmol kg^{-1} on day 21 reflecting the greater antidiuresis on that day. The mean plasma sodium and potassium concentrations achieved were also lower on day 21, as was the packed cell volume. Thus the renal responsiveness to vasopressin was greater following 14 days during which an oestrogen and a gestagen were taken than after 7 days during which no ovarian steroids were administered.

Boyce N *et al.* (2001). *J Physiol* **531.P**, 161*P*.

Forsling ML *et al.* (1996). *J Endocrinol* **148**, 457–464 .

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All procedures accord with current local guidelines and the Declaration of Helsinki.

O34

Incremental graded exercise test to exhaustion in trained and sedentary men: integrated neuroendocrine and metabolic response

A.M. Diego Acosta, J.C. García Romero, V.J. Fernández-Pastor, S. Perán and J.R. Alvero

Exercise Physiology, School of Sport's Medicine, University of Málaga, Spain

A group of long-distance runners and a group whi practice no sport on regular basis, with an age of 20–24 years, carried out a maximum effort test to evaluate the two main stress-activated

systems: sympathetic with its endocrine medullar component and the pituitary–adrenal axis, as well as insulin and HGH hormones related to some metabolites used as fuel during the exercise and recovery period.

Prior, informed consent was obtained from each subjects and the project was approved by the Investigation and Ethics Committee of the Faculty of Medicine. A test was designed on a treadmill with a 3 % gradient and 2 km h^{-1} progressive increases in speed every 10 min, starting at 6 km h^{-1} until exhaustion; for blood samples collection an antecubital vein was catheterized with a system enabling replacement of blood volume extracted with physiological saline solution. Plasma hormone concentrations was determined by RIA, catecholamines by HPLC and metabolites by enzymatic methods.

The results showed maximal plasma concentrations of ACTH, cortisol (C), β-endorphin (β-end) and human growth hormone (HGH) at the end of the exercise test in the trained group ($P < 0.01$, Student's t test for paired data). Control subjects exhibited a higher responsiveness compared with trained of catecholamines. Insulin declined during exercise until the end of exercise with the minimal value and maximal for lactate concentrations ($P < 0.05$). Glucose levels of the control group did not vary during exercise; on the contrary, glucose levels in runners increased at minute 20 of exercise coinciding with HGH and noradrenaline increase. In the control group there were no variations in β-hydroxybutyrate or acetoacetate but in runners there was a significant increase at the end of the exercise and at minute 3 of recovery period of this metabolites (Rudolph *et al.* 1998).

HGH, ACTH, C and β-end increases are physiological responses to stress in the critical zone next to exhaustion and end of effort test that involve metabolic adaptations during exercise and recovery. The increase in C and β-end delays fatigue and allows a greater workload according to the degree of training of subjects (Diego Acosta *et al.* 1995). FFA concentrations levels 67 % below baseline values and ketone body changes were statistically significant, indicating the use of this alternative pathway to obtain energy in trained subjects. Linear regression analyses and statistically significant correlation between hormones, metabolites and fitness ($r = 0.723$: $P < 0.05$ to $r = 0.956$: $P < 0.001$) showed organic adaptations to endurance of trained subjects with homeostatic compromise which necessarily implies an integrated response of the studied parameters.

Diego Acosta AM *et al.* (1995). *Olympic Scientific Congress* **2**, 67–71.

Rudolph D *et al.* (1998). *J Sports Sci* **16**, 121–128.

All procedures accord with current local guidelines and the Declaration of Helsinki.

P145

5-HT_{1A} and 5-HT_2 receptor activation reduces NMDA-stimulated LH secretion in prepubertal male and female rats

L. Pinilla, M.L. Barreiro, M. Tena-Sempere and E. Aguilar

Physiology Section, Faculty of Medicine, University of Cordoba, 14004 Córdoba, Spain

The hypothalamic control of gonadotrophin secretion is exerted through the pulsatile release of luteinizing hormone-releasing hormone (LHRH), which in turn is regulated by many neurotransmitters and neuropeptides such as noradrenaline, endogenous opioids, gamma-aminobutyric acid, nitric oxide, galanin, excitatory amino acids (through ionotropic receptors such as *N*-methyl-D-aspartic acid (NMDA) or kainate (KA) receptors) and serotonin. Given that physiological interactions between different neurotransmitters involved in the control of LHRH/LH secretion have been described, the objective of the present experiments was to analyse the effects of activation of different 5-hydroxytryptamine (5-HT) receptor subtypes on gonadotrophin secretion and their role in the NMDA-stimulated LH release.

We analysed gonadotrophin secretion after manipulation of the serotoninergic and aminoacidergic systems and their interaction in 5-, 16- and 23-day-old male and female rats. The animals were decapitated 15 min after the last injection and blood samples were collected. To this end, serum LH and follicle-stimulating hormone (FSH) concentrations were measured in rats treated with 5-hydroxytryptophan methyl ester (a precursor of 5-HT synthesis) plus fluoxetine (a blocker of 5-HT reuptake), D,L-*p*-chlorophenylalanine methyl ester (a blocker of 5-HT synthesis), *R*(+)-8-hydroxy-propylaminotetralin hydrobromide (8-OH-DPAT, an agonist of 5-HT_{1A} receptors), (±)2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) and α-methyl-5-hydroxytryptamine (agonists of 5-HT_2 receptors), and 1-phenylbiguanide (an agonist of 5-HT_3 receptors). In addition, the effects of 8-OH-DPAT and DOI on NMDA-stimulated LH secretion were analysed.

