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Programma

Lunedì 23-03-2009

13,00-13,45 Iscrizione, affissione di tutti i poster e PP delle comunicazioni orali

13,45-14,00 Apertura dei lavori

Comunicazioni Orali: Moderatori *Anna Spada e Roberto C.Melcangi*

14,00-14,15 **Biamonte F. et al.** INTERACTIONS BETWEEN NEUROACTIVE STEROIDS, AGE AND REELIN GENE DOSAGE REGULATE PURKINJE CELL SURVIVAL DURING DEVELOPMENT.

14,15-14,30 **Giatti S. et al.** Ro5-4864, A LIGAND OF TRANSLOCATOR PROTEIN-18kDa (TSPO), IS NEUROPROTECTIVE IN AN EXPERIMENTAL MODEL OF DIABETIC NEUROPATHY.

14,30-14,45 **Deledda C. et al.** NEUROTROPHIC EFFECTS OF EXENDIN-4 IN A NEURONAL CELL MODEL.

14,45-15,00 **Bo' E. et al.** NPY AND LEPTIN ARE TARGETS FOR TRIBUTYLTIN DURING DEVELOPMENT AND IN ADULTHOOD

15,00-15,15 **Busnelli M. et al.** IDENTIFICATION OF 'FUNCTIONAL SELECTIVE' OXYTOCIN/VASOPRESSIN COMPOUNDS USING A BRET TECHNIQUE

15,15-15,30 **Scicchitano B. et al**. MOLECULAR MECHANISMS REGULATING SKELETAL MUSCLE HOMEOSTASIS: EFFECTS OF V1a VASOPRESSIN RECEPTOR OVEREXPRESSION

15,30-15,45 **Petrocchi P. et al.** EFFECT OF VGF-DERIVED PEPTIDES, TLQP-21, ON HORMONE GENE EXPRESSION AND SECRETION IN GH3 CELL LINE

15,45-16,00 **Mostallino M.C. et al.** SYNAPTIC AND EXTRASYNAPTIC GABA RECEPTOR DURING PREGNANCY AND POST PARTUM

16,00-16,30 Caffè

16,30-17,30 Poster 1-8 Moderatori: *Roberta Possenti e Vittorio Locatelli*

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Cantalupo E. et al. CORTICOTROPIN RELEASING FACTOR (CRF) INHIBITS CELL GROWTH OF HUMAN NEUROBLASTOMA AND MEDULLOBLASTOMA CELL LINES VIA THE ACTIVATION OF CRF- RECEPTOR TYPE 1 AND P18INK4C mRNA INCREASE.

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Petrella C. et al. *IN VITRO* AND *IN VIVO* PHARMACOLOGICAL ROLE OF TLQP-21, A VGF-DERIVED PEPTIDE, IN THE REGULATION OF RAT MOTOR GASTRIC FUNCTIONS

Sornelli F. et al. NGF AND BDNF AS SIGNALLING MOLECULES IN THE ADIPOSE TISSUE OF STRESSED AND DIABETIC RODENTS

Petrella C. et al. ROLE OF THE CANNABINOID SYSTEM IN THE EXOCRINE PANCREAS

Martedì 24-03-2009

Comunicazioni Orali: Moderatori *Marcella Motta* e *Giancarlo Panzica*

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9,15-9,30 Campolongo P. et al. ENDOCANNABINOID IN THE RAT BASOLATERAL AMYGDALA ENHANCE MEMORY CONSOLIDATION AND ENABLE GLUCOCORTICOID MODULATION OF MEMORY

9,30-9,50 Calamandrei G. et al. BEHAVIOURAL AND NEUROENDOCRINE EFFECTS OF THE ORGANOPHOSPHOROUS INSECTICIDE CHLORPYRIFOS

9,50-10,10 Manni L. A ROLE FOR ACUPUNCTURE IN THE TREATMENT OF NEURO-ENDOCRINE DISEASES.

10,10-10,30 Parmigiani S. et al. PERSONALITY TRAITS AND ENDOCRINE RESPONSE AS POSSIBLE ASYMMETRY FACTORS OF AGONISTIC OUTCOME IN KARATE ATHLETES

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11,00-12,00 Lettura magistrale: Moderatori *Marcella Motta* e *Giancarlo Panzica*

Enrico Alleva. ANIMAL MODELS IN BEHAVIORAL NEUROENDOCRINOLOGY: STRENGHTS, LIMITS AND TRANSLATIONAL VALUE

12,00-13,30 Poster 9-22 Moderatori: *Gemma Calamandrei e Alessandro Peri*

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Marolda R et al. SP PROTECTS CEREBELLAR GRANULE CELLS AGAINST β –AMYLOID–INDUCED APOPTOSIS

De Marinis E. et al. A NEW ACTION OF 17β-ESTRADIOL IN NEUROBLASTOMA CELLS: THE OVER- EXPRESSION OF NEUROGLOBIN, AN ENDOGENOUS NEUROPROTECTANT

Amadoro G. et al. ENDOGENOUS AB CAUSES CELL DEATH VIA EARLY TAU HYPERPHOSPHORYLATION

Tirassa P. et al. MODULATION OF HIPPOCAMPAL PLASTICITY BY INDUCING NGF AND BDNF SYNTHESIS VIA ELECTRICAL STIMULATION OF CERVICAL VAGUS NERVE

Pesaresi M. et al. DIABETES SIGNIFICANTLY DECREASES THE mRNA LEVELS OF 18.5

kDa AND 21.5 kDa ISOFORMS OF MYELIN BASIC PROTEIN PRESENT IN THE SPINAL CORD AND TESTOSTERONE OR DIHYDROPROGESTERONE TREATMENT COUNTERACTS THIS EFFECT

Manciocco A. et al. BEHAVIOURAL AND NEUROSTEROID BASED ANALYSIS TO MONITORING WELFARE STATE IN FARM PIGS RAISED IN AN ENRICHED ENIVIRONMENT

Romano E. et al. HETEROZYGOUS REELER MICE EXHIBIT RESCUE OF BEHAVIOURAL PHENOTYPE FOLLOWING NEONATAL TREATMENT WITH ESTRADIOL

Panelli R. et al. AROMATASE BLOCKAGE IN PREPUBERTAL MALE RATS BY ANASTROZOLE: EFFECTS ON SOCIAL PLAY

Gioiosa L. et al. EFFECTS OF LOW DOSES OF BISFENOL-A AND ALFA-ETHINYLESTRADIOL ON PARENTAL AND REPRODUCTIVE BEHAVIOUR IN MALE AND FEMALE MICE

Miceli D. et al. PERINATAL EXPOSURE TO BISPHENOL-A AFFECT SEXUAL BEHAVIOR IN ADULT MALE AND FEMALE MOUSE

D'andrea I. et al. COMMUNAL NESTING, AN EARLY SOCIAL ENRICHMENT, SHAPES SOCIAL BEHAVIOR AND COPING RESPONSE TO SOCIAL STRESS IN ADULT MOUSE

Macrì S. et al. NEONATAL MILD AND SEVERE STRESS RESPECTIVELY RELATE TO ADULT RESILIENCE AND VULNERABILITY IN MICE.

Berry A. et al. GREATER RESISTANCE TO OXIDATIVE STRESS AND TO STRESSFUL STIMULI IN THE P66SHC-/- MOUSE, A MODEL OF DELAYED AND HEALTHY AGING

13,30-14,30 Pranzo

14,30-15,30 Assemblea GISNe e Chiusura lavori

http://www.dafml.unito.it/gisne/index.html

Lunedì 23 Marzo

Presentazioni orali

INTERACTIONS BETWEEN NEUROACTIVE STEROIDS, AGE AND REELIN GENE DOSAGE REGULATE PURKINJE CELL SURVIVAL DURING DEVELOPMENT.

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It has been known for some time that the *reeler* mutation in the heterozygous state causes a loss of Purkinje cells (PCs) in the adult mouse cerebellum. This PC loss is more evident in male than female mice. Using stereological analysis we have assessed total PC numbers in the cerebella of wild type (+/+), heterozygous (rl/+) and reeler (rl/rl) male and female mice during early postnatal development. PC numbers are reduced in rl/+ males at postnatal day 15, with no parallel loss of granule cells or inferior olivary neurons. Early postnatal administration of the estrogen receptor (ER) agonist 17β-Estradiol (17b-E) in the cisterna magna increases PC numbers in male rl/+ but has no effect in female rl/+ or male/female +/+ mice; conversely, administration of the ER antagonists 4-OH-Tamoxifen or ICI 182,780 selectively reduce PC numbers in female +/+ and rl/+, but has no effect in male rl/+ or +/+ mice. We hypothesize that PC loss in the male rl/+ may be due to an imbalance of cerebellar testosterone (T) and/or 17b-E metabolism, since at P5 rl/+ male cerebella compared to +/+ males show increased levels of T and 17b-E, associated with decreased levels of dihydrotestosterone. Decreased reelin function and neuroactive steroids imbalance may thus interact during neurodevelopment to influence PC survival. Our results thus support the male rl/+ mouse as a model of developmental interaction between genetic vulnerability and sex hormone levels on PCs, which could be relevant for neurodevelopmental disorders of genetic origin that are characterized by gender differences and cerebellar involvement.

This work was supported by grants from and from NAAR-AUTISM SPEAKS (Grant number 4919) and from the Fondation Jerôme Lejeune.

Ro5-4864, A LIGAND OF TRANSLOCATOR PROTEIN-18kDa (TSPO), IS NEUROPROTECTIVE IN AN EXPERIMENTAL MODEL OF DIABETIC NEUROPATHY.

Giatti S. [1#], Pesaresi M. [1], Maschi O. [2], Bianchi R. [3], Cavaletti G. [4], Caruso D. [2], Melcangi R.C. [1]

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An important complication of diabetes is the peripheral neuropathy, which involves a spectrum of structural, functional and biochemical alterations in peripheral nerves [1]. Slowing in nerve conduction velocity (NCV) and reduction of Na^+,K^+-ATPase activity are early disorders of nerve function that can be caused by hyperglycemia, impairment in neurotrophic support, increase in ROS production, etc. Moreover, recent evidences obtained in our laboratory have shown that the levels of neuroactive steroids are reduced in sciatic nerve of rat raised diabetic by a single injection of streptozotocin (STZ) [3,7]. These observations are in agreement with the protective effects exerted by neuroactive steroids, like progesterone (PROG), testosterone (T) and their derivatives in experimental model of diabetic neuropathy induced by STZ. It is ascertain that the treatment with neuroactive steroids is able to improve thermal sensitivity, nerve conduction velocity and Na^+,K^+-ATPase activity, impaired in STZ rats. Moreover, diabetes significantly reduces the expression of important myelin protein, such as glycoprotein zero and peripheral myelin protein 22, and neuroactive steroids are able to counteract this reduction and consequently improve the morphological integrity of myelin membranes [5,8]. On this basis, an interesting therapeutic strategy could be to increase the levels of neuroactive steroids directly in the nervous system, avoiding in this way, possible endocrine side effects exerted by these molecules. With this perspective, ligands of translocator protein-18 kDa (TSPO) may represent an interesting option. TSPO is mainly present in the mitochondrial outer membrane; it promotes the translocation of cholesterol to the inner mitochondrial membrane [4] where resides the enzyme cytochrome P450 side chain cleavage that transforms cholesterol into pregnenolone (PREG), with a subsequent increase of steroid levels [2,6]. On this basis, in the diabetic model of STZ rat, we have assessed whether a chronic treatment with a TSPO ligand, such as Ro5-4864, could increase the low levels of neuroactive steroids observed in sciatic nerve and consequently to be protective in this experimental model. Data obtained by liquid chromatography-tandem mass spectrometry show that the treatment with Ro5-4864 is able to significantly stimulate the levels of PREG and PROG reduced by diabetes in the sciatic nerve. Interestingly, we also observed protective effects exerted by Ro5-4864. In particular, the treatment with this TSPO ligand is effective in counteracting the decrease of NCV and Na^+,K^+-ATPase activity, and is able to reduce thermal threshold, impaired in STZ rats. Altogether, the data here reported show for the first time that a TSPO ligand is effective in reducing the severity of diabetic neuropathy through a local increase of neuroactive steroid levels.

REFERENCE LIST:

[1] Biessels GJ, Cristino NA, Rutten GJ, Hamers FP, Erkelens DW, Gispen WH. Neurophysiological changes in the central and peripheral nervous system of streptozotocin-diabetic rats. Course of development and effects of insulin treatment. Brain. 1999 Apr;122 (Pt 4):757-68.

[2] Brown RC, Papadopoulos V. Role of the peripheral-type benzodiazepine receptor in adrenal and brain steroidogenesis. Int Rev Neurobiol. 2001;46:117-43. Review.

[3] Caruso D, Scurati S, Maschi O, De Angelis L, Roglio I, Giatti S, Garcia-Segura LM, Melcangi RC. Evaluation of neuroactive steroid levels by liquid chromatography-tandem mass spectrometry in central and peripheral nervous system: effect of diabetes. Neurochem Int. 2008 Mar-Apr;52(4-5):560-8.

