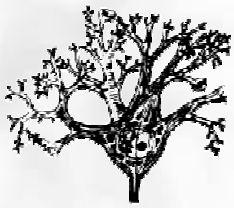

Gruppo Italiano
Studio Neuromorfologia
G.I.S.N.

Accademia delle Scienze
Bologna

21 - 22
Novembre 2008

18° Convegno Nazionale

Gruppo Italiano per lo Studio della Neuromorfologia (G.I.S.N.)

Accademia delle Scienze
Via Zamboni, 31
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Con il patrocinio ed il supporto della Società Italiana di Anatomia ed Istologia (SIAI)

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THE G.I.S.N. RESEARCH GROUPS: UNIVERSITA' CATTOLICA DEL S.CUORE

Fabrizio Michetti
Università Cattolica del S. Cuore, Roma, Italy

The research group of the Institute of Anatomy and Cell Biology of the Università Cattolica del S. Cuore in Rome (chairman: Fabrizio Michetti) is involved in research essentially addressing neurodegeneration and neurorepair processes, with special emphasis to the role of neural proteins. The evaluation of neural proteins as biomarkers of brain injury is also actively investigated.

In particular, the administration of the neurotoxicant trimethyltin (TMT) offers an animal model of neurodegeneration characterized by massive neuronal loss and gliosis in the limbic system (mainly in the hippocampus) accompanied by significant behavioural alterations, including cognitive deficit. TMT-induced neurodegeneration in cell and hippocampal organotypic cultures is also actively investigated by this group. The main goal of this research line, which has also investigated and currently investiagates the TMT-induced cellular and molecular mechanisms leading to neurodegeneration (including gene expression profile, apoptosis, mitochondrial involvement in cell death) deals with the verification of the hypothesis that definite calcium-binding proteins (calretinin-CR and parvalbumin-PV) exert a neuroprotective role against the intracellular calcium overloading accompanying TMT-induced neuron death. A series of data published in recent years by this research group, indicating that definite neuron subpopulations containing CR or PV are selectively spared by the neurotoxicant, and that TMT-induced neuron death is due to intracellular calcium dysregulation, which is substantially reduced in CR- and PV-containing neurons, support this hypothesis, which nevertheless still deserves additional investigation to be conclusively demonstrated.

The evaluation of the S100B protein as a marker of active brain injury, and the study of the active role possibly exerted by this protein in brain injury constitutes another main research topic of this group. After the first demonstration, obtained by this research group since the beginning of the Eighties, that the levels of S100B in the cerebrospinal fluid constitute an index of active brain injury, a series of data, published from this group and from other laboratories more directly involved in clinical studies, confirmed the reliability of this biomarker, which is currently investigated in a wide variety of pathological conditions (from stroke to schizophrenia) and of biological fluids (cerebrospinal fluid, blood, urine, amniotic fluid, saliva). The possibility that S100B, released in biological fluids, also actively participates in the cascade of events leading to brain injury is currently investigated by this group.

The main facilities, and the related experimental techniques, that this group makes available to collaborating G.I.S.N. groups include: Affymetrix microarray system ,laser-capture microdissector, real-time PCR lightcycler, confocal microscope, transmission and scanning electron microscopes, animal care house and experimental surgery platform (stereotaxic neurosurgery).

Human Adult Stem Cells and Cardiovascular Regenerative Medicine: Rescuing infarcted myocardium and conduction system

Carlo Ventura

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The use of human adult stem cells for cardiac cell therapy is hampered by an extremely low yield of spontaneous cardiovascular commitment *in vitro* and *in vivo*. Cell lineage specification is fashioned at multiple interconnected levels and is controlled by a complex interplay between cell signaling, nucleosomal assembly, the establishment of multifaceted transcriptional motifs and the temporal and spatial organization of chromatin in loops and domains. We have developed a mixed ester of hyaluronan with butyric and retinoic acid (HBR) and provide evidence that it acted as a novel cardiogenic/vasculogenic agent in human mesenchymal stem cells (hMSCs) isolated from bone marrow (BMhMSCs), and alternative sources, including dental pulp (DPhMSCs), and fetal membranes of term placenta (FMhMSCs). HBR remarkably enhanced the gene expression of vascular endothelial growth factor (VEGF), KDR (a major VEGF receptor), and hepatocyte growth factor (HGF). Notably, HBR increased the secretion of the angiogenic, mitogenic, and antiapoptotic factors VEGF, and HGF, priming stem cell differentiation into endothelial cells. HBR also increased the transcription of the cardiac lineage-promoting genes GATA-4 and Nkx-2.5, and the yield of cardiac marker-expressing cells. These responses were significantly more pronounced in FMhMSCs than in DPhMSCs or BMhMSCs. Transplantation of FMhMSCs into infarcted rat hearts was associated with increased capillary density at the infarct border zone, near-normalization of left ventricular function, and significant decrease in scar tissue. Transplantation of HBR-preconditioned FMhMSCs further enhanced capillary density and the yield of human von Willebrand factor (hvWF)-expressing cells, additionally decreasing the infarct size. Some engrafted, HBR-pretreated FMhMSCs, were also positive for connexin 43, and cardiac troponin I. Thus, cardiac rescue by HBR-exposed FMhMSCs was mainly mediated by a large supply of angiogenic and antiapoptotic factors and involved increased FMhMSC differentiation into vascular cells. Concerning the conduction system, it is now becoming evident that hMSCs form purkinje fibers in fetal sheep heart and that hMSCs-derived cardiomyocytes may also be applied as bio-pacemakers. These findings may contribute to further development in cell therapy of heart failure. Within this context, FMhMSCs may hold promise for allogenic, “off the shelf” strategies of cardiovascular rescue.