Neither the activation nor the blockade of the serotoninergic system modified LH (0.1 ng ml^{-1}) secretion. Basal gonadotropin secretion remained unchanged in 23-day-old male and female rats after selective activation of 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors. The stimulatory effect of NMDA on LH secretion was blocked in both sexes after activation of the serotoninergic system, through specific 5-HT_{1A} and 5-HT_2 receptors agonists.

Although basal LH secretion is not under the control of the serotoninergic system in prepubertal male and female rats, the specific activation of 5-HT_{1A} and 5-HT_2 receptors decreased the stimulatory effect of NMDA on LH secretion.

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All procedures accord with current National and local guidelines.

P146

The antiprogestin RU486 reduces basal and LHRH-stimulated LH secretion and LHRH self-priming elicited by activation of PKC signalling pathway in an oestrogen-dependent manner

M.C. Bellido, R. Aguilar, J.C. Garrido and J.E. Sánchez-Criado

Department of Cell Biology, Physiology and Immunology, University of Córdoba, 14004 Córdoba, Spain

To explore the involvement of pituitary progesterone receptor (PR) in basal and stimulated LH secretion and in luteinizing hormone releasing hormone (LHRH) self-priming elicited through PKC signalling pathway and the role of the oestrogen (E) environment, eight randomly selected hemipituitaries from adult female rats in pro-oestrus or from 2 weeks ovariectomized (OVX) rats under ether anaesthesia were incubated, in the absence of P, over 3 h in DMEM. In the first experiment, hemipituitaries were incubated continuously with: medium alone, LHRH (10 nM), the PKC stimulator phorbol ester 12-myristate 13-acetate (PMA; 100 nM), LHRH + the PKC inhibitor staurosporine (100 nM), LHRH+RU486 (10 nM) and PMA+RU486. Control pituitaries were incubated with staurosporine or RU486. In the second experiment, hemipituitaries from control rats in pro-oestrus or OVX rats were incubated, 1 h apart, with LHRH to determine the

LHRH self-priming and this was compared with the priming effect of PMA. Also, the effect of staurosporine and RU486 on LHRH self-priming and PMA priming was studied. Medium was aspirated every hour to determine LH accumulation in the medium and LHRH self-priming. Rats were humanely killed.

Both LHRH and PMA stimulated LH secretion; staurosporine reduced basal and LHRH-stimulated LH secretion and RU486 reduced basal and LHRH- and PMA-stimulated LH secretion from proestrous pituitaries. The stimulating effect of LHRH and PMA on LH secretion was significantly reduced in OVX rats. Both LHRH and PMA induced LHRH priming. Staurosporine reduced LHRH self-priming while RU486 reduced both LHRH self-potentiation and PMA priming. The magnitude of these effects was blunted in OVX rats. These results indicated that the PKC signalling pathway in the gonadotrope mediated basal and LHRH-stimulated LH secretion and LHRH self-priming in an oestrogen-dependent manner through ligand-independent activation of PR.

All procedures accord with current National and local guidelines.

P147

Differential effects of selective oestrogen receptor modulators on luteinizing hormone releasing hormone-induced luteinizing hormone secretion and inositol phosphate accumulation in female rat pituitary glands

P. Guelmes, P. Abreu, J.E. Sánchez-Criado, L. Prieto, R. Alonso and G. Hernández

Department of Physiology, Universities of La Laguna and Córdoba, Spain

In females, oestradiol (E_2) sensitizes pituitary gonadotrophs and potentiates luteinizing hormone releasing hormone (LHRH) self-priming, which is partially dependent on phospholipase C (PLC) activation. We found previously that, in cyclic female rats, LY117018 (LY), raloxifene (RA), tamoxifene (TX), and ICI 182,780 (ICI) impaired LHRH-induced luteinizing hormone (LH) secretion. However, TX also displayed oestrogen agonist activity on LHRH self priming (Sánchez-Criado *et al.* 2002). The aim of the present study was to compare the effect of different selective oestrogen receptor modulators (SERMs) on LHRH induced-LH secretion and pituitary inositol phosphate (IP) accumulation, as an indicator of PLC activity.

Two hundred and fifty adult female Sprague-Dawley rats were housed under a 14 h:10 h light-dark cycle. Animals were ovariectomized under I.P. ketamine/xylacine (100 mg/10 mg per kg body wt) anaesthesia and, after 2 weeks of recovery, they were S.C. injected with vehicle (VEH), E_2 (40 μg), and/or LY, RA, TX (3 mg of each per rat per day), and ICI (0.25 mg per rat per day). Animals were killed 2 days later (1600 h), and hemipituitaries cultured overnight in DMEM containing E2 (10 nM) and/or LY, RA, TX (100 nM), ICI (10 nM), plus 5 μCi of 2-H^3-*myo*-inositol. Glands were then incubated with LiCl (10 nM) and exposed to LHRH (10 nM) or VEH for 40 min. Media and pituitaries were assayed for LH and IP accumulation, respectively. Experimental procedures were according to the EU guidelines for care and use of laboratory animals.