[4] Casellas P, Galiegue S, Basile AS. Peripheral benzodiazepine receptors and mitochondrial function. Neurochem Int. 2002 May;40(6):475-86. Review.

[5] Leonelli E, Bianchi R, Cavaletti G, Caruso D, Crippa D, Garcia-Segura LM, Lauria G, Magnaghi V, Roglio I, Melcangi RC. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. Neurosci 2007; 144:1293-1304.

[6] Papadopoulos V, Amri H, Li H, Yao Z, Brown RC, Vidic B, Culty M. Structure, function and regulation of the mitochondrial peripheral-type benzodiazepine receptor. Therapie. 2001 Sep-Oct;56(5):549-56. Review.

[7] Roglio I, Giatti S, Pesaresi M, Bianchi R, Cavaletti G, Lauria G, Garcia-Segura LM, Melcangi RC. Neuroactive steroids and peripheral neuropathy. Brain Res Rev. 2008 Mar;57(2):460-9. Epub 2007 May 6. Review.

[8] Roglio I, Bianchi R, Giatti S, Cavaletti G, Caruso D, Scurati S, Crippa D, Garcia-Segura LM, Camozzi F, Lauria G, Melcangi RC. Testosterone derivatives are neuroprotective agents in experimental diabetic neuropathy. Cell Mol Life Sci. 2007 May;64(9):1158-68.

NEUROTROPHIC EFFECTS OF EXENDIN-4 IN A NEURONAL CELL MODEL.

Deledda C[1]., Luciani P.[1], Benvenuti S.[1], Cellai I.[1], Squecco R.[3], Monici M.[2], Luciani G.[3], Cialdai F. [2], Francini F.[3], Peri A.[1]

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Glucagon-like peptide-1 (GLP-1) is an endogenous insulinotropic peptide secreted from the gastrointestinal tract in response to food intake. It enhances glucose-dependent insulin secretion, and lowers blood glucose and food intake in patients with type 2 diabetes mellitus (T2DM). Exendin-4 (EXE) is a long-acting GLP-1 receptor (GLP-1R) agonist and the first of the new class of drugs approved to treat T2DM. GLP-1Rs are also expressed in the nervous system where receptor activation elicits neurotrophic actions. Moreover, effects on neuronal proliferation and differentiation have been previously reported in the rat pheochromocytoma cell line PC12. The aim of this study was to investigate, in the human neuroblastoma cell line SY5Y, whether EXE was able 1) to induce differentiation and 2) to protect from oxidative insults.

In SY5Y we detected GLP-1R mRNA expression and we demonstrated that this receptor is functionally active. To investigate differentiation, morphological changes induced by EXE were determined. A well known differentiating agent, i.e. retinoic acid (RA), was used as control. 0.3µM EXE-treatment (24h) determined a significant increase in the number of neurite-like protrusions compared to RA, but their length was lower. Fluorescent immunostaining analysis performed by epifluorescent-microscopy revealed dramatic changes in intracellular actin and tubulin distribution that were more evident in EXE treated cells. However, the electrophysiological characterization, achieved by the whole cell patch clamp technique both in current- and voltage-clamp mode, showed that 48-hr EXE treatment doesn't cause a substantial change in membrane resting potential and resistance respect to untreated cells, whereas RA appears more effective in causing a resting membrane depolarization and a decrease in membrane resistance. In addition, cell surface areas estimated by measuring cell capacitance were not significantly different after 48 hr EXE treatment, whereas RA caused a significant increase. These results suggest that the differentiating effects of EXE might be limited to the early steps of neuritogenesis. With regard to neuroprotection, EXE treatment protected SY5Y cells against H_2O_2 induced apoptosis as assessed both by trypan blue test and MTS assay and counteracted the activation of caspase-3. An important actor in the polimerization of actin is cofilin, which has been also reported to be involved in the early steps of apotosis. Western blot analysis of total and phosphorylated (i.e. inactivated) cofilin revealed that EXE treatment induced a shift toward the inactivated form in agreement with its modulation property on apoptosis.

In summary our results suggest that EXE exerts neurotrophic activity in a human neuronal model, possibly promoting early neuritogenic events. Moreover our data confirm the neuroprotective actions elicited by EXE, that are paralleled by an important cytoskeleton rearrangement in which cofilin might play a central role.

NPY AND LEPTIN ARE TARGETS FOR TRIBUTYLTIN DURING DEVELOPMENT AND IN ADULTHOOD

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Several environmental chemicals, collectively referred to as 'endocrine disrupting chemicals' (EDCs), can interfere with complex endocrine signalling pathways and cause adverse consequences. Tributyltin (TBT) belongs to a family of pesticides used in antifouling paints for ship hulls and toxic to fish, molluscs and other aquatic organisms. TBT can act as xenoandrogen inducing virilization of prosobranch females by imposex development and a marked decrease of fecundity. Recent studies have proposed that TBT may act as obesogen, activating RXR and PPAR receptors and inducing adipogenetic genes synthesis for adipocites diffrentiation.
The aim of this study was to test the hypothesis that exposure to TBT can alter not only the peripheral deposition of fat, but also the central mechanisms controlling animal food intake. Therefore, we used an experimental mouse model of adult or perinatal TBT exposure. TBT, diluted in olive oil, was orally administered at a dose of 0,025µg/g/day/body weight to adult animals (male and female mice of C57/BL6 strain) for 4 weeks or to pregnant females from gestational day 8 (G8) to pups postnatal day 21 (P21), day of pups' sacrifice. The animals were perfused with paraformaldehyde 4% in phosphate buffer. Brains were dissected and frozen for further immunocytochemical analysis, whereas the body fat was removed and weighted.
TBT-treated adult of both sexes show statistically significant reduction of food intake in comparison to controls, but no differences were found in body weight and fat deposition. In addition, in treated animals we observed a great reduction of blood leptin levels compared to controls, probably due to the androgenic action of TBT. Therefore these results suggest the induction of an indirect obesogenic effect: treated animals show a reduction in food intake but the body weight doesn't change and blood leptin levels collapse in comparison to controls. This effect indicates a possible alteration of brain circuits controlling food intake, perhaps directly linked to the androgenic nature of TBT.
In pups, treated pre- and postnataly, we observed a significant reduction in body weight in comparison to controls and high leptin levels at P21. Again, we have here a result that can be interpreted as due to alteration of homeostatic mechanisms that can influence central nervous circuits. Finally these results agree with recent studies demonstrating that newborn male rats treated with leptin may develop leptin resistance and an increase of body weight in adult life.
Brain sections were stained for NPY immunocytochemistry. The NPY is one of the main controllers of neural circuits dedicated to food assumption. The main cluster of NPY positive elements is located within the arcuate (ARC) nucleus and project to several nuclei, including the paraventricular nucleus (PVN) and the dorsomedial nucleus (DM). Due to the decrease of circulating levels of leptin, in adult TBT-treated animals we expected a parallel increase in NPY immunoreactivity. However, the computerized quantitative analysis of NPY immunoreactivity distribution demonstrated a statistically significant reduction of NPY expression in PVN, DM, and Arc nuclei of treated-male mice in comparison to controls. Therefore, also these results are indicating that adult exposure to TBT is destroying the normal regulation of nervous circuits involved in the control of food intake.

Acknowledgement This work was supported by Region Piemonte and University of Turin.

IDENTIFICATION OF 'FUNCTIONAL SELECTIVE' OXYTOCIN/VASOPRESSIN COMPOUNDS USING A BRET TECHNIQUE

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The human oxytocin receptor (OTR) is a promiscuous receptor functionally coupled to various G-proteins ($G\alpha_{q\text{-}11}$, $G\alpha_i$, and $G\alpha_h$). The multiple receptor coupling is particularly relevant because it may results in the activation, in the same cell, of several signalling pathways. Moreover, specific ligands may possess different intrinsic efficacy on the different signalling pathway, a phenomenon referred to as "agonist-directed trafficking of receptor stimulus", "biased agonism" or "functional selectivity". The existence of these analogues is consistent with a multistate model of receptor activation in which ligands can induce specific receptor conformations capable of differentially promoting the coupling of a single receptor to different G-proteins.

To explore the 'coupling specificity' of OTR we performed a BRET assay measuring G protein activation, in which the energy transfer occurs between the human G proteins alpha subunits ($G\alpha_q$ and $G\alpha_{i1}$) fused to Renilla Luciferase and the γsubunit of the hetherotrimeric G-proteins fused to the GFP10 and we tested a series of peptidic OT analogues (agonists and antagonists).

Our preliminary results indicate that the agonists cause a *decrease* in BRET in cells expressing $G\alpha_q$ or $G\alpha_{i1}$, confirming that the OTR is functionally coupled to both G□ subunits. On the contrary, when we tested several $G\alpha_q$ antagonists, we found that they produced an *increase* in the BRET ratio in cells expressing $G\alpha_q$, suggesting different rearrangements of the OTR/G protein complex with respect to that of produced by agonists.

These results indicate that this G protein BRET assay can be successfully used to screen for OTR agonists and antagonists coupling selectivity.

MOLECULAR MECHANISMS REGULATING SKELETAL MUSCLE HOMEOSTASIS: EFFECTS OF V1a VASOPRESSIN RECEPTOR OVEREXPRESSION

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The neurohypophyseal nonapeptide arg-vasopressin (AVP) and related peptides constitute a novel family of positive regulators of terminal differentiation of myogenic cell lines and primary satellite cells (1, 2, 3). By interacting with V1a type receptor (4), AVP induces activation of phospholipases C and D, increases cytosolic Ca^{2+} concentrations, regulates cAMP levels by activating type IV cAMP specific phosphodiesterase, and up-regulates Myf-5 and myogenin expression (3,5,6,8). In a chemically defined medium, which eliminates the interference of serum components, AVP activates CaMK and calcineurin signalling pathways (9,10). The stimulation of the CaMK pathway induces the cytosolic compartmentalization of histone deacetylase 4, which in turn activates the transcription of muscle specific genes. The activation of calcineurin is responsible for the dephosphorylation of the NFAT transcription factor and its translocation into the nucleus (10). The combined activation of both pathways by AVP results in the formation of multifactor complexes on the promoter of muscle specific genes, stimulates muscle differentiation and is required for the full expression of the differentiated and hypertrophic phenotype (10).

To better clarify the physiological role of AVP in skeletal muscle, we analyzed the AVP effects on muscle regeneration induced after a cardiotoxin–induced experimental lesion. Modulation of the expression of the endogenous V1aR was observed during regeneration, suggesting a role for AVP signaling in this process. To increase skeletal muscle sensibility to circulating AVP, in the absence of systemic effects related to the administration of the hormone itself, we over-expressed a construct carrying the V1a AVP receptor under control of a muscle –specific promoter (MLC-V1aR) in mouse tibialis anterior muscle by electroporation-mediated gene delivery *in vivo*. The local over-expression of the MLC-V1aR in injured muscle results in enhanced regeneration. V1aR over-expressing muscle exhibits: early activation of satellite cells and regeneration markers, accelerated differentiation, increased cell population expressing hematopoietic stem cell markers and its conversion to the myogenic lineage. We demonstrate that V1aR over-expressing muscle increases calcineurin and IL-4 expression levels, and induces the phosphorylation of FOXO trascription factors, inhibiting the expression of atrophic genes. This study highlights a novel *in vivo* role for the AVP-dependent pathways which may represent a potential gene therapy approach for many diseases affecting muscle homeostasis.