GASTROINTESTINAL MOTILITY AT THE CELLULAR LEVEL: MUSCLE, NERVES AND INTERSTITIAL CELLS OF CAJAL

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La motilita gastrointestinale a livello cellulare: musculi, nervi e cellule interstiziali di Cajal

The function of the gastrointestinal tract is controlled by a dynamic interaction between different cell types that interact either directly or through a large number of signaling molecules. Enteric neural integrity is essential for normal gastrointestinal motility, as is a constant communication between the enteric and the central nervous system. Enteric neurons are classified into intrinsic primary afferent neurons (IPANs), interneurons, motor neurons, and intestinofugal neurons. A fourth class of enteric neurons, intestinofugal afferent neurons (IFANs), have their cell bodies within the myenteric plexus but send their processes out of the gut wall. Smooth muscle cells form an electrical syncytium within the gut and are innervated, directly or indirectly through interstitial cells of Cajal (ICC) by neurons. Not only are smooth muscle cells the final effector cells that result in gastrointestinal motility, they also have an active role in the control of motility through mechanosensitive ion channels. The basic electrical rhythm of the gut, the slow wave, originates from a complex network of cells known as ICC. ICC generate and propagate the slow wave, control the smooth muscle membrane potential gradient, are mechanosensors and are required for cholinergic and nitrergic neurotransmission. Other cellular elements such as the immune system and enteric glia are now increasingly understood to be actively involved in the modulation of intestinal function. In summary, complex interactions between these cell types results in effective coordination of motility, secretion and blood flow in the gastrointestinal tract. Much progress has been made in the recent years on the understanding of these complex interacting networks. Loss of subsets of enteric nerves, of ICC, malfunction of smooth muscle and alteration in immune cells have been identified as the basis of many motility disorders. We now need to understand the initial factors triggering these changes and how to intervene to prevent, halt and reverse them.

GIOVANNI MINGAZZINI AND CLINICAL ANATOMY OF NERVE CENTRES

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GIOVANNI MINGAZZINI E L'ANATOMIA CLINICA DEI CENTRI NERVOSI

Due to their clinical and functional co-relations, neuroanatomy is still a fundamental discipline to fully tackle the neuroscience field, today one of the most stimulating lines of research that compares many challenges of clinical medicine,. The orientation of Giovanni Mingazzini's studies, from the beginning of his scientific undertaking and then along his gradual heading towards more modern perspectives, are a good reflection of the transformation of thought, at first "freniatrico" and then neurological. This development influenced the work of important figures between the end of the 18th century and the beginning of the 19th. The techniques of fine anatomical investigation, pillars of first Normal and then Pathological Anatomy, extended the boundaries of morphology that together with physiology led the way to Neuropathology, able to examine even the psychic and mental phenomenon through by a practical localisation of nerve centres. History teaches that the promoters of certain new ideas apparently fade behind the strength of the ideologies of their successors, but even today the experience of Mingazzini, his school and the arguments of his time, offer historic anchorage not to be let go of in the uncertainties of "nerve and mental pathology". A look to diverse psychiatric trains of thought and to neurology tendencies at the beginning of the 20th Century certainly could not ignore the arrival of ever more sophisticated instruments, which at the time allowed for important advancements in the diagnostics of nervous system illnesses, heralding new models for understanding, not just treating, neurological and psychiatric pathologies.

MAGNETIC RESONANCE IMAGING FOR THE STUDY OF THE 'IN VIVO' HUMAN BRAIN: ANATOMICAL AND FUNCTIONAL TECNIQUES

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La Risonanza Magnetica per lo studio del cervello umano in vivo: tecniche anatomiche e funzionali

Advances in non-invasive brain scanning and imaging technologies over the last two decades have opened up promising new methods of work for researchers and clinicians. Neuroimaging techniques have enabled scientists to look for the first time into the human brain *in vivo,* both anatomically and functionally. The advent of computational neuroanatomy has allowed for morphometric evaluations over the whole brain. For example, structural Magnetic Resonance Imaging (MRI) brain scans can be analyzed by Voxel-based Morphometry (VBM), an objective and automatic procedure that identifies regional differences in relative gray matter density; it allows every point in the brain to be considered in an unbiased way, with no *a priori* regions of interest. Alternatively, when the focus is on anatomical connectivity, MR images can be sensitized to the diffusion of water molecules within the voxel, and from these images it is possible to compute the local direction of greatest diffusion through Diffusion Tensor Imaging (DTI), a techniques that allows us to visualize the location, the orientation, and anisotropy of the brain white matter tracts. Besides, functional magnetic resonance imaging (fMRI) let us literally watch the brain while it works: it measures the haemodynamic response related to neural activity, showing which areas of the brain are active at any given time. fMRI has revealed exciting insights into the spatial and temporal changes underlying a broad range of brain functions, including perceptual and motor abilities, normal and abnormal language processes and higher cognitive processes.