E_2 enhanced LHRH-induced LH secretion and pituitary IP accumulation. Both effects were inhibited by the four SERMs. However, TX and ICI were unable to block the effect of E_2 on LHRH-induced accumulation of IP. Furthermore, ICI alone potentiated pituitary accumulation of IP in response to LHRH, thus micmicking the effect of E_2.

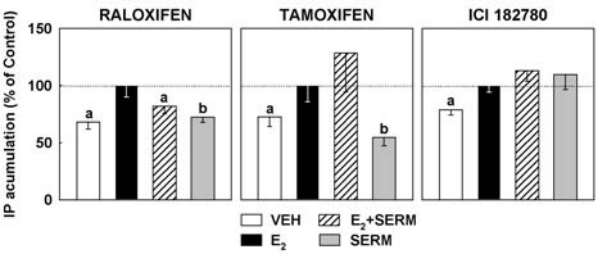

Figure 1. Effect of E_2 and different SERMs on LHRH-induced IP accumulation in female rat pituitary glands. Data (means and S.E.M.) represent the percentage of total IP compared to E_2 treated groups (100 %), and were analysed by two-way ANOVA. Each experiment ($n = 7$) was repeated three times. (a) $P < 0.05$; (b) $P < 0.01$ *vs.* E_2.

These results indicate that structurally different SERM molecules may exert selective agonist/antagonist activity on gonadotrophs. Intriguingly, ICI, a 'pure' antiestrogen, behaved as an oestrogen agonist on LHRH-induced PLC activation. Since this compound completely abolishes oestrogen receptor (ER) immunostaining in rat gonadotrophs (Sánchez-Criado *et al.* 2002), the above observations may be suggestive of an alternative pathway of oestrogen action in the rat gonadotroph that is independent of classical ER activation.

Sánchez-Criado JE *et al.* (2002). *Neuroendocrinology* **76**, 203–213.

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All procedures accord with current National and local guidelines.

P148

Oestrogen-induced galanin gene expression in the female rat anterior pituitary is dependent on a hypothalamic mechanism

J.G. Hernández-Jiménez*, R. Hill, G. Hernández*, P. Guelmes*, J.E. Sánchez-Criado†, R. Alonso* and F.J. López

*Departments of Physiology, Universities of *La Laguna and †Córdoba, Spain and Ligand Pharm. Inc., San Diego, USA*

Galanin (GAL) participates in the regulation of the hypothalamic–pituitary–gonadal axis. On the other hand, oestrogen dramatically stimulates GAL gene expression in the anterior pituitary of mice and rats (Shen *et al.* 1999). In these studies, we have evaluated whether oestrogen effects on anterior pituitary GAL gene expression require the physical connection between the pituitary and the hypothalamus.

Fifty-day-old Sprague-Dawley female rats were housed under a 14 h–10 h light–dark cycle and treated according to the European Community Guidelines for Care and Use of Laboratory Animals; all the results are expressed as means ± S.E.M.; $n = 5$. They were ovariectomized under I.P. ketamine–xylazine anaesthesia ((100 mg–10 mg) kg^{-1} b.w.), and allowed to recover for 2 weeks. Rats were treated orally for either 24 h (h) or 4 days (d) with oestrone sulphate (E1S) or vehicle (0.5 % carboxymethyl-cellulose), and killed humanely. Changes in anterior pituitary GAL gene expression were measured by ribonuclease protection assay.

E1S increased GAL mRNA levels in a time- and dose-dependent fashion. Maximal effects were reached 12 h after a single E1S dose

at doses greater than 3 mg kg^{-1}, with 14.72-fold increase (ED_{50} = 2.17 ± 1.24 mg kg^{-1}). After 4 days of treatment (killing conducted 24 h after the last dose), maximal increases in GAL gene expression were reached at the 10 mg kg^{-1} E1S dose, with a 99.77-fold increase (ED_{50} = 2.93 ± 1.25 mg kg^{-1}). The effects of E1S on GAL gene expression were also studied in ovariectomized rats bearing a pituitary transplant under the kidney capsule. GAL mRNA levels increased in both the native and the transplanted pituitaries after E1S treatment. However, while in the native pituitaries GAL gene expression increased 7.68- and 23.23-fold after 24 h and 4 days treatment, respectively, in the transplanted pituitaries elevations of only 2.11- and 2.40-fold were observed for the 24 h and 4 day regimens. Serum PRL levels indicated that the transplanted pituitaries were fully functional.

We conclude that oestrogen action requires a physical connection between the pituitary and the hypothalamus to induce maximal pituitary GAL gene expression. Therefore, although the nature of a hypothetical hypothalamic mechanism(s) and its precise action are unknown, these data suggest that oestrogen-induced GAL gene expression results from a combined action of the steroid on both the hypothalamus and the anterior pituitary.

Shen ES *et al* (1999). *Endocrinology* **40**, 2628–2631.