1. Teti A, et al. 1993 *Am J Physiol* 265 (C34):C113–C121
2. Nervi C, et al. 1995 *Cell Growth Diff* 6:81–89
3. Minotti S, et al. *Cell Growth Diff* 9:155–164
4. Alvisi M, et al. *Differentiation* 2008; 76 (4): 371-380.
5. Naro F, et al. 1997 *J Cell Physiol* 171:34–42
6. Naro F, et al.1999 *Mol Biol Cell* 10:4355–4367
7. Coletti D, Silvestroni L, Naro F, Molinaro M, Adamo S, Palleschi S *2000 Exp Cell Res* 256:94–104
8. Nervi C, et al. 1995 Cell Growth Diff 6:81–89
9. Scicchitano BM et al. *Mol Biol Cell*. 2005 Jun 1
10. Scicchitano B.M.et al. *Mol.Endocrinology* 16:1407-1416,2002

EFFECT OF VGF-DERIVED PEPTIDES, TLQP-21, ON HORMONE GENE EXPRESSION AND SECRETION IN GH3 CELL LINE

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Vgf (nonacronymic), originally identified as a nerve growth factor responsive gene, is expressed in neurons within the central and peripheral system and in various endocrine cells. Vgf encodes for a 617 amino acid protein in rat and mouse, and 615 in human. Specific prohormone convertase PC1/3 and PC2 cleave the precursor protein bearing a number of peptides that are stored in dense core granules and secreted through the regulated pathway (Levi 2004).
VGF is highly expressed in female rat pituitary gonadotrope and lactotrope cells. The level of vgf expression changes during lactation in hypothalamic region (Mahata 1993), changes in rat pituitary gland through the estrous cycle phases (Ferri 1996) and during the reproductive different seasons in female sheep (Brancia 2005).
Recently, we have identified and characterized in rat brain extracts, a new VGF-derived peptide designated as TLQP-21. This peptide is present in the rat brain and exerts different biological activity in vivo: from regulation of energy homeostasis increasing energy expenditure (Bartolomucci 2006) and gastroenteric function (Severini in press) to protection of primary cultures of rat cerebellar granule cells from neurotoxin effect induced by serum and potassium deprivation (Severini 2008) and inflammatory pain modulation with an increase in licking response following peripheral injection of TLQP-21 (Rizzi 2008).
We have investigated as in vitro model the GH3 cell line, a rat tumour cell line that present somatotrope and lactotrope phenotype that in presence of EGF and Estrogens inhibit GH expression and stimulates PrL expression and secretion.
GH_3 cell line treated for 6 days with Epidermal Growth Factor (EGF), increased prolactin and VGF gene expression during differentiation of this cell line toward the mammotrope phenotype, as demonstrated by immunochemical and western blot analysis. We have evaluated the possible paracrine effect of VGF derived peptide. We show that TLQP-21 increased GH_3 proliferation and differentiation by MTT proliferation assay and morphological analysis. It increases the prolactin expression without modifying GH expression as demonstrated by rtPCR and western blots. Although no activation of classic MAPK was evidenced, TLQP-21 induced a significant increase in intracellular calcium, as measured by FURA-2AM. Taken together, the present results demonstrated that TLQP-21 contributes to mammotrope differentiation of GH_3 cell line.. Preliminary results shows that in vivo rtPCR of pituitary gland from control, pregnant and lacting female rat increases VGF expression during lactation. Further experiments are in progress to evaluate the TLQP-21 activity in primary cell culture of pituitary cells. In conclusion, we hypothesize that TLQP-21 could be exert in pituitary gland a neuroendocrine role directed on lactotrope cells.

Bartolomucci et al. PNAS 103:14584-14589, 2006
Brancia et al. J of Endocrinol. 186:97-107, 2005
Ferri et al. Endocrinology136:2244-2251, 1995
Levi et al. Cell Mol Neurobiol 24:517-533, 2004
Mahata et al. Journal Neuroendocrinol. 5:323-330, 1993
Severini et al. J of Neurochem 104:534-544, 2008

SYNAPTIC AND EXTRASYNAPTIC $GABA_A$ RECEPTOR DURING PREGNANCY AND POST PARTUM

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Pregnancy is a mammalian process characterized by changes in mood and anxiety levels as well as marked fluctuations of plasma and brain hormonal levels [1]. Previous studies demonstrated that, in pregnant rats, the increase of neuroactive-steroid (NS) concentrations are accompanied by plastic changes of the expression of specific subunits of the gamma-aminobutyric acid type A (GABA-A) receptors in rat brain [1-3]. In the hippocampus, granule cells of the dentate gyrus (DG) exhibit two components of inhibitory GABAergic transmission that together regulate the excitability of this brain region: a phasic component, mediated by synaptic GABA-A receptors composed of alpha, beta, and gamma2 subunits, and a tonic component, highly sensitive to NS, that is mediated by ambient GABA acting on extrasynaptic GABA-A receptor composed by alpha4, beta, and delta subunits (4). Aim of this work was to evaluate whether these two components of the GABAergic transmission might be altered during pregnancy and immediately after delivery. Whole cell patch clamp recordings of GABAergic currents were obtained in the voltage clamp mode from hippocampal slices prepared from rats in estrus (E), at day 15 (P15) or 19 (P19) of pregnancy, or at 2 days after delivery. Phasic and tonic GABAergic currents were recorded from both DG granule cells and CA1 pyramidal neurons. In DG granule cells, the application of exogenous GABA or the neuroactive steroid 3alpha,5alpha-THP induced an increase of tonic current that was significantly greater at P19 than in estrus. This effect was completely reversed 2 days after delivery. Neither tonic nor phasic currents were affected by pregnancy or after delivery in CA1 pyramidal cells. Immunohistochemical analysis revealed a marked increase in the abundance of the delta subunit of the GABA-A receptors and a concomitant decrease in that of the gamma2 subunit in the hippocampus at P19 with respect to estrus. In contrast, 2 days after delivery was associated with a pronounced reduction in the abundance of delta subunit and an enhancement of that of gamma2 subunit. Moreover, the expression of the alpha4 subunit did not change during pregnancy but was increased 2 days after delivery. Treatment of rats from P12 to P18 with the 5alpha-reductase inhibitor finasteride, but not clomiphene citrate, prevented the changes in tonic current and in delta and gamma2 subunit expression normally apparent at P19.These data suggest that the number of extrasynaptic GABA-A receptors is increased in DG granule cells during late pregnancy as a consequence of the associated marked fluctuations in the brain levels of neuroactive steroids. This regulation of extrasynaptic GABA-A receptor-mediated tonic current may in turn be crucial for the changes in mood and for the anxiolytic effect associated with pregnancy [5] as well as for the arousal state typical of the period immediately preceding delivery and early postpartum.

Rerefence List

1. Concas et al., PNAS (USA), 95:13284-13289, 1998.
2. Brussaard et al., Neuron, 19: 1103-1114, 1997.
3. Follesa et al., Eur. J. Neurosci.: 10:2905-2912, 1998.
4. Farrant and Nusser, Nat Rev Neurosci 6:215-229, 2005.
5. de Brito Faturi et al., Pharmacol Biochem Behav 85:569-574, 2006.

Martedì 24 Marzo

Presentazioni orali

DEVELOPMENT OF A MOUSE MODEL OF DEPRESSIVE DISORDERS COMBINING NEONATAL STRESS AND DIETARY L-TRYPTOPHAN DEPLETION

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Depression, a pathology characterised by mood and neuro-vegetative disturbances, depends on a multi-factorial contribution of individual predisposition and environmental factors. Most of present mouse models of depression are devalued due to the fact that – in order to induce depressive-like symptoms – they rest only on one pathogenetic factor, be the latter genetic or environmental, thereby holding limited construct validity (they fail to mimic the pathology in its etiology). In order to overcome these limitations, we developed a novel mouse model in which the genetic (reduced serotonin levels during the early stages of postnatal life) and the environmental (early-life stress mimicked through neonatal corticosterone administration, isomorphic to maternal neglect) components of depression are mimicked. Our aim was to demonstrate that these manipulations, in mice, contribute to the emergence of neuro-behavioral abnormalities resembling human depression. The early environmental component is mimicked through the supplementation of corticosterone in the maternal drinking water while the genetic/innate factor is mimicked through maternal access to an L-tryptophan deficient diet. This diet reduces circulating levels of serotonin (one of the core etiological factors in depression) through the removal of its precursor (L-tryptophan).

Four groups of CD1 mouse dams (N=12-13 per group) were exposed to Animal Facility Rearing conditions (AFR group), given access to the L-tryptophan restricted diet between postnatal day (P)0-8 (T group), to corticosterone dose between P1-8 (C group) or both (TC group). We evaluated the short-term effects of these manipulations on maternal care through a detailed ethogram (3 daily, 75-min sessions, between P0-10) and their long-term effects on anxiety- and depressive-like behaviour in adolescent and adult offspring. To assess offspring emotionality, the following behavioural paradigms were used: novelty seeking, aimed at addressing the preference for a novel environment over a familiar one; elevated O-maze, evaluating the exploration of an environment imposing on the animal an approach-avoidance conflict; forced swimming test, measuring the willingness to escape an aversive inescapable situation.

Active maternal care steadily declined in all groups throughout lactation and was significantly higher in AFR dams compared to C, T and TC dams. Thus, AFR dams displayed significantly more active nursing (arched back nursing and licking) than all treated dams. Time-budget wise, while T dams showed more activity out of the nest, C and TC dams showed increased resting time. Pups' body weight increased steadily in all groups between P 11-23. However, C and TC pups were significantly lighter than both T and AFR.

Novelty seeking: All subjects showed a preference for the novel compartment; such preference steadily declined throughout the session in all groups. However, the preference for the novel compartment declined more rapidly in TC subjects compared to the other groups. Additionally, whereas all subjects showed a preference for the novel compartment throughout the entire session, TC subjects showed no such preference during the last five minutes of observation. Elevated 0-maze: Although differences failed to reach statistical significance, T adult subjects showed a tendency towards reduced time spent in the open sectors of the 0-maze compared to AFR subjects, which were in turn indistinguishable from C and TC subjects. Forced swimming test: As expected, mice showed an increment in the time spent floating as the session progressed. However, such increment varied depending on age and treatment. Specifically, whereas adolescent C and TC mice showed reduced floating compared to both AFR and T individuals, adult T subjects spent more time in floating behavior compared to AFR and TC mice.

The observation that a combination of reduced serotonergic tone and increased early stressors (be the latter in the form of circulating corticosterone, reduced maternal care or both) results in heightened emotionality supports the notion that these two factors contribute to induce disturbances isomorphic to human depression. The route of administration and the possibility to control the independent variables predisposing to depressive-like symptoms disclose novel avenues towards the development of valid animal models.

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ENDOCANNABINOIDS IN THE RAT BASOLATERAL AMYGDALA ENHANCE MEMORY CONSOLIDATION AND ENABLE GLUCOCORTICOID MODULATION OF MEMORY

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Extensive evidence indicates that the basolateral complex of the amygdala (BLA) modulates the consolidation of memories for emotionally arousing experiences, an effect that involves the activation of the glucocorticoid system. As the BLA expresses high densities of cannabinoid CB1 receptors, the present experiments investigated whether the endocannabinoid system in the BLA influences memory consolidation and whether glucocorticoids interact with this system. The CB1 receptor agonist WIN55,212-2 (5-50 ng/0.2 μL per side), infused bilaterally into the BLA of male Sprague-Dawley rats immediately after inhibitory avoidance training, induced dose-dependent enhancement of 48-h retention. Conversely, the CB1 receptor antagonist AM251 (0.07-0.28 ng/0.2 μL per side) administered posttraining into the BLA induced inhibitory avoidance retention impairment. Furthermore, intra-BLA infusions of a low and non-impairing dose of AM251 (0.14 ng/0.2 μL per side) blocked the memory enhancement induced by concurrent administration of WIN55,212-2. Delayed infusions of WIN55,212-2 or AM251 administered into the BLA 3 h after training or immediate posttraining infusions of these drugs into the adjacent central amygdala did not significantly alter retention performance. Lastly, intra-BLA infusions of a low and otherwise non-impairing dose of AM251 (0.14 ng/0.2 μL per side) blocked the memory-enhancing effect induced by systemic administration of corticosterone (3 mg/kg, sc). These findings indicate that endocannabinoids in the BLA enhance memory consolidation and suggest that CB1 activity within this brain region is required for enabling glucocorticoid effects on memory consolidation enhancement.

BEHAVIOURAL AND NEUROENDOCRINE EFFECTS OF THE ORGANOPHOSPHOROUS INSECTICIDE CHLORPYRIFOS

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Prolonged exposure to environmental contaminants at apparently non-toxic doses might represent a major risk factor for delayed adverse effects on neurobehavioral and neuroendocrine functions. Chlorpyrifos (CPF), one of the most widely used organophosphorous insecticide, elicits developmental neurotoxicity at doses well below the threshold for systemic toxicity, exerting subtle but disruptive effects on neural cell development (1). In the past years we have studied extensively the neurobehavioural effects of developmental exposure to CPF in the mouse species, focusing on end points related to social behaviours in adolescence and adulthood. In male mice prenatal and neonatal exposure to CPF enhanced reactivity to novel environmental cues and had pro-aggressive effects at adolescence and adulthood in a social interaction test. In adult females, prenatal CPF increased social responsiveness, while neonatal CPF enhanced maternal responding towards foster pups. In a recent study we show that neonatal exposure to CPF alters maternal behaviour profile and reduces motivation to build and defend the nest from a male intruder (2,3,4). The behavioural changes induced by developmental CPF occur in the absence of significant inhibition of brain acetylcholinesterase, and are paralleled by permanent alteration in the expression of hypothalamic neuropeptides: specifically, oxytocin levels are enhanced in both sexes, vasopressin levels are decreased in males, whereas no effects of CPF are evident as for prolactin expression (5). A recent study has also shown that this same protocol of exposure to CPF also alters thyroid morphology and levels of T3 and T4 hormones at adulthood (6). These results confirm that developmental exposure to CPF induces long-lasting alterations in sexual-dimorphic responses of the rodent social repertoire, possibly by interfering with hypothalamic neuroendocrine mechanisms regulating social and maternal responses. As recent epidemiological studies show that organophosphorous pesticides, and CPF in particular, might alter neuropsychological maturation of exposed human infants (7), the effects so far reported in animal models suggest that neuroendocrine mechanisms might be potential targets for these neurotoxic compounds in humans too.