SESSIONE I

SYSTEMATIC, CHEMICAL AND DEVELOPMENTAL NEUROMORPHOLOGY

NEUROMORFOLOGIA DESCRITTIVA, CHIMICA E DELLO SVILUPPO

OLFACTORY ENSHEATHING CELLS: AN OPTIMAL SUBSTRATE FOR HIPPOCAMPAL NEURONS. A STUDY *IN VITRO*

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GLIA OLFATTIVA: UN OTTIMO SUBSTRATO PER I NEURONI IPPOCAMPALI.
UNO STUDIO *IN VITRO*

Olfactory ensheathing cells (OECs) are generated by the nasal olfactory mucosa and migrate from the olfactory nerve to the first two layers of the olfactory bulbs. With the properties to both astrocytes and Schwann cells, OECs are also responsible for secreting growth factors as NGF, BDNF, bFGF, and neurotrophin 3/4/5. In addition to neurotrophic factors, OECs cells express laminin, fibronectin, S100, cell adhesion molecule (N-CAM).

Over the past 10 years OECs have emerged as a leading reparative candidate. When OECs are transplanted into the injured spinal cord, they have shown significant promise in regeneration of spinal cord lesions. It has been demonstrated that OECs integrate extensively in astrocytic environments of glial scars, while Schwann cells do not integrate. OECs are therefore better candidates than any other types of cells for transplant-mediated repair of CNS damage.. As previous investigations reported the positive effect of OECs on the hypothalamic neuronal survival in cultures and as OECs represent a source of trophic factors, in this study we aim to evaluate the efficacy of OECs on the hippocampal neurons in vitro. Primary neuronal cultures were obtained from postnatal rat (P2) hyppocampus and treated with trypsin and grown on poly-L-lisine–coated coverslips in DMEM/FCS. OECs were prepared from postnatal rat (P2) olfactory bulbs and, after incubation with trypsin, they were mechanically dissociated and plated on coverslips containing the hypothalamic cultures previously described. Some coverslips of hippocampus neurons were considered as controls. Conditioned medium from OECs cultures was used to feed some hippocampal neurons coverslips. After a week, both hippocampus cultures and co-cultures were processed by immunohistochemical procedure, using poly and monoclonal antibodies (PGP 9.5 and S-100) as different neuronal and glial markers. Our results show that in co-cultures of hippocampal neurons and OECs the number of neurons was significantly increased in comparison with control cultures. Moreover, these neurons exhibited a dense axonal outgrowth. OEC-conditioned media stimulated the neuronal survival.

In conclusion, our data suggest that OECs, as are source of growth factors, exert a positive effects on survival and growth of hippocampal neurons “in vitro”. OECs might be considered a better approach for functional restoration and for neural plasticity.

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY (SPECT), IN VIVO MOLECULAR IMAGING TECHNIQUE OF CORTICAL (ALZHEIMER'S DISEASE) AND SUBCORTICAL FORMS OF NEURODEGENERATIVE DEMENTIA (PARKINSON'S DISEASE; DEMENTIA WITH LEWY BODIES).

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TOMOGRAFIA AD EMISSIONE DI FOTONE SINGOLO (SPECT), UNA TECNICA DI IMAGING MOLECOLARE IN VIVO DELLE FORME CORTICALI (MALATTIA DI ALZHEIMER) E SOTTOCORTICALI (MALATTIA DI PARKINSON CON DEMENZA, DEMENZA CON CORPI DI LEWY) DELLE DEMENZE DEGENERATIVE.

Dementia is a term used to describe various different brain disorders that have in common a loss of brain function that is usually progressive and eventually severe.

The clinical identification and differential diagnosis of dementias is more important, especially now that several medications for the treatment of mild to moderate Alzheimer's disease (AD) are available.

The identification and differential diagnosis of AD is especially challenging in its early stages, because of the difficulty in distinguishing it from the mild decline in memory that can occur with normal aging and from mild cognitive manifestations of other neuropsychiatric conditions, such as depression, as well as other causes of dementia.

Alzheimer's disease can be diagnosed definitively only by histopathologic examination of brain tissue, but the important rule of Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) has been largely demonstrate.

Many neurodegenerative diseases produce significant brain-function alterations detectable with PET or SPECT even when structural images with CT or MRI reveal no specific abnormalities.

SPECT is currently more used than PET in neurodegenerative syndromes, mainly in the study of dementia and movement disorders; it is highly sensitive method for the early diagnosis of underlying neurodegeneration and the functional changes in the basal ganglia and it is less expensive than PET.

The specificity, and diagnostic accuracy of the metabolic and perfusional pattern of bilateral temporo-parietal hypoperfusion allow differentiation between AD and other degenerative dementia.

However the differential diagnosis between Alzheimer's disease and