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P149

An ICI 182,780-sensitive oestrogen receptor, associated with the plasma membrane, participates in oestradiol-related prevention of amyloid β peptide$_{1–40}$-induced toxicity in a cholinergic cell line

B. Guerra*, R. Marin*, A. Morales*, M. Díaz† and R. Alonso*

*Departments of *Physiology and †Animal Biology, University of La Laguna, Tenerife, Spain*

It has been proposed that extracellular neuritic plaques formed by amyloid β peptide (Aβ) deposits may contribute to degenerative changes found in Alzheimer's disease (AD). Neuroprotective effects of 17β-oestradiol (E2) have been described in cellular and animal models of injury that partially mimic the neurotoxic events of AD. However, the mechanisms underlying oestrogen-related neuroprotection remain unclear. We have shown previously (Guerra *et al.* 2001) that the 1–40 fragment of human Aβ induces massive cell death in a murine cholinergic cell line (SN56) which expresses functional oestrogen receptors (ERs) (Martin *et al.* 2001; Martinez-Morales *et al.* 2001). This effect is prevented by E2 in a dose-dependent manner through a mechanism mediated by ER. We now report that these effects are also observed after short exposures to E2 or the impermeant conjugate oestradiol-peroxidase (E-HRP) and are partially dependent on an ICI 182,780-sensitive ER associated with the plasma membrane.

Using confocal microscopy on SN56 cells fixed under non-permeabilized conditions and exposed to a polyclonal antibody (MC-20) directed to ERα, we have detected an ER at the plasma membrane level. Western blot analyses of purified cell membrane fractions revealed the presence of two forms of ER (67 and 80 kDa). Fifteen minutes exposure to either E2 or E-HRP prevented Aβ-induced cell death. This effect was reduced by both the ER antagonist ICI 182,780 or MC-20 antibody (see Fig. 1).

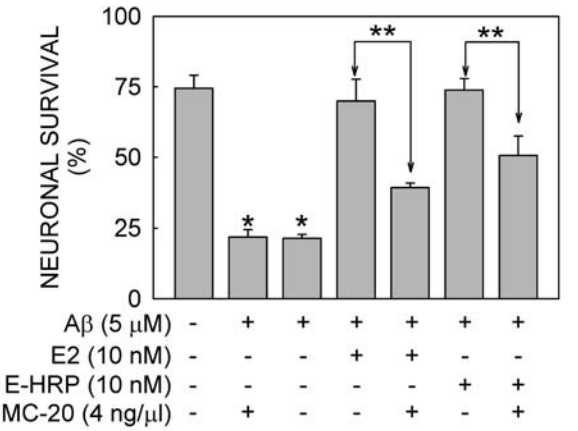

Figure 1. Specific MC-20 anti-ERα antibody inhibits neuroprotective actions of either E2 or E-HRP. SN56 cells were pre-incubated with MC-20 antibody (1:50) prior to treatment with steroids for 15 min. Cells were washed and exposed to 5 μM A$\beta_{1–40}$ for 24 h. Cell survival, as percentage of untreated controls, was reduced in presence of Aβ (70–80 %, *$P < 0.0001$). Both E2 and E-HRP prevented Aβ-induced cell death ($P < 0.001$), an effect that was reduced by pretreatment with MC-20 antibody (**$P < 0.01$). Data are expressed as means ± S.E.M. and were analysed by one-way ANOVA followed by Tukey's *post hoc* test to compare between groups. Statistical significance is given by P values from 0.05 and below.

Using E-HRP and E2 coupled to BSA and a fluorescence probe (E-BSA-FITC), we found binding sites for E2 at the surface of SN56 cells. Binding of both conjugates was blocked by pre-treatment with either E2 or ICI 182,780. Pre-exposure to increasing concentrations of MC-20 antibody significantly decreased E-BSA-FITC labelling. These results suggest that the ER observed at the plasma membrane domain shares structural similarities with its intracellular counterpart, and that it might participate in membrane-mediated neuroprotective oestrogen actions in SN56 cells.

Guerra B *et al.* (2001). *Rev Neurol* **33**, 89.

Marin R *et al.* (2001). *Neuroscience* **107**, 447–454.

Martinez-Morales JR *et al.* (2001). *Neuroscience* **103**, 1027–1033.

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All procedures accord with National and local guidelines.

P151

Influence of dietary fat composition on aminopeptidase activity in the frontal cortex and hypothalamus of rat brain

I. Prieto, A.B. Segarra, I. Banegas, F. Alba*, F. Vives* and M. Ramirez

*Unit of Physiology, University of Jaen, Spain and *Institute of Neuroscience Federico Oloriz, University of Granada, Spain*

Dietary fat induces changes in brain and peripheral tissue membrane fatty acid (FA) composition (Youdim *et al.* 2000). These changes are mainly due to the polyunsaturated FA (PUFA) composition of the diets. This affects membrane fluidity, eicosanoid synthesis, enzymatic activities associated to membrane, and has possible health implications (Paxinos &

Watson, 1998). Aminopeptidase activities (AP) play a major role in controlling the function of neuropeptides. The aim of this study was to analyse the effect of several fats used in the diet on brain AP activities.

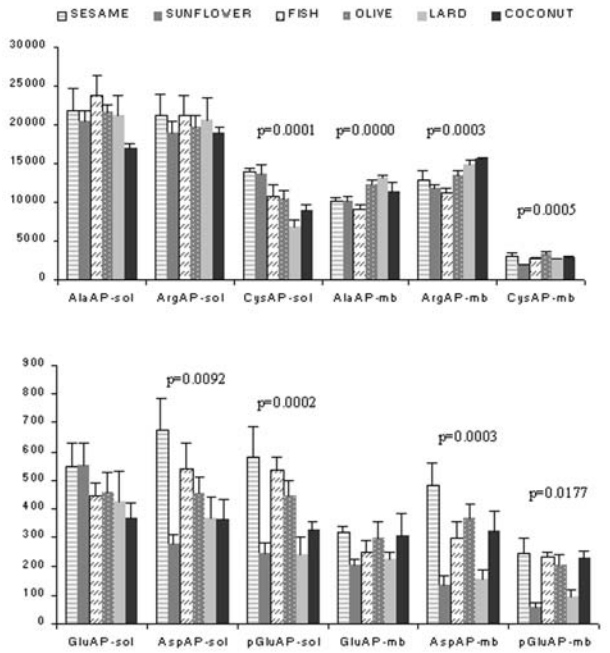

Figure 1. Soluble (sol) and membrane-bound (mb) aminopeptidase activities in frontal cortex (pmol (mg protein)$^{-1}$ min^{-1}). $n = 8$, means ± S.E.M., p = difference between bars.