1. Dam K, Seidler FJ, Slotkin TA..*Brain Res Dev Brain Res* 1999; 116:9-20.
2. Ricceri L, Markina N, Valanzano A, Fortuna S, Cometa MF, Meneguz A, Calamandrei G.. *Toxicol Appl Pharmacol.* 2003 Sep 15;191(3):189-201.
3. Ricceri L, Venerosi A, Capone F, Cometa MF, Lorenzini P, Fortuna S, Calamandrei G. *Toxicol Sci.* 2006;93(1):105-13
4. Venerosi A, Cutuli D, Colonnello V, Cardona D, Ricceri L, Calamandrei G. *Neurotoxicol Teratol.* 2008 30(6):468-74.
5. Tait S, Ricceri L, Venerosi A, Maranghi F, Mantovani A, Calamandrei G *Environ Health Perspect.* 2009; 17: 112-6.
6. De Angelis S, Tassinari R, Maranghi F, Chiarotti F, Ricceri L, Venerosi Pesciolini A, Gilardi E, Moracci G, Calamandrei G, Olivieri A, Mantovani A *Toxicol Sci*. 2009, in press.
7. Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. *Pediatrics*. 2006;118(6):1845-59.

A ROLE FOR ACUPUNCTURE IN THE TREATMENT OF NEURO-ENDOCRINE DISEASES.

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Acupuncture is a therapeutic technique, part of the Traditional Chinese Medicine, a system with empirical basis, which has been used in the treatment and prevention of diseases for centuries in China and more recently also in Western Countries. It has been suggested that manual acupuncture (MA) or electro-acupuncture (EA), with repetitive muscle contraction, results in the activation of physiological processes similar to those resulting from physical exercise [1]. Both acupuncture and physical exercise release endogenous opioids, though other neurotransmitter and neuro-modulator systems may be involved in the described acupuncture action on stress, pain, neuro-endocrine and autonomic activity and immune systems [2]. It has recently been proposed that at least some of the effects attributed to acupuncture are mediated by neurotrophic factors, such as the nerve growth factor (NGF).

Specific data on the correlation between NGF and acupuncture comes from our work on experimental models of polycystic ovary (PCO) in rat [3-6] and more recently on pain response [7]. PCO syndrome (PCOS) is a common condition characterized by menstrual abnormalities and features of hyperandrogenism, with a prevalence of 5-10% of women in their reproductive age [8]. It is a complex endocrine and metabolic disorder associated with ovulatory dysfunction, hyperandrogenism, abdominal obesity and insulin resistance. The syndrome is associated with peripheral and central factors that influence sympathetic nerve activity [9-11]. It has recently demonstrated that EA and physical exercise improve metabolic disturbance and modulate gene expression of sympathetic markers, with a parallel improvement of ovarian morphology in a model of rat PCOS [9, 10]. NGF is important in the ovarian physiology not only as a regulator of sympathetic innervation, but also in follicular function and development [12-14]. Both the local (ovary) and central (hypothalamic) levels of NGF are deregulated in rat PCO [11]. This support the hypothesis that the NGF/NGF receptor system expressed in the peripheral organ and in the CNS, in particular by hypothalamic neurons, can be active in the pathogenesis of EV-induced PCO.

We used the estradiol-valerate (EV) model of rat PCOS, that is characterized by the development of ovarian characteristic of the human syndrome together with hypertension and increased sympathetic and HPA axis activity. PCO and control rats were then with acupuncture or allowed to experience 5 weeks of voluntary physical exercise. We demonstrated that both acupuncture and physical exercise decreased the ovarian NGF and NGF-receptor content and modulated the ovarian adrenergic responsiveness, normalizing the expression of α_1 and β_2 adrenergic receptors. This molecular changes are associated with the improvement of ovarian morphology. Moreover, our preliminary data indicate that EA is able to modulate the NGF/NGF receptor content and the expression of the catecholamine biosynthesis enzyme tyrosine hydroxylase in the hypothalamus of healthy rodents. This suggest the possibility to affect neuro-endocrine activity and the sympathetic drive towards peripheral organs through the sensory stimulation achieved by means of EA.

In conclusion, experimental observations in rat models of PCOS suggest that both low-frequency EA and physical exercise might affect the neuro-endocrine milieu and the autonomic activity acting at local as well as at systemic levels. Our studies point to a role for NGF as a mediator of physical therapy effects on a major neuro-endocrine disease as well as a role for acupuncture in the regulation of NGF-mediated sensory and autonomic response.

References

1. Andersson, S*, et al.*, Med Hypotheses, 1995. **45**(3): p. 271-81.
2. Kaptchuk, TJ, Ann Intern Med, 2002. **136**(5): p. 374-83.
3. Manni, L*, et al.*, Am J Physiol Regul Integr Comp Physiol, 2006. **290**(3): p. R826-35.
4. Manni, L*, et al.*, Reprod Biol Endocrinol, 2005. **3**: p. 21.
5. Manni, L*, et al.*, Auton Neurosci, 2005. **118**(1-2): p. 79-87.
6. Manni, L*, et al.*, J Neuroendocrinol, 2005. **17**(12): p. 846-58.
7. Aloe, L*, et al.*, Neurosci Lett, 2008.
8. Diamanti-Kandarakis, E, Ann N Y Acad Sci, 1997. **816**: p. 177-93.
9. Manneras, L*, et al.*, Am J Physiol Regul Integr Comp Physiol, 2009.
10. Manneras, L*, et al.*, Endocrinology, 2008. **149**(7): p. 3559-68.
11. Stener-Victorin, E*, et al.*, J Neuroendocrinol, 2008. **20**(3): p. 290-8.
12. Dissen, GA*, et al.*, Endocrinology, 2000. **141**(12): p. 4736-50.
13. Dissen, GA*, et al.*, Endocrinology, 2000. **141**(3): p. 1073-1082.
14. Mayerhofer, A*, et al.*, Endocrinology, 1996. **137**(12): p. 5662-70.

PERSONALITY TRAITS AND ENDOCRINE RESPONSE AS POSSIBLE ASYMMETRY FACTORS OF AGONISTIC OUTCOME IN KARATE ATHLETES

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Individual variations of plasma levels of hormones testosterone (T) and cortisol (C), before (pre) and after (post) Kumite (real fight) and Kata (ritualized fight) were measured in male karate athletes and analysed in relation with the agonistic outcome (i.e. winning or losing the real fight) and personality trait measures. T and C increased only during Kumite contest and pre and post-competition C levels were higher in losers than winners. Losers showed higher levels of harm avoidance and anxiety as well as lower levels of novelty seeking than winners. Importantly, novelty seeking negatively correlates with pre C and the higher the level of risk assessment, of emotionality and insecurity indexes the higher the pre C level. In conclusion, personality traits might be an important factor of asymmetry between athletes influencing both the probability of winning or losing an agonistic interaction and the different anticipatory endocrine response to the incipient fight.

POSTERS

ENDOGENOUS AB CAUSES CELL DEATH VIA EARLY TAU HYPERPHOSPHORYLATION.

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Alzheimer's disease (AD) is characterized by Ab overproduction and tau hyperphosphorylation. We report that an early, transient and site-specific AD-like tau hyperphosphorylation at Ser262 and Thr231 epitopes is temporally and causally related with an activation of the endogenous amyloidogenic pathway that we previously reported in hippocampal neurons undergoing cell death upon NGF withdrawal (Matrone et al., 2008b). Such tau hyperphosphorylation, as well as apoptotic death, is (i) blocked by 4G8 and 6E10 Aḅantibodies or by specific b and /or g-secretases inhibitors; (ii) temporally precedes tau cleavage mediated by a delayed (6-12h after NGF withdrawal) activation of caspase-3 and calpain-I; (iii) under control of Akt-GSK3b–mediated signalling. Finally, we show that such site-specific tau hyperphosphorylation causes tau detachment from microtubules and an impairment of mitochondrial trafficking.

In conclusion, our results make available useful informations about the temporal sequence linking endogenous Ab, tau hyperphosphorylation/cleavage and apoptotic signalling in the same neuronal model under physiological conditions as seen in the AD brain. In addition, the present work provides a further elucidating step in this scenario whereby, although Aḅpeptide appears to be the primary cause of AD-like neuronal degeneration, tau plays a crucial role as modulator of disease progression. Finally, we give suggestions about a potential therapeutical strategy to prevent or delay the onset of AD pathogenesis raising the possibility that lowering tau hyperphosphorylation, even in the presence of amyloid, might slow-down the disease.

GREATER RESISTANCE TO OXIDATIVE STRESS AND TO STRESSFUL STIMULI IN THE P66$^{SHC-/-}$ MOUSE, A MODEL OF DELAYED AND HEALTHY AGING

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Evidence is mounting that reactive oxygen species (ROS) produced as a consequence of stressful challenges could interact with the proper functioning of the hypothalamic-pituitary-adrenal (HPA) axis resulting in greater vulnerability to aging and neurodegeneration. P66$^{Shc-/-}$ mice are highly resistant to oxidative stress and previous data indicate this mutant to be characterised by a delay in the aging process, as shown by a smoother age-dependent change in the behavioural profile. Here we tested the hypothesis that these mutants might be less susceptible to the effects of stressful procedures involving an increase in ROS, such as restraint stress or lipopolysaccharide (LPS) treatment. Although adrenocortical reactivity in response to restraint stress or LPS did not differ as a function of the genotype, a hyperdrive of the HPA axis was revealed following treatment with a synthetic glucocorticoid agonist. When measuring changes in hippocampal oxidative status following LPS, only wild-type (WT) subjects showed increased levels of F2-isoprostanes, an index of lipid peroxidation. At the same time, the neurotrophin brain-derived-neurotrophic factor was selectively increased in WT subjects, while levels of prostaglandin E_2 were reduced in the mutants, possibly as a result of reduced neuronal excitation in response to stress in the latter group. Overall, the greater resilience to stress-induced changes in the p66$^{Shc-/-}$ mutants might underlie the better health status and the greater longevity characterizing these mice.

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DESACYL-GHRELIN AND SYNTHETIC GH-SECRETAGOGUES MODULATE THE PRODUCTION OF INFLAMMATORY CYTOKINES IN MOUSE MICROGLIA CELLS STIMULATED BY β-AMYLOID FIBRILS

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Data from Alzheimer's disease (AD) patients and AD animal models demonstrate the accumulation of inflammatory microglia at sites of insoluble fibrillar β-amyloid protein (fAβ) deposition. It is known that fAβ binds to CD36, a type B scavenger receptor also involved in internalization of oxidized LDL, and initiate a signalling cascade that regulates microglial recruitment, activation and secretion of inflammatory mediators leading to neuronal dysfunction and death. The recent demonstration of a binding site for the growth hormone secretagogues (GHS) on the CD36 prompted us to ascertain whether ghrelin and synthetic GHS could modulate the synthesis of inflammatory cytokines in fAβ-activated microglia cells. We demonstrate that N9 microglia cells express the CD36 and are a suitable model to study the activation of inflammatory cytokines synthesis. In fact, in N9 cells exposed to $fA\beta_{25-35}$ for 24 hrs, the expression of IL-1β and IL-6 mRNA significantly increased. Interestingly, 10^{-7}M desacyl-ghrelin, hexarelin and EP80317, in the nanomolar range effectively counteracted $fA\beta_{25-35}$ stimulation of IL-6 mRNA levels, whereas ghrelin was ineffective. Similarly, the effects of $fA\beta_{25-35}$ on IL-1β mRNA levels were attenuated by desacyl-ghrelin, hexarelin and EP80317, but not ghrelin. Since we have observed that the specific GHS receptor, GHS-R1a, is not expressed in N9 cells, the actions of GHS should be mediated by different receptors. Reportedly, hexarelin and EP80317 are capable of binding the CD36 in mouse macrophages and reducing atherosclerotic plaque deposition in mice. We conclude that desacyl-ghrelin, hexarelin and EP80317 might interfere with fAβ activation of CD36 in microglia cells.

CORTICOTROPIN RELEASING FACTOR (CRF) INHIBITS CELL GROWTH OF HUMAN NEUROBLASTOMA AND MEDULLOBLASTOMA CELL LINES VIA THE ACTIVATION OF CRF-RECEPTOR TYPE 1 AND $P18^{INK4C}$ mRNA INCREASE.