Diets were prepared with fats of different degrees of saturation and essential FA composition. The AP activities analysed were: AlaAP, ArgAP, CysAP, pGluAP, GluAP and AspAP, assayed in both soluble (sol) and membrane-bound (MB) fractions obtained from the frontal cortex and hypothalamus of adult male rats. Male Wistar rats were divided into six groups ($n = 8$) and received for 16 weeks a synthetic diet, adequate with respect to all essential nutrients, and with different source of fat (10%): sesame (S), sunflower (SF), fish (F), olive (O), coconut (C) oil or lard (L). After the feeding period animals were humanely killed and the brains were removed and the hypothalamus and frontal cortex dissected in agreement with the stereotaxic atlas of Paxinos and Watson (1998). Sol and MB fractions were obtained from these samples (Prieto *et al.* 2001). AP activities and proteins were measured as previously described (Prieto *et al.* 2001). Data were analysed by one-way analysis of variance. *P* values below 0.05 were considered significant. The experimental procedures for animal use and care were in accordance with European Communities Council Directive 86/609/EEC. Results are shown in Figs 1 and 2.

Several enzymatic activities exhibit significant differences between groups ($P < 0.001$). The frontal cortex (Fig. 1) differs from the hypothalamus (Fig. 2) in the behaviour of several of the AP studied. In frontal cortex, except for sol AspAP and pGluAP, in which the lowest levels were obtained with SF, there is a general tendency of sol AP to decrease with the degree of saturation of the diet. In contrast, MB AlaAP and MB ArgAP showed their highest levels using saturated fat in the diet. The rest of MB AP (CysAP, GluAP, AspAP and pGluAP) showed a heterogeneous pattern with the lowest levels of activity when SF and L were used in the diet. In hypothalamus, sol and MB AlaAP, ArgAP and CysAP exhibited a similar pattern of activity to that in frontal cortex. However, the rest of the AP exhibit a heterogeneous pattern with the highest levels of activity when S was used in the diet.

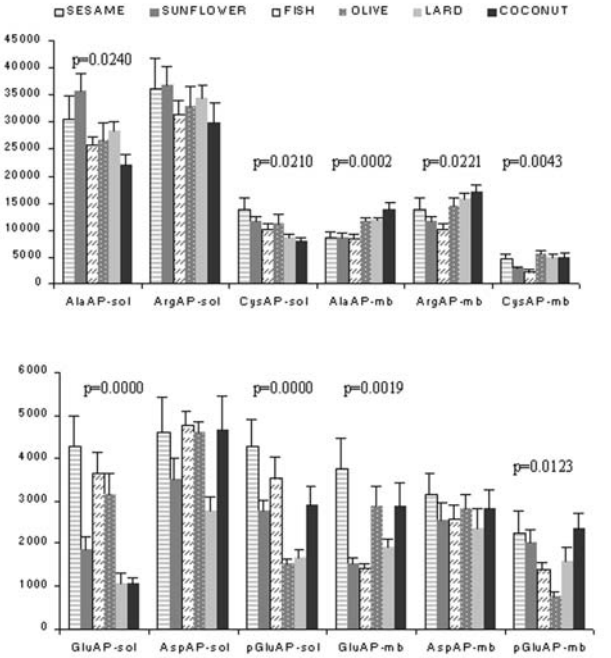

Figure 2. Soluble (sol) and membrane-bound (mb) aminopeptidase activities in hypothalamus (pmol (mg protein)$^{-1}$ min^{-1}). $n = 8$, means ± S.E.M., p = difference between bars.

The present data suggest that several AP involved in neuropeptide metabolism, may be modified depending on the fat used in the diet. This may have therapeutic importance in pathologies such as hypertension, Alzheimer's and Parkinson's disease in which, brain AP activities may be involved.

Paxinos G & Watson C (1998). *The Rat Brain in Stereotaxic Coordinates.* Academic Press, New York.

Prieto I *et al.* (2001). *Regul Pept* **101**, 189.

Youdim KA *et al.* (2000). *Int J Neuroscience* **18**, 383.