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Corticotropin releasing factor (CRF), the major regulator of the synthesis and release of proopiomelanocortin gene products of the anterior pituitary, plays a key role in the regulation of endocrine, autonomic and behavioral responses to stress. The actions of CRF are mediated through specific seven membrane spanning domains receptors that are coupled to adenylate cyclase. Two types of CRF receptors, CRF type 1 receptor (CRF-R1) and CRF type 2 receptor (CRF-R2) encoded by two distinct genes, have been identified so far. These receptors display different distribution within several tissues, reflecting distinct biological functions. Recently, the expression of CRF receptors, both CRF-R1 and -R2 types, in human cancers originating from tissues which may or may not express CRF receptors has been reported (1); the latter were found in pituitary adenomas, pancreatic tumors and in tumors originating from the central and peripheral nervous system. Moreover, several cancer cell lines, particularly endometrial adenocarcinoma, breast cancer cells, neuroblastoma, small cell lung cancer and melanoma cell lines also express CRF receptors. These findings suggest that CRF may play a role in the control of neoplastic cell growth, although the biology of CRF in human cancer tissue is still poorly characterized. We have previously shown that CRF is able to inhibit the proliferation of a human endometrial adenocarcinoma cell line, Ishikawa (IK) cells (2), as well as the proliferation of MCF-7 human breast cancer cells (3) but, to date, there is little information on the CRF ability to inhibit growth of cell lines derived from other tumor types. In this light, here we investigated the possible antiproliferative effects of CRF on cell lines derived from the nervous system, such as the human neuroblastoma cell lines IMR-32 (which expresses functional CRF type 1 receptors) and SK-N-SH, as well as the medulloblastoma cell line Daoy. The treatment of all cell lines with 100 nM CRF induced a time-dependent inhibition of cell growth, with a maximal effect after 8 days (IMR 32: 47 ± 5 % inhibition, $P < 0.01$ *vs* control, SK-N-SH: 54 ± 7 % inhibition, $P < 0.01$ *vs* control, Daoy: 39 ± 3 % inhibition, $P < 0.05$ *vs* control). This effect was counteracted by the CRF receptor antagonist astressin. Moreover, RT-PCR revealed that both SK-N-SH and Daoy cells express CRF-R1 receptors. The measurement of CRF-induced intracellular cAMP accumulation, suggested the down-stream involvement of cAMP-PKA pathway in the effect of this peptide on cell growth. The antiproliferative effect of CRF is not a consequence of the induction of apoptosis, as shown by staining experiments with the fluorescent dye Hoechst 33258. Finally, CRF increased the accumulation of the tumor suppressor gene $p18^{INK4C}$ mRNA, in neuroblastoma cells, in a time–dependent manner. The results presented here suggest that CRF may be involved in the control of cell growth in the nervous system tumors *via* the activation of its specific receptors and, at least in part, *via* the increase in p18 gene expression.

1. Reubi JC, Waser B, Vale W, Rivier J : Expression of CRF1 and CRF2 receptors in human cancers. J Clin Endocrinol Metab 2003, 88: 3312-3320.

2. Graziani G, Tentori L, Portarena I, Barbarino M, Tringali G, Pozzoli G, Navarra P: CRH inhibits cell growth of human endometrial adenocarcinoma cells via CRH-receptor 1-mediated activation of cAMP-PKA pathway. Endocrinology 2002, 143: 807-811.

3. Graziani G, Tentori L, Muzi A, Vergati M, Tringali G, Pozzoli G, Navarra P: Evidence that corticotropin-releasing hormone inhibits cell growth of human breast cancer cells via the activation of CRH-R1 receptor subtype. Mol Cell Endocrinol 2007, 264: 44-49.

ANTINEOPLASTIC EFFECTS OF SOMATOSTATIN ANALOGUES IN PRIMARY HUMAN PITUITARY ADENOMA CELL CULTURES

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Somatostatin (SST) is a regulatory peptide and exerts its biologic effects through five different high affinity G protein-coupled receptors (SSTRs). SSTRs are widely expressed in various human tissues including normal and neoplastic pituitary gland, where SST is involved in inhibition of hormone secretion and cell proliferation.
Accordingly, SST analogues, that bind with high affinity SSTR2 and 5, are able to effectively control hormonal hypersecretion and tumor growth in the majority of patients affected by GH-secreting adenomas (GH-omas). Nevertheless, about one third of these patients is resistant to treatment. Furthermore, resistance to SST analogues is the rule in Non Functioning Pituitary Adenomas (NFPA), despite the fact that these tumors usually express SSTRs. Thus, the identification and characterization of SST analogues with a different receptor selectivity appears an important step forward in optimizing the pharmacological treatment of pituitary tumors.
The aim of the present study was the preparation and characterization of primary cell cultures derived from GH-omas and NFPA and the evaluation of the effects on cell proliferation and viability induced by treatment with the following molecules: BIM-23206 (SSTR5 selective agonist and hereafter indicated as AGO5), BIM-23A387 [SSTR2 and Dopamine Receptor 2 (D2R) selective agonist, CH], BIM-23120 (selective agonist for SSTR2, AGO2) and KE108 (universal SSTR agonist, PAN). Next, we wanted to correlate the biological effects of these molecules with the expression level of SSTRs and of the *seladin-1* gene in tumoral tissues. This gene was identified several years ago and its expression was found to be reduced in brain areas susceptible to damage in Alzheimer's disease, whereas its over expression confers resistance to apoptosis by inhibiting the activation of caspase-3.
We have previously shown that the expression of *seladin-1* was significantly lower in GH-omas than in NFPA and we suggested that the reduced effectiveness of SST analogues in the treatment of NFPA could be related, at least in part, to the anti-apoptotic activity of this gene.
In the present study, we have first found that all SSTR subtypes are expressed in the cell populations obtained from GH-omas and NFPA and that expression remains unchanged in the early populations (from P0 to P2): in NFPA cells SSTR2 is the most expressed receptor subtype, followed by SSTR1 and SSTR4, whereas SSTR3 and SSTR5 display the lowest level of expression. In GH-oma cells, with the exception of SSTR4, all SSTRs receptors are expressed, and the highest levels were detected for SSTR2 and SSTR1.
The levels of D2R were higher in NFPA than in GH-omas.
Both in GHomas and NFPA none of the treatments induced any effect on the inhibition of cell proliferation determined by cell count. With regard to cell viability, detected by MTS assay, PAN in GH-omas and PAN and AGO2 in NFPA had a significant inhibitory effect.
In any case, the effect obtained with PAN in NFPA was much lower than that observed with the same molecule in GH-oma cells.
Moreover, as previously demonstrated in tissue samples, we found that in primary cell cultures obtained from NFPA the expression of the anti apoptotic gene *seladin-1* was significantly higher than in GH-oma cells and these data confirmed that seladin-1 could be one of the factors that negatively affect pharmacological treatment in NFPA. Accordingly, we can hypothesize a possible therapeutic strategy that provides the use of specific antagonists of seladin-1 in these tumors in order to determine a permissive effect on the activity of SST analogues.

A NEW ACTION OF 17β-ESTRADIOL IN NEUROBLASTOMA CELLS: THE OVER-EXPRESSION OF NEUROGLOBIN, AN ENDOGENOUS NEUROPROTECTANT

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17β-estradiol (E2) orchestrates cellular mechanisms involved in the development and differentiation of various neuron populations, modulation of synaptic plasticity and neuronal excitability, and induction of neuronal survival and axonal outgrowth [1, 2, 3]. Moreover, a growing body of evidence supports the idea that E2 may be beneficial in Alzheimer's disease and other neurodegenerative processes [4, 5].

Recently, a novel O_2-binding heme-protein, neuroglobin (Ngb), has received great attention as a new neuroprotectant. In particular, *in vivo* studies show that Ngb expression in neurons is up-regulated after anoxia-reperfusion. Ngb-mediated neuroprotection has been also related to the antiapoptotic action and to the ability of this globin to scavenge reactive nitrogen and oxygen species [6, 7].

Here, we report that E2 induces a time- and dose-dependent increase of Ngb levels in neuroblastoma SK-N-BE cells. This effect is specific for E2 being not induced by male sex hormones (*i.e.*, testosterone and dihydro-testosterone). Furthermore, the use of estrogen receptors (ER) specific ligands and inhibitors clearly indicate the ER-dependence of this E2-induced effect. Finally, the involvement of rapid and transcriptional ER activities has also been assessed. These results suggest that the E2-dependent increase of Ngb could represents a novel mechanism underlying the neuroprotective effects of estrogens.

References:

[1]. Lee AW, Pfaff DW. Hormone effects on specific and global brain functions. J Physiol Sci. 2008; 58:213-220.

[2]. Jasnow AM, Mong JA, Romeo RD, Pfaff DW. Estrogenic regulation of gene and protein expression within the amygdala of female mice. Endocrine. 2007; 32:271-279.

[3]. Melcangi RC, Magnaghi V, Galbiati M, Martini L. Formation and effects of neuroactive steroids in the central and peripheral nervous system. Int Rev Neurobiol. 2001; 46:145-176.

[4]. Vegeto E, Benedusi V, Maggi A. Estrogen anti-inflammatory activity in brain: a therapeutic opportunity for menopause and neurodegenerative diseases. Front Neuroendocrinol. 2008; 29:507-519.

[5]. Nilsen J. Estradiol and neurodegenerative oxidative stress. Front Neuroendocrinol. 2008; 29:463-475.

[6]. Nienhaus K, Nienhaus GU. Searching for neuroglobin's role in the brain. IUBMB Life. 2007; 59:490-497.

[7]. Greenberg DA, Jin K, Khan AA. Neuroglobin: an endogenous neuroprotectant. Curr Opin Pharmacol. 2008; 8:20-24.

THE NOVEL NEUROENDOCRINE PEPTIDES “NERP-1” AND “NERP-2”: CHANGES IN ALTERED WATER BALANCE

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The *vgf* gene is selectively expressed in specific neuronal and endocrine cells, and is known to be involved in the regulation of food intake and/or energy expenditure by means of several, only partly identified VGF precursor derived peptides. Two novel, C-terminally amidated VGF peptides named NERP-1 and NERP-2 were recently shown to suppress plasma vasopressin release (Yamaguchi et al, 2007). Of these, at least NERP-1 immunoreactivity was found in several locations in man and rat (Rindi et al, 2007; Cocco et al, 2007). To address response and modulation profiles of NERP peptides, ELISA assaies were set up with antibodies to their respective C-terminal amidated decapeptides (NERP-1 assay: ID_{50} = 0.8 pmol/ml, <0.0001% cross-reactivity of either des-amidated, or C-terminally Glycine-extended peptide; rat NERP-2 assay: ID_{50} = 0.5 pmol/ml). Rats (Sprague-Dawley, 500g) were divided in 3 groups: (i) controls, with free access to water; (ii) water deprived, 48h; (iii) salt-loaded (2% NaCl in their drinking water, 7 days). At sacrifice (ether overdose), parallel samples of plasma and hypothalamus, and the whole pituitary were taken. Hypothalamic samples included the suprachiasmatic, supraoptic and paraventricular nuclei. In view of its high content of other VGF peptides (VGF C-terminus and TLQP-peptides), the median eminence was removed (under stereomicroscopical control) and extracted separately. Tissues were homogenized in PBS (with protease inhibitors), hence heat treated (in boiling water, 15 min). NERP-1 immunoreactivity was more abundant in pituitary, compared to hypothalamus (~80-120 *versus* ~30-45 pmol/g, respectively), with lower amounts of NERP-2 (20-50 *versus* 15-25 pmol/g). In NaCl loaded rats, NERP-1 immunoreactivity distinctly increased in hypothalamic and median eminence extracts (~50-200%), while decreased in pituitary (~40-60%), with lower changes in water deprived animals. As to NERP-2, a comparable, clear-cut increase was found in hypothalamus and median eminence (>100%), but also in pituitary samples. Pending confirmation of the molecular species involved, preliminary data on plasma indicate a decrease in both NERP-1 and -2 in both experimental groups, compared to controls.

Hence, a distinct response was apparent for NERP peptides in the hypothalamus, including the median eminence. Intracerebroventricular injection of either NERP-1 or NERP-2 was shown to inhibit stimulated vasopressin release from the posterior pituitary, by a possible hypothalamic mechanisms acting in the supraoptic / paraventricular nuclei (Toshinai & Nakazato, 2009). However, the response we found in the median eminence may point to the relevance of NERP peptides in other pathways and mechanisms, possibly involving parvocellular vasopressinergic and/or other neurons. The apparently diverging changes seen for NERP-1 and NERP-2 in the (whole) pituitary warrant further investigation, with characterization of the molecular species involved and their possible differential actions. Indeed, it would not be surprising if at least a degree of divergent bioactivity and significance became apparent, given the profound sequence dis-similarity between NERP-1 and NERP-2 peptides.

COMMUNAL NESTING, AN EARLY SOCIAL ENRICHMENT, SHAPES SOCIAL BEHAVIOR AND COPING RESPONSE TO SOCIAL STRESS IN ADULT MOUSE

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Early experiences produce persistent changes in behavior and brain function. Being reared in a Communal Nest (CN), consisting of a single nest where three mothers keep their pups together and share care-giving behavior from birth to weaning, provides an highly stimulating social environment to the developing pup. CN characterizes the natural ecological niche of the mouse species and represents a form of early social enrichment. In the CN, both mother-offspring and peer-to-peer interactions are markedly increased. At adulthood, CN mice show higher propensity to interact socially with conspecifics and more elaborate social competencies compared to mice reared in standard laboratory conditions (SN). In particular, CN mice play the role of either the dominant or the subordinate starting from the first agonistic encounter, while SN mice need five social encounters to fully show their social role. Furthermore, CN mice display high levels of aggressive behavior only when appropriate in an eco-ethological perspective, i.e. when they have to set up or defend their own territory. With regard to emotional behavior and hypothalamic-pituitary-adrenal (HPA) axis activation, CN mice are less vulnerable to anhedonia following psychosocial stress and display a reduced activation of the HPA axis after acute or prolonged exposure to social challenge. The present findings show that being reared in a CN plays a crucial role in structuring adult social competencies in the mouse. Overall, the social environment to which an organism is exposed during critical developmental periods, exerts a major effects in shaping social behavior at adulthood. *Supported by EU, project INTELLIMAZE contract n 037965.*

EFFECTS OF LOW DOSES OF BISFENOL-A AND ALFA-ETHINYLESTRADIOL ON PARENTAL AND REPRODUCTIVE BEHAVIOUR IN MALE AND FEMALE MICE

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Bisphenol A (BPA) is a man made chemical compound used in the production of polycarbonate, epoxy resins, lining food storage cans and dental sealants. Due to its estrogenic activity, it is able to interact and modulate the neuroendocrine system. In this connection, a critical time windows for the development of an organism is fetal life, when even small changes in the levels of hormones, such as estradiol, or estrogen-mimicking chemicals, such as BPA, may lead to changes in brain structure and function, and in sexual differentiation processes. A number of studies showed that in mice, exposure to low doses of BPA during pregnancy induces alterations in body growth rate of the offspring (both females and males pups), accelerates puberty in females, reduces the spermatogenesis in males, alters exploration and locomotor activity and learning, often showing sex specific effects (reviewed in Palanza et al. 2008).
In this experiment, we directly treated pregnant female mice with BPA or alpha-ethinylestradiol (AEE; used as a positive control for the estrogenic activity). Pregnant CD1 dams were trained to drink corn oil with or without low doses of BPA (2 or 10 µg/Kg/day) and AEE (0,004 and 0,04 µg/Kg/day) comparable with those occurring in the environment, during the last week of pregnancy. We determined spontaneous maternal behaviour of the treated dams as well as early development, subsequent reproduction and parental behaviour in the offspring. Results showed no significant effect of BPA or AEE on maternal behaviour and early offspring development. When 3 months-old, male and female mice prenatally exposed to BPA or AEE were paired with mice of the same group treatment group and assessed for sexual behaviour, reproductive success and parental responses. Sexual behaviour and reproductive success were not affected by prenatal exposure to different doses of BPA and AEE, whereas males exposed to BPA and AEE spent more time in performing parental cares as compared to control males.
These data confirm that prenatal exposure to low doses of environmental estrogens, during early phases of central nervous system sexual differentiation, can produce long-term effects on behaviour in adult animals.

Reference:
Palanza, P., L. Gioiosa, et al. (2008). "Effects of developmental exposure to bisphenol A on brain and behavior in mice." Environ Res **108**(2): 150-7.

ESTRADIOL REGULATES VASOPRESSIN IMMUNOREACTIVITY IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI: ROLE OF ESTROGEN RECEPTORS IN PHYSIOLOGICAL AND SALT LOADED CONDITIONS.

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Arginine-vasopressin (AVP) is a neurohypophyseal hormone acting as a regulator of the fluid balance and also as a CNS neurotransmitter or neuromodulator. The vasopressin system is constituted of 2 cellular systems: magnocellular and parvocellular neurons. Magnocellular and parvocellular neurons of the paraventricular nucleus (PVN) express both estrogen receptors (ER α and ER β), whereas magnocellular neurons within the supraoptic nucleus (SON) only express ER β. In vitro studies in SK-N-SH neuroblastoma cells, transfected with AVP promoter, have shown that the AVP gene is regulated by estradiol via the two estrogen receptors (ERs). However the two ERs exert a differential regulation of the AVP promoter. ER α increases AVP promoter activity after estrogen stimulation. In contrast, ER β activates AVP gene transcription in a constitutive manner and induces a decrease of the promoter activity after estrogen stimulation (1). In this study we have examined the role of ERs on AVP regulation in SON and PVN magnocellular neurons by using an *in vivo* approach. Ovariectomized adult female rats were treated for 3 days with either vehicle, 17β-estradiol (50 µg/Kg), the selective ER α agonist PPT (1 mg/Kg), or the selective ER β agonist DPN (1 mg/kg) in physiological or salt loaded (NaCl 2%) conditions.

Results

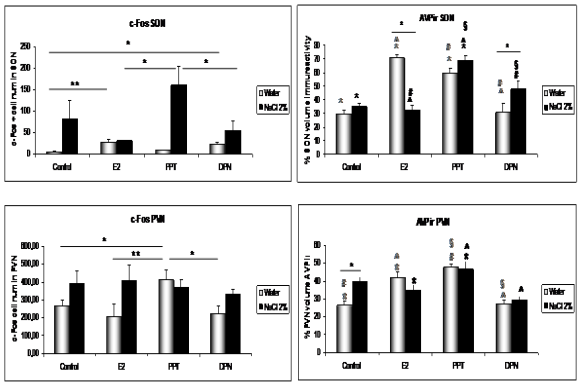
The figures show the number of c-Fos immunoreactive cells in the SON and the PVN and the volome fraction of AVP immunoreactivity in the SON and the PVN.

In not salt loaded conditions:

• E2: increased the number of activated cells (detected by means of *c-fos* immunoreactivity) in SON, but not in PVN and increased AVP immunoreactivity in SON and PVN.

• PPT: increased the number of activated cells in PVN but not in SON and increased AVP immunoreactivity in SON and PVN.

• DPN: increased the number of activated cells in SON but not in PVN and did not affect AVP immunoreactivity neither in PVN nor in SON.



In salt loaded condition:

• E2: did not change the number of activated cells in SON and decreasd AVP immunoreactivity in comparison to not salt loaded E2-treated animals.

• PPT: increased the number of activated cells and AVP immunoreactivity in SON.

• DPN: did not change the number of activated cells nor AVP immunoreactivity in SON.

Conclusions

These findings indicate that estradiol acts with regional specificity to activate magnocellular neurons and that both ER α and ER β are involved in this hormonal action.

Refence

(1) Shapiro R.A., Xu C., Dorsa D.M. (2000), Differential Transcriptional Regulation of Rat Vasopressin Gene Expression by Estrogen Receptor a and b. Endocrinology. 141, 4056-4064

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NEONATAL MILD AND SEVERE STRESS RESPECTIVELY RELATE TO ADULT RESILIENCE AND VULNERABILITY IN MICE.

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Neonatal stimulation has been shown to adjust cognitive abilities, stress reactivity, fear responses and immune activity in adult mammals of many species. However, whereas severe stressors often relate to the emergence of pathology, mild neonatal stimulation has been traditionally associated with 'positive' effects or resilience. External stressors stimulate the hypothalamic pituitary adreno-cortical (HPA) axis to induce a corticosterone secretion in mouse dams, which, in turn is directly transmitted to the progeny through lactation. Such corticosteroid transfer may offer a unitary mechanism whereby low corticosterone exposure may favour resilience and high corticosterone increase vulnerability to pathology. In this study we investigated this hypothesis by comparing the long-term effects of a neonatal exposure to low (33 mg/l) and high doses (100 mg/l) of corticosterone – during the first 10 days of life through supplementation in the maternal drinking water – with animal facility reared mice. Offspring cognitive abilities, central brain derived neurotrophic factor (BDNF) regulation and plasma levels of auto-antibodies (aAbs) directed to serotonergic and dopaminergic targets were assessed in adulthood. Cognitive performance was addressed through the attentional-set-shifting task, a rodent analogous of the Wisconsin Card Sorting Task (WCST). The WCST is used, in humans, to assess the ability to perform shifts between cognitive attentional sets. This ability is usually impaired in neuropsychiatric and in prefrontal cortex impaired patients. These patients generally display poor performance when required to form new rules and discard those previously acquired (stuck-in-set-perseveration). While low levels of neonatal corticosterone reduced adult mice perseverative responses and increased aAbs levels directed to serotonin transporters, high doses of neonatal corticosterone reduced hippocampal BDNF levels and aAbs directed to dopamine transporters. Reduced perseverative responding and increased aAbs levels towards serotonin transporters may be associated with resiliency whereby the former suggests improved cognitive skills and the latter apparently relates to reduced serotonin reuptake through diminished transporters activation. Conversely, resembling the consequences of psychiatric disorders on CNS neurotrophic regulation, and mimicking the effects of early maternal deprivation, elevated doses of neonatal corticosterone down-regulated BDNF expression. Therefore, although preliminary, these results support the view that both adaptive plasticity and pathological outcomes may depend on maternal circulating corticosterone levels during lactation and that these effects may follow a U-shaped profile. Additionally, this study allows to hypothesize a parsimonious descriptive model – explaining both resilience and vulnerability to pathology – through the postulation of a single common mediator (circulating levels of corticosterone).

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BEHAVIOURAL AND NEUROSTEROID BASED ANALYSIS TO MONITORING WELFARE STATE IN FARM PIGS RAISED IN AN ENRICHED ENIVIRONMENT

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By improving conditions of housing and care, it can be possible to meet the animals' basic welfare needs and to maximize, at the same time, performance and meat quality. At this aim, there is a large body of literature supporting the hypothesis that the enrichment of the physical environment can improve the welfare of farm animals. As a matter of fact, the European Directive (2001/93/EC) underlines that "pigs must have permanent access to sufficient quantity of material to enable proper investigation and manipulation activities". Nevertheless, it should be noted that the majority of available studies have focused on the introduction of enrichments in the pens for short periods of time.

A holistic monitoring approach to determine the level of psychological and physical well-being includes behavioural (i.e. posture, vocalisations, play), physiological (i.e. body weight, body temperature, heart rate) and biochemical (i.e. haematological and immune parameters, neurosteroids, corticosteroids) measures. Fluctuations in these indices are normal within certain boundaries, but more extreme departures are indicative of changes of the level of welfare. Recently, the level of dehydroepiandrosterone sulphate (DHEA-S) in serum of cows and pigs has been reported to be a new potential biochemical measure of their state of welfare. Nevertheless, little and controversial literature is available on this issue.

The aim of this study was to evaluate the effects of a range of environmental enrichments in a commercial line of pigs over the growth period (three to six months of life) by evaluation of behavioural and biochemical indicators. Twenty-eight Large White/Landrace x Pietrain crossed pigs were used. At the beginning of the study, the subjects were grouped in two housing conditions. Fourteen animals (seven castrated males and seven intact females) were housed in a pen containing physical objects (chains inserted in rubber hoses, non-toxic plastic balls, rubber hoses and spring ropes) (ENRICHED GROUP) and fourteen animals (seven castrated males and seven intact females) were housed in standard housing condition (STANDARD GROUP). Each pen contained a total of 50 individuals, with a sex ratio of female : male = 1:7. Tap water and food were provided ad libitum. The presence of *abnormal behaviours*, including *body biting*, *tail biting*, *ear biting* and *bell noising* was recorded by at first hand observations. Biochemical measures, including the level of DHEA-S and haematological and immune parameters were obtained by blood collection. All data were collected at arrival (Time 0), 15 days later (Time 1) and 75 days later (Time 2).

The data analysis is still in progress, therefore only partial results concerning the Time 1 are presented. With respect to the *abnormal behaviours*, as a whole, their frequency was four times higher in the STANDARD GROUP than in the ENRICHED GROUP. A gender difference also appeared, with the males raised in standard condition showing *body biting* and *ear biting* with 90 % and 40% of occurrence higher, respectively, than in males housed in the presence of an enriched environment. It should be also noted that *tail biting* was never showed by any individual housed in the enriched pen. The *bell noising* was strongly reduced in the ENRICHED GROUP and the standard housed females showed this behaviour at the 80% more than the enriched counterpart. Furthermore, it could be noted that an age-related profile appeared in the use of the objects. In fact, this was particularly prominent in animals at Time 1 than before or later on.

In regard to DHEA-S levels, a significant difference between animals from the two rearing conditions was found. Enriched pigs, as a whole, presented a 40% decrease in serum DHEA-S concentration. Interestingly, this profile was limited to the male group.

The combination of behavioural and biochemical indicators represents an effective approach to an accurate and valid monitoring of the welfare state of farm animals.