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P152

Effect of intracerebroventricular injection of LPS on activity and expression of tyrosine hydroxylase in the rat hypothalamus

M.C. González*, P. Abreu*, P. Barroso-Chinea† and T. González-Hernández†

*Departments of *Physiology and †Anatomy, School of Medicine, University of La Laguna, Tenerife, Spain*

The administration of lipopolysaccharide (LPS) by different routes is used as an animal model for different types of infection or inflammation. LPS challenge is interpreted by the brain as a stressor causing changes in central neurotransmitter systems. The present study examines the effect of intracerebroventricular (I.C.V.) administration of LPS (5 mg/4 ml) or sterile saline (vehicle) on tyrosine hydroxylase (TH) activity in the median eminence (ME) and on expression of TH mRNA and TH protein

in the arcuate nucleus of the hypothalamus. Animals were anaesthetised using ketamine (100 mg kg^{-1})–xylacine (10 mg kg^{-1}) and humanely killed. Enzyme activity was measured at 3, 6 and 12 h after injection of saline or LPS, and TH expression was analysed at 6 and 12 h. Intracerebroventricular injection of vehicle induced a light but not significant reduction of the TH activity in the ME at 6 and 12 h in relation to intact animals. LPS injected in the third ventricle decreased significantly TH activity at 6 h ($P < 0.04$, ANOVA followed by Tukey's *post hoc* test) but not at 3 and 12 h after injection as compared to sham operated animals. However, an *in situ* hybridization study to examine the mRNA TH expression in the arcuate nucleus showed that saline injection had no effect but LPS induced a reduction of 80.5 % in the number of TH+mRNA cells at 6 h and of 44 % at 12 h. In addition, immunohistochemical study for detection of TH expressing cells showed that LPS also reduced protein expression in the arcuate nucleus in a 89 % at 6 h and 44 % at 12 h. Saline injection had no effect on TH expression. Parallel sections were stained with Nissl staining and no differences in the total number of cells were found. These results show that the I.C.V. administration of LPS reduces transiently the dopaminergic activity in the mediobasal hypothalamus through a transcriptional action more than a direct action on the TH activity.

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All procedures accord with current National and local guidelines.

P153

Effect of low doses of LPS on the somatotropic axis

T. Priego*, M. Granado*, I. Ibáñez*, A.I. Martín†, A. López-Calderón* and M.A. Villanúa*

**Department of Physiology, Faculty of Medicine, Complutense University, 28040 Madrid and †Department of Morphology and Physiology, Europea University, 28670 Madrid, Spain*

Lipopolysaccharide (LPS) is a component of the wall of gram-negative bacteria which induces an inflammatory response. During inflammation the adrenal axis is activated, and in most cases this increase is accompanied by the inhibition of the somatotropic axis. However, the mechanism by which inflammation inhibits the growth hormone and insulin-like growth factor-I (GH–IGF-I) axis is not well known. The aim of this study was to analyse the effect of low LPS doses on pituitary GH secretion and serum IGF-I levels.

Rats received two LPS injections (at 17.30 and at 08.30 h the following day) of different dosages (0, 5, 10, 50 or 100 μg kg^{-1}, I.P.). Four hours after the last injection rats were humanely killed. Serum GH and IGF-I concentrations were analysed by RIA and pituitary mRNA levels of GH were analysed by Northern Blot. Comparison of means was performed with Duncan's multiple range test.

LPS administration decreased serum IGF-I levels in a dose-dependent way ($P < 0.01$). In contrast, serum GH concentrations increased after 5 and 10 μg kg^{-1} of LPS administration ($P < 0.05$ and $P < 0.01$ respectively). In addition, pituitary GH mRNA was increased with the LPS dosages of 10 and 50 μg kg^{-1} ($P < 0.05$). In order to elucidate if the increase in GH secretion is secondary to the IGF-I decrease or a specific LPS-effect, a second experiment was performed. Primary pituitary cell cultures (200 000 cells well^{-1}) were incubated with LPS (0, 0.1, 10, 100, 1000 ng well^{-1}) for 4 h. GH release to the culture medium was analysed by RIA. The *in vitro* studies showed that LPS induced an increase of GH release after the addition of 0.1 and 10 ng well^{-1} of LPS in the culture media ($P < 0.05$). The data indicate that LPS in low doses directly stimulates pituitary GH release, whereas it decreases circulating IGF-I by a GH-independent mechanism.

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All procedures accord with current National guidelines.

P154

NO mediates the inhibitory effect of chronic inflammation on pituitary GH gene expression

I. Ibáñez*, T. Priego*, M. Granado*, A.I. Martín†, M.A. Villanúa* and A. López-Calderón*

**Department of Physiology, Faculty of Medicine, Complutense University, 28040 Madrid and †Department of Morphology and Physiology, Europea University, 28670 Madrid, Spain*

Experimental arthritis is an animal model of chronic inflammation that is associated with growth inhibition and a decrease in pituitary growth hormone (GH) secretion. Nitric oxide (NO) is an important mediator in inflammatory processes. Several studies suggest that NO can mediate the inhibition of pituitary GH secretion during chronic inflammation. For this purpose we administered aminoguanidine, a selective inhibitor of inducible nitric oxide synthase (iNOS), to arthritic rats.

Experimental arthritis was induced by a subcutaneous injection of Freund adjuvant to male Wistar rats. Control rats were injected with paraffin oil. Twenty days after adjuvant injection, arthritic and control animals were divided into two groups; one was treated with aminoguanidine (200 mg kg^{-1} S.C. daily, during 8 days), and the second group was injected daily with 250 μl of saline S.C. On day 28 after adjuvant injection, all rats were humanely killed by decapitation and serum concentration of nitrites + nitrates was determined by the Griess method. Hypothalamic somatostatin and pituitary GH gene expression were measured by Northern blot hybridization. Comparison of means was performed with Duncan's multiple range test.

Aminoguanidine treatment decreased the inflammatory symptoms and the serum concentration of nitrites + nitrates in arthritic rats ($P < 0.01$). Chronic arthritis induced a significant decrease ($P < 0.01$) in pituitary GH mRNA, whereas it increased ($P < 0.01$) the hypothalamic somatostatin mRNA. Aminoguanidine administration did not modify pituitary GH or hypothalamic somatostatin gene expression in control rats. In contrast, aminoguanidine treatment was able to prevent the decrease in pituitary GH mRNA and the increase in hypothalamic somatostatin mRNA in arthritic rats. All these data suggest that the increased released of NO during chronic inflammation plays an inhibitory role in pituitary GH gene expression.

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All procedures accord with current National guidelines.

P155

Agouti-related peptide, neuropeptide Y and somatostatin-producing neurones are targets for ghrelin actions in the rat hypothalamus

Luisa M. Seoane†, Miguel López†, Sulay Tovar‡, Felipe F. Casanueva*, Rosa Señarís* and Carlos Diéguez*

Department of Physiology, Faculty of Medicine, San Francisco s/n, Santiago de Compostela, Spain

Ghrelin, the endogenous ligand of the growth hormone (GH) secretagogue receptor acts at a central level to elicit growth hormone release and to regulate food intake. In order to elucidate the neural circuit that exerts its effects, we measured the expression of hypothalamic neuropeptides involved in weight regulation and growth hormone secretion, following ghrelin administration.

Chronic intracerebroventricular cannulae were implanted under ketamine-xylazine anaesthesia into adult male rats, fed or fasted for 72 h, were treated centrally (I.C.V.) with one, single dose of ghrelin (5 mg). After 2, 4 and 6 or 8 h, the animals were humanely killed and agouti-related peptide, melanin-concentrating hormone, neuropeptide Y, prepro-orexin, growth hormone-releasing hormone and somatostatin mRNA levels were measured by *in situ* hybridization.

We found that ghrelin increased agouti-related peptide (60% *vs.* control) and neuropeptide Y (80% *vs.* control) expression in the arcuate nucleus of the hypothalamus of fed and fasted rats. In contrast, no change was demonstrated in the mRNA levels of the other neuropeptides studied at any time evaluated. Finally, we examined the effect of ghrelin on growth hormone-releasing hormone and somatostatin mRNA levels in GH-deficient (dwarf) rats. Our results show, that ghrelin increases somatostatin mRNA levels in the hypothalamus of these rats (increase is 40% *vs.* control).

Data are expressed as a percentage *vs.* control. Comparison between the different groups was assessed by ANOVA.

This study furthers our understanding of the molecular basis and mechanisms involved in ghrelin effect on food intake and GH secretion.

This work was supported by Fondo de Investigaciones Sanitarias, Spanish Ministry of Health, Spanish Ministry of Education, DGICYT, and European Union.

All procedures accord with current National and local guidelines.

P156

Orexin A suppresses *in vivo* growth hormone secretion

Sulay Tovar‡, L.M. Seoane†, M. López†, R. Señarís*, F.F. Casanueva* and C. Diéguez*

Department of Physiology, Faculty of Medicine, San Francisco s/n, Santiago de Compostela, Spain

Orexins are a newly described family of hypothalamic neuropeptides. Based on the distribution of orexin neurons and their receptors in the brain, it has been postulated that they could play a role in the regulation of neuroendocrine function. Growth hormone (GH) secretion is markedly influenced by nutritional status and body weight. To investigate the role orexin A plays in the neuroregulation of GH secretion we have studied its effect on spontaneous GH secretion as well as GH responses to GHRH and ghrelin in freely moving rats. Finally, we also assessed the effect of orexin A on *in vitro* GH secretion.

Chronic intracerebroventricular and intravenous cannulae were implanted under ketamine–xylazine anaesthesia. We administered orexin A (10 μg, I.C.V.) or vehicle (10 μl, I.C.V.) to freely moving rats. Spontaneous GH secretion was assessed for 6 h with blood samples taken every 15 min. The animals were humanely killed. Administration of orexin A led to a decrease in spontaneous GH secretion in comparison with vehicle-treated rats, as assessed by mean GH levels (4.2 ± 1.7 *vs.* 9.4 ± 2.2 ng ml^{-1}; $P < 0.05$), mean GH amplitude (3.6 ± 0.5 *vs.* 20.8 ± 5.6 ng ml^{-1}; $P < 0.01$) and area under the curve (848 ± 379 *vs.* 1957 ± 458 ng ml^{-1} (4 h)$^{-1}$; $P < 0.05$). In contrast, orexin A failed to modify *in vivo* GH responses to GHRH (10 μg kg^{-1}; I.V.) although it markedly blunted GH responses to ghrelin (40 μg kg^{-1}; I.V.) (mean peak GH levels: 331 ± 71 ng ml^{-1}, vehicle, *vs.* 43 ± 11 ng ml^{-1} in orexin A-treated rats: $P < 0.01$). Data are expressed as means ± S.E.M. Comparison between the different groups was assessed by the Mann-Whitney test. These data indicate that orexin A plays an inhibitory role on GH secretion and may act as a bridge among the regulatory signals that are involved in the control of growth and nutritional status.

This work was supported by Fondo de Investigaciones Sanitarias, Spanish Ministry of Health, Spanish Ministry of Education, DGICYT, and European Union.

All procedures accord with current National and local guidelines.