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SP PROTECTS CEREBELLAR GRANULE CELLS AGAINST β–AMYLOID–INDUCED APOPTOSIS

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Substance P (SP) is an 11-aa neuropeptide, member of the tachykinins (TK) family, broadly distributed in the central nervous system and having a functional role both in physiological and pathological conditions. Recently, a consistent SP reduction in the cerebral cortex, hippocampus, basal ganglia and cerebrospinal fluid of AD patients was reported, indicating a possible neuroprotective role of SP in the progression of this disease. Aim of the present study was to evaluate the SP neuroprotective potential against apoptosis induced by the neurotoxic beta-amyloid peptide (Aβ) in cultured rat cerebellar granule cells (CGCs). We found that SP, at a concentration of 200nM, protected CGCs against high concentrations of both $A\beta_{25-35}$ and $A\beta_{1-42}$-induced apoptotic death. Indeed SP was able to reduce the increased Aβ - induced apoptosis as revealed by live/dead cell assay, caspase(s)-induced PARP-1 cleavage and Hoechst staining, hence providing evidence for a possible neuroprotective effect of SP in brain. Recent data have pointed out the involvement of Akt activation in the antiapoptotic mechanism of action exerted by SP, both in colonic epithelial cells and in CGCs. In the present study, we demonstrated that Aβ-induced inhibition of phospho-Akt in CGCs was reversed by SP pre-treatment, suggesting that the neuroprotective effect of SP towards Aβ toxicity is strictly mediated by the Akt pathway. In conclusion, the present work provides evidences for the signalling pathways that are activated by SP to execute its protective function against A in CGCs, indicating that this peptide could have a potential therapeutic relevance in the Alzheimer's disease and other age-related neurodegenerative disorders.

PERINATAL EXPOSURE TO BISPHENOL-A AFFECT SEXUAL BEHAVIOR IN ADULT MALE AND FEMALE MOUSE

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In mammals, endogenous estrogens are crucial for the differentiation of sexual brain system during the perinatal period. The estrogenic environment during pregnancy and lactation affects directly sexual behavior in rodents. In this work, we tested the hypothesis that exposure to exogenous estrogenic compounds during perinatal and/or postnatal periods could affect the adult sexual behavior in mice of both sexes. Bisphenol-A (BPA) is a chemical compound present in the environment that is able to bind to estrogen receptors and, potentially, to interfere with the normal cellular development in target organs and tissues. Therefore, BPA may interfere with the processes of sexual differentiation of brain and behavior.
Different doses of bisphenol-A (5, 10, 20 or 40 μg/kg/day) were orally administered to two different groups of pregnant CD1 female mice. To test the organizational effect of BPA, a first group (Gr1) was treated from prenatal day 10 (PrND10) to postnatal day 8 (PDN8), while a second group (Gr2) was additionally treated from PND31 to PDN60 to observe the activational effect. The offspring of the treated mothers were tested during the 9^{th} – 10^{th} week of life (PND 60-70) by measuring several aspects of the sexual behavior: mount, body sniffing, ano-genital sniffing, self- and allo-grooming for males, and receptivity, body and ano-genital sniffing, rejection for females. Results show not significant differences for sexual behavior patterns in females of both groups; on the contrary, exposure of mother to BPA modified the behavior of Gr1 and Gr2 males. We found that the administration of the lower doses of BPA in the Gr1 altered the male ano-genital sniffing behavior (5, 10 and 20 μg/kg/day), the body sniffing behavior (10 μg/kg/day) and allo-grooming behavior (5 μg/kg/day). In the Gr2, we observed statistically significant differences in mount and allo-grooming behavior (40 μg/kg/day). This experiment shows that perinatal exposure to BPA (at levels that are under the safe daily limit for human, 50 μg/kg/day) may irreversibly influence male sexual behavior. These effects are depending by the dose and also by further exposure in adulthood. In addition, they confirm that precocious exposure to endocrine disrupting chemicals, in particular to BPA, may have a high impact on the organization of specific neural pathways that can later affect complex behaviors and functions related to reproduction.

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AROMATASE BLOCKAGE IN PREPUBERTAL MALE RATS BY ANASTROZOLE: EFFECTS ON SOCIAL PLAY

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Sex steroids are crucial for the ontogenesis of sexually dimorphic behaviours. In particular, in laboratory rodents the aromatization of testosterone into estradiol at the central level is necessary for the masculinization of brain and behaviour. In the rat the temporal window for the organizational action of sex steroids is mainly the perinatal period. However, some plasticity is still present even during the prepubertal period (Della Seta et al., 2006). In the present work we studied the effects of the prepubertal administration of the aromatase blocker, anastrozole, on juvenile play behaviour of male rats, a good predictor of adult social behaviour.

We treated 3 groups of prepubertal rats - from 23 to 32 days of age - as follows:

a) anastrozole, 50 µg/day in 100 µl 20% sucrose solution (per os) and placebo pellets; b) positive control, anastrozole 50 µg/day and 3,5 µg/day 17ß estradiol, slow release pellets; c) negative control, sucrose solution and placebo pellets. At 45 days of age play behaviour of juvenile rats was observed in their home cage and videorecorded for 10 min. Behavioural analysis was carried out using Noldus Observer software on the basis of an established ethogram (Poole and Fish, 1975; Dessì-Fulgheri et al 2002).

By means of a PCA analysis 2 main factors were identified, explaining 41.1% of total variance. 1, social play (23.7%); 2, exploration (17.4%). The analysis revealed that: a) hormonal manipulations in the prepubertal period may have long term effects; b) only one group of behaviours (factor 1) is influenced by anastrozole i.e. social play, and the effect is, contrary to our expectation, an increase of aggressive components of play. As far as point a) is concerned, our results confirm that the organization window for sex steroids is open until puberty. For point b) our hypothesis is that the blockage of the aromatase pathway may enhance the alternative 5α reductase pathway, resulting in an increase of 5α dihydrotestosterone at the central level. This is in line with Meaney (1988) who observed an increase of play fighting after DHT administration.

In summary, the results of the present experiment prove that the organization of male play behavior may still be influenced by the alteration of the estrogenic milieu, well beyond the perinatal period.

Della Seta, D., Minder, I., Belloni, V., Aloisi, A. M., Dessì-Fulgheri, F., Farabollini, F. (2006). "Pubertal exposure to estrogenic chemicals affects behavior in juvenile and adult male rats". Hormones and Behavior 50:301-307.

Dessì-Fulgheri, F., Porrini, S., Farabollini, F. (2002). "Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats". Environmental Health Perspectives 110: 403-407.

Meaney, M. J. (1988). "The sexual differentiation of social play". Trends in Neurosciences 11: 54-58.

Poole, T. B., Fish, J. (1975). "An investigation of playful behavior in Rattus norvegicus and Mus musculus (Mammalia)". Journal of Zoology 175: 61-71.

DIABETES SIGNIFICANTLY DECREASES THE mRNA LEVELS OF 18.5 kDa AND 21.5 kDa ISOFORMS OF MYELIN BASIC PROTEIN PRESENT IN THE SPINAL CORD AND TESTOSTERONE OR DIHYDROPROGESTERONE TREATMENT COUNTERACTS THIS EFFECT.

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Diabetic neuropathy is one of the most complications of diabetes leading to degeneration of axons and myelin in peripheral nervous system (PNS). We have demonstrated that the gene expression of two of the most important myelin proteins such as glycoprotein zero and peripheral myelin protein 22, is affected in the sciatic nerve of streptozotocin (STZ)- induced diabetic neuropathy [6]. Interestingly, neuroactive steroids, such as progesterone (PROG), dihydroprogesterone (DHP) and dihydrotestosterone (DHT) (i.e., the derivative of testosterone, T), are able to counteract this impairment [6,7]. Compared to complications in the PNS, diabetic complications in the CNS has been studied relatively little. For this reason we considered the myelin basic protein (MBP), the only structural protein found so far to be essential for the formation of CNS myelin [1], analyzing, by RNase protection assay, the mRNA levels of its two isoforms (i.e., 18.5 kDa and 21.5 kDa) in three structures of CNS (i.e., cerebral cortex, cerebellum and spinal cord) of STZ-treated rat. Data obtained indicate that, after 3 month of diabetes, the gene expression of both MBP isoforms is significantly decreased in all CNS structures considered. Neuroactive steroids have been also reported to modulate the expression of myelin proteins in CNS [3,4,5], consequently we have then analyzed whether PROG, DHP, THP, T, DHT and 3alpha-diol may counteract the decrease of MBP observed in CNS structures of diabetic animals. We observe that chronic treatment for 1 month with T or PROG is able to increase the mRNA levels of both MBP isoforms in spinal cord. This effect is not observed in cerebral cortex and in cerebellum. Interestingly also DHP, the 5alpha-reduced metabolite of P, is able to induce a stimulatory effect whereas DHT, THP and 3alpha-diol were ineffective. Consequently, we have analyzed by real time PCR, the gene expression of the enzyme converting T and PROG into their corresponding 5alpha-reduced metabolites (i.e., the enzyme 5alpha-reductase). Data obtained have indicated that, diabetes induces a significant decrease of 5alpha-reductase mRNA levels in spinal cord. This result is in agreement with data obtained by liquid chromatography-tandem mass spectrometry indicating that in spinal cord the levels of DHT and DHP are significantly decreased by diabetes [4]. Interestingly, the treatment with PROG counteracts the reduction of 5alpha-reductase. Thus, it is possible to hypothesize that the effect of PROG on the mRNA levels of MBP isoforms is due to its conversion into DHP. Altogether, the present data report for the first time an impairment of MBP gene expression due to diabetes at the level of spinal cord and that, neuroactive steroids, such as T and DHP, may counteract this effect.

REFERENCE LIST

[1] Boggs JM. Myelin basic protein: A multifunctional protein. Cell Mol Life Sci. 2006; 63: 1945-61.

[2] Caruso D, Scurati S, Maschi O, De Angelis L, Roglio I, Giatti S, Garcia-Segura LM, Melcangi RC. Evaluation of neuroactive steroid levels by liquid chromatography-tandem mass spectrometry in central and peripheral nervous system: effect of diabetes. Neurochem Int. 2008 Mar-Apr;52(4-5):560-8. Epub 2007 Jun 19.

[3] Ghoumari AM, Ibanez C, El-Etr M, Leclerc P, Eychenne B, O'Malley BW, Baulieu EE, Schumacher M. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. J Neurochem. 2003; 86:848-5

[4] Labombarda F, Gonzalez S, Gonzalez Deniselle MC, Garay L, Guennoun R, Schumacher M, De Nicola AF. Progesterone increases the expression of myelin basic protein and the number of cells showing NG2 immunostaining in the lesioned spinal cord. J Neurotrauma. 2006 Feb;23(2):181-92.

[5] Labombarda F, González SL, Lima A, Roig P, Guennoun R, Schumacher M, de Nicola AF. Effects of progesterone on oligodendrocyte progenitors, oligodendrocyte transcription factors, and myelin proteins following spinal cord injury. Glia. 2008 Dec 2. [Epub ahead of print]

[6] Leonelli E, Bianchi R, Cavaletti G, Caruso D, Crippa D, Garcia-Segura LM, G. Lauria, Magnaghi V, Roglio I and Melcangi RC. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. Neuroscience. 2007; 144:1293–1304.

[7] Roglio I, Bianchi R, Giatti S, Cavaletti G, Caruso D, Scurati S, D. Crippa D, Garcia-Segura LM, Camozzi F, Lauria G and Melcangi RC. Testosterone derivatives are neuroprotective agents in experimental diabetic neuropathy. Cell Mol Life Sci. 2007; 64:1158–68.

In vitro and *in vivo* pharmacological role of TLQP-21, a VGF-derived peptide, in the regulation of rat motor gastric functions

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Vgf gene expression has been detected in the central and peripheral nervous system and in various endocrine and neuroendocrine cells in the gastrointestinal (GI) tract. Aim of this study was to investigate the pharmacological activity of different VGF-derived peptides. Among these, TLQP-21, corresponding to the 556-576 fragment of the protein was the unique active peptide and its pharmacological profile was further studied. The effects of TLQP-21 were examined *in vitro* evaluating muscle contraction of rat isolated GI preparations and *in vivo* in rat gastric emptying. In rat longitudinal forestomach (RLF), TLQP-21 (100 nM-10 μM) concentration-dependently induced muscle contraction (in female rats, EC_{50} = 0.47 mM, E_{max}: 85.7 ± 7.9 and in male rats, 0.87 mM, E_{max}: 33.4 ± 5.3; n = 8), by release of PGE_2 and PGF_{2a} from the mucosal layer. This effect was significantly ($p<0.05$) antagonized by indomethacin and both COX-1 (S560) and COX-2 (NS398) selective inhibitors. TLQP-21, i.c.v. injected (2-32 nmol per rat) significantly ($p<0.05$) decreased gastric emptying by about 40%. This effect was significantly ($p<0.05$) blocked by i.c.v. injection of indomethacin, suggesting that, also *in vivo,* this peptide acts in the brain stimulating PGs release. The present results demonstrate that this VGF-derived peptide plays a central and local role in the regulation of rat gastric motor functions.