P157

Expression and homologous regulation of GH secretagogue receptor mRNA in rat adrenal gland

M.L. Barreiro, L. Pinilla, E. Aguilar and M. Tena-Sempere

Physiology Section, Faculty of Medicine, University of Cordoba, 14004 Córdoba, Spain

Growth hormone secretagogues (GHSs) are a family of synthetic peptidyl and non-peptidyl compounds with biological effects in several endocrine and non-endocrine target tissues, including activation of the hypothalamic–pituitary–adrenal axis. The physiological relevance of the GHS-signalling system was recently substantiated by the identification of the specific receptor for GHSs, namely GHS-R, and its endogenous cognate ligand, ghrelin. We have recently reported evidence for the novel expression and functional role of GHS signalling system in a major steroidogenic tissue, the testis (Tena-Sempere *et al.* 2002). In the present study, we aimed at evaluating the expression and homologous regulation of the GHS-receptor gene in rat adrenal. For comparative purposes, homologous regulation of GHS-receptor gene expression in rat pituitary and testis was also assessed. The animals were humanely killed and then we prepared tissue incubations.

Our RT-PCR analysis demonstrated expression of the GHS-R mRNA in adult rat adrenal gland. To note, expression of this message in adrenal tissue appeared to be under the regulation of homologous signals *in vitro*, as short-term incubation of adrenal samples in serum-free medium induced a significant increase ($P < 0.01$, ANOVA followed by Tukey's test) in GHS-R mRNA levels, that was inhibited by exposure to different doses of GHRP-6 (10^{-9} to 10^{-5} M) or ghrelin (10^{-7} M). In contrast, a strikingly different pattern of homologous regulation was observed in the pituitary and testis, where serum-free medium incubation decreased GHS-R mRNA levels, which were restored to basal *in vivo* values by stimulation with GHRP-6 and/or ghrelin.

In conclusion, our study provides novel evidence for the expression and regulation of the GHS-R gene in rat adrenal, and evidences clear-cut differences in the regulation of GHS-R mRNA expression by homologous signals between pituitary, testis and adrenal gland in the rat.

Tena-Sempere M *et al.* (2002). *Endocrinology* **143**, 717–723.

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All procedures accord with current National and local guidelines.

P158

A high tryptophan nocturnal meal increases activity in rats

S. Esteban, S. Aparicio, M. Comas, C. Garau, C. Roca, S. Tejada, A. Gamundi and M.C. Nicolau

Laboratori de Fisiologia, Universitat de les Illes Balears, 07071 Palma de Mallorca, Spain

Tryptophan is the precursor in the synthesis of serotonin (5-HT) and melatonin. It has been shown that the increase in brain tryptophan and/or in 5-hydroxytryptophan (5-HTP) causes a parallel increase in slow wave sleeping time. It is also well know that melatonin synthesis occurs only during night time and causes marked increases in motor activity in rats. Thus, two opposite effects could be expected after a meal with high tryptophan contents.

To elucidate this question, fourteen 200–240 g male Wistar rats were kept individually housed in cages equipped for activity recording and submitted to a 1 h–23 h L–D photoperiod (lights on at 08.00 h) in an isolated, thermostated (22 ± 2 °C) chamber. After acclimatizing, the animals received three different diet schedules, each during three consecutive days: (1) commercial chow (0.2 % tryptophan) for the whole day; (2) chow enriched with 2 % tryptophan from 01.00 to 13.00 h, replaced by standard chow during the following period 13.00 to 01.00 h; and (3) standard chow from 01.00 to 13.00 h and tryptophan enriched one from 13.00 to 01.00 h. The experiments were performed under approval of the Ethical Committee of the University of Balearic Islands.

When the tryptophan was administered during night time a mild increase (17 ± 3 %, mean ± S.E.M.) in activity counts was observed, while no effect was found during day time. In another experiment, rats received either L-tryptophan (300 mg kg^{-1}) or vehicle (methylcellulose) by oesophageal cannula either at 08.30 or at 20.30 h. After nocturnal tryptophan administration, rats showed also increased activity (45 ± 5 %, 4 h after injection, $P < 0.05$, ANOVA), but again no effects after diurnal one. After these experiments, tryptophan (300 mg kg^{-1}) was administered through an oral cannula and we used the accumulation of 5-HTP (after decarboxylase inhibition with NDS 1015, 100 mg kg^{-1}, I.P.) as a measure of tryptophan hydroxylation rate in the rat brain *in vivo*. Animals were humanely decapitated 4 h later and their brain was homogenized for 5-HTP, 5-HT and 5-hydroxyindolacetic acid (5-HIAA) electrochemical HPLC detection. A diurnal cycle in tryptophan hydroxylase activity was observed. Tryptophan induced an increase in daytime 5-HTP (103 ± 16 % $P < 0.05$, ANOVA–Scheffé's test), 5-HT (78 ± 11 %, $P < 0, 05$) and 5HIAA (188 ± 31, $P < 0.01$), but this increase was much lower when administered at 20.30 h, in 5-HTP accumulation (36 %, n.s.), 5-HT content (19 %, n.s.) and 5-HAA (5 %, n.s.).

In summary, the results indicate that nocturnal tryptophan administration produces a short-term activation of motor activity in rats and this action could be mediated by melatonin.

This work was supported by a DGICYT grant AGL2000-0182-P4-03.

All procedures accord with current National and local guidelines.