ROLE OF THE CANNABINOID SYSTEM IN THE EXOCRINE PANCREAS

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Clinical cases of pancreatitis are reported in heavy marijuana smokers. Cannabinoids (CBs), which include the bioactive constituents of the marijuana plants, activate two types of G-protein-coupled receptors, cannabinoid type 1 (CB1r) and type 2 (CB2r). In the enteric nervous system there is evidence of the presence of endocannabinoids and of both CB1 and CB2 receptors in different species, including humans. The pathophysiological role of the endogenous cannabinoid system in the pancreas is controversial. The aim of this study was to investigate: 1) the role of the cannabinoid system in the regulation of exocrine pancreatic secretion, by studying the effects of the synthetic CB1r- and CB2r-agonist, WIN55,212, on amylase secretion from isolated lobules and acini of guinea pig and rat, 2) the expression of CB-receptors in rat pancreatic tissue by immuno-chemistry and Western blot analysis both in basal condition and after cerulein (CK)-induced pancreatitis. In pancreatic lobules of both guinea pig and rat, WIN55,212 significantly inhibited amylase release stimulated by KCl depolarization through inhibition of pre-synaptic acetylcholine release, but did not modify basal, carbachol- or CK-stimulated amylase secretion. The effect of WIN55,212 was significantly reduced by pre-treatment with selective CB1r- and CB2r- antagonists, which when given alone, did not affect the KCl-evoked response. Conversely, WIN55,212 was unable to affect basal and CK- or carbachol-stimulated amylase release from pancreatic acini of both guinea pig and rat. Immunofluorescent staining of rat pancreatic tissues showed that CB1r and CB2r are expressed in lobules and in acinar cells, and their presence was also confirmed in acinar cells by Western-blotting analysis. After CK-induced pancreatitis, the expression of CB1r in acinar cells was not changed, whilst a down-regulation of CB2r was observed. In conclusion, the present study shows that WIN55,212 inhibits amylase release from guinea pig and rat pancreatic lobules and, for the first time, that CB receptors are expressed in the rat pancreatic lobules, where they mediate an inhibitory control of cholinergic transmission. In contrast, although CB1r and CB2r are present in rat pancreatic acinar cells both in basal conditions and after CK-induced pancreatitis, they result inactive on amylase secretion, leaving their functional role to be established, both in physiological and pathological conditions. A more extended knowledge of biological and molecular alterations linked to acute pancreatitis and the role of the CB-system in this life-threatening condition will open the way for new therapies and our results could lay a basis for testing the potential therapeutic value of cannabinoid antagonists in this pathological condition.

HETEROZYGOUS REELER MICE EXHIBIT RESCUE OF BEHAVIOURAL PHENOTYPE FOLLOWING NEONATAL TREATMENT WITH ESTRADIOL

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Males are affected more frequently than females (ratio of 4:1) by autistic spectrum disorders (ASD), which are characterized by impaired social skills, repetitive behaviors, impairments in planning and attention. Twin studies support a strong genetic component. According to the “extreme-male brain” theory, this skewed sex ratio can be explained by elevated fetal testosterone levels that lead to increased risk of developing social and communication deficits. A neurobiological interpretation of this theory posits that gonadal hormones modulate the penetrance of genetic mutations associated with autism. Male heterozygous reeler (rl/wt) mice show a decreased number of Purkinje cells (PC) in the cerebellum at postnatal day 15 (P15), while female rl/wt do not show any PC loss. It has been previously reported that treatment at P5 into the cisterna magna of mice, with the estrogen receptor agonist 17-beta-Estradiol (17betaE2) increases PC numbers in male rl/wt but has no effect in female rl/wt or male/female wt mice. PC loss in the male rl/wt was hypothesized to depend on an early imbalance of estrogen/testosterone tissue levels. We report here data gathered through a longitudinal behavioral analysis in these mice. A genotype-by-estradiol interaction was found for number of *ultrasonic vocalizations* in response to separation from the mother at P7. Basal levels in rl/wt females were significantly lower than in wt. Neonatal estradiol reverted this profile. In males, no genotype-dependent effect appeared. In the *homing test* on P9, a significant lower percentage of rl/wt mice reached the nest area than corresponding wt pups. In the absence of motor impairments, this suggests a genotype-dependent alteration in sensitivity to or in central processing of social stimuli. Remarkably, this deficient profile was reverted by neonatal estradiol. In a *social task* performed in adulthood, early estradiol administration increased time spent in proximity to a novel social stimulus by wt males and by rl/wt female mice. When adult male mice were assessed in an *attentional set-shifting task*, involving the formation of new rules to obtain a palatable reward, rl/wt subjects showed a higher number of perseverative responses. Neonatal estradiol abolished the differences between rl/wt and wt mice. Our results support the male rl/wt mouse as a model of neuropathological, and behavioral alterations related to autistic spectrum disorder. The molecular pathways involved in mediating the action of sex steroids on PCs could represent candidate signaling pathways in ASD and potentially lead to new therapeutic targets.

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NGF AND BDNF AS SIGNALLING MOLECULES IN THE ADIPOSE TISSUE OF STRESSED AND DIABETIC RODENTS

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The adipose tissue is the body's largest endocrine and paracrine organ producing multiple signaling proteins collectively designated adipokines (Bulcão C., et al., 2006: Curr. Diabetes. Rev. 2, 19-28). There is a growing evidence showing that neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are also produced and released by adipose tissues (Ryan V.H., et al., 2008: Horm. Metab. Res. 40, 1-8) , but their role is largelly uknown. We have recently investigated this aspect in white (WAT) and brown adipose tissues (BAT) in stressed mice and diabetic rats. Stress was produced in adult male mice of Swiss CD-1 strain by social isolation for 8 weeks and diabetes was induced in adult Sprague Dawley male rats intravenous injection of streptozotocin (STZ). Stressed and diabetic animals and their respective controls were sacrificed with an overdose of Nembutal, and WAT of the epicardial region, and BAT of the interscapular region were isolated and used for NGF and BDNF immunoenzymatic assay following the manufacturer's instructions. We found that the concentration of NGF in BAT increased significantly in stressed mice as compared to control tissue ($p<0.05$), while in WAT the increase was less significant ($p<0.075$). In diabetic rodents the presence of NGF in WAT and BAT was markedly enhanced ($ps<0.05$). Diabetes causes also a mild increase in BDNF in both WAT and BAT ($p=0.08$ in post-hocs). BDNF presence was not altered in stressed mice.
Since mast cells release NGF and BDNF and express NGF-receptors, samples of adipose tissues were used for histological and immunohistochemical studies. It was found that mast cells are present in adipose tissue. Mast cells released-NGF in stressed and diabetic rodents may serve to regulate the sympathetic innervation of adipose tissue and to regulate neurendocrine signals. Moreover, the adipo-derived NGF may be implicated in extraneuronal functions, including cardiometabolic disorders, as recently suggested (Manni L., et al., 2007: Neurosci. Lett. 426, 39-44; Sornelli F, et al. General Physiology and Biophysics, in press).

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Modulation of hippocampal plasticity by inducing NGF and BDNF synthesis via electrical stimulation of cervical vagus nerve

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Electrical stimulation of the cervical vagus nerve (CVS) is an approved treatment for epilepsy and depression, and is currently under investigation as a possible therapy in conditions of brain trauma and diseases associated with cognitive impairment. However, less is known about the mechanism by which CVS influences brain functions.
We have performed experiments to analyse whether and how the neurotrophins Nerve Growth Factor (NGF) Brain Derived Neurotrophic Factor (BDNF), which regulate neuronal survival and plasticity in adult brain and exert a neuroreparative action, may be involved in the mechanism mediating the central effects of CVS.
We found that a single series of stimulation induces the expression of NGF and BDNF in the hypothalamus, hippocampus and cortex relatively to the conditions and parameters of stimulations. Chiefly, a selective increase of NGF in hippocampus is obtainable by stimulating the vagal nerve at the cervical levels (CVS) at a stimulation frequency of 5Hz. The increase of NGF was accompanied by changes in the neurotrophin receptor and induction of GABA synthesis.
Three days after CVS, the hippocampal GABA expression levels remain higher than in the controls, and a large number of newly formed cells (Bdru+), also expressing neuronal markers, were detected in the dental gyrus. Parallely, the synthesis of the Neural cell adhesion molecule (NCAM), a prominent modulator of synaptic plasticity, was increased in a frequency-dependent manner in the hippocampus of CVS rats. Changes in the distribution and expression of TrkA and TrkB, but not in neurotrophin levels were detected.
These findings demonstrate that vagal afferents modulate neurotrophin synthesis in brain and that a selective induction of hippocampal NGF is obtainable by varying the parameters of stimulation. Furthermore, our results suggest the possible involvement of NGF in the mechanism by which CVS enhances neuronal plasticity in the hippocampus.

DIVERGING EFFECTS OF CORTISTATIN AND SOMATOSTATIN ON THE PRODUCTION AND RELEASE OF PROSTANOIDS FROM RAT CORTICAL MICROGLIA AND ASTROCYTES

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Cortistatin (CST) is a recently discovered cyclic neuropeptide cloned from human, rat and mouse tissues [1] and belonging to the family of somatostatin(SS)-like Cys-Cys loop peptides. CST displays an aminoacid sequence identical to SS in the portion of the molecule interacting with SS receptors; such identity accounts for the fact that CST binds all the SS receptor subtypes with affinity similar to that of SS, and that several physiological effects of CST are mediated *via* the activation of these receptors [2]. On the other hand, recent studies have shown that CST displays properties different and even antagonists with respect SS, such as the induction of slow-wave sleep and the reduction of locomotor activity [3]. Moreover, CST has been shown to signal through receptors distinct from those for SS, namely the putative CST receptor MrgX2 [4] and the GHSR-1a ghrelin receptor [5]. Although CST expression was initially reported to be restricted to the cerebral cortex and hippocampus, it is currently known that the peptide has wide distribution in many organs, notably including the immune system. CST -but not SS- was shown to be expressed and produced by different human cell types of the immune-inflammatory lineage, suggesting a possible role for CST in the regulation of immune-inflammatory responses [6]. In fact, in different models of immune-inflammatory systemic disorders, such as endotoxemia and inflammatory bowel disease, CST down-regulates the inflammatory response mediated by activated macrophages, decreases neutrophils and monocytes levels into inflamed organs, while increasing anti-inflammatory signals, i.e. IL-10 [7; 8]. This evidence candidates CST as an endogenous immuno-modulatory agent with potential therapeutic applications; however, its role in the control of immune-inflammatory responses within the CNS compartment remained so far unexplored. In this study we compared the effects of CST and SS on the production and release of prostanoids from primary cultures of rat cortical microglia and astrocytes. The levels of immuno-reactive PGE2 measured in the incubation medium of cultured cells, either under basal conditions or after stimulation by Interleukin-1 Beta (IL-1β), were taken as an index of cyclo-oxygenase (COX) activity. In astrocytes, CST did not modify basal PGE_2 release in the range of concentrations 1-100 nM, while it was able to partially counteract IL-1β-stimulated PGE_2 production in 24-h experiments; such reduction reached statistical significance at 10 and 100 nM concentrations. At variance with astrocytes, CST reduced in a significant manner basal release of PGE_2 from microglia; the drug was also able to completely counteract, at 10 and 100 nM concentrations, the increase in prostanoid release induced by the cytokine. In the same experimental paradigm, SS did not inhibit either basal or stimulated PGE_2 release from both astrocytes and microglia. In order to better understand the molecular mechanisms underlying the inhibition of prostanoid production elicited by CST in microglial cells, we investigated the effects of CST on COX-2 gene expression by real-time polymerase chain reaction (RT-PCR). The treatment with 100 nM of CST fully reverts the increase in COX-2 expression caused by a 4-h exposure to IL-1β. In conclusion, findings presented in this work suggest a role for CST as an endogenous factor with a immuno-modulating anti-inflammatory profile within the CNS. In fact, the microglia is classically considered a sub-population of CNS-resident macrophages, and usually the COX-2 pathway is activated in parallel and/or downstream to the pathways that are activated in the regulation of immune-inflammatory responses.

References

[1] de Lecea L, Criado JR, Prospero-Garcia O, et al. 1996. A cortical neuropeptide with neuronal depressant and sleep-modulating properties. Nature 381: 242-245.

[2] Siehler S, Nunn C, Hannon J, et al. 2008 Pharmacological profile of somatostatin and cortistatin receptors. Mol Cell Endocrinol. 286: 26-34.

[3] Spier AD, de Lecea L. 2000. Cortistatin: a member of the somatostatin neuropeptide family with distinct physiological functions. Brain Res Rev 33: 228-241

[4] Robas N, Mead E, Fidock M. 2003. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. J Biol Chem 278: 44400-4

[5] Broglio F, Papotti M, Muccioli G, Ghigo E. 2007. Brain-gut communication: cortistatin, somatostatin and ghrelin. Trends Endocrinol Metab., 18, 246-51

[6] van Hagen PM, Dalm VA, Staal F, Hofland LJ. 2008. The role of cortistatin in the human immune system. Mol Cell Endocrinol. 286: 141-7

[7] Gonzalez-Rey E, Varela N, Sheibanie AF, et al. 2006. Cortistatin, an antiinflammatory peptide with therapeutic action in inflammatory bowel disease. Proc Natl Acad Sci U S A 103: 4228-33

[8] Gonzalez-Rey E, Chorny A, Robledo G, Delgado M. 2006b. Cortistatin, a new antiinflammatory peptide with therapeutic effect on lethal endotoxemia.J Exp Med., 203, 563-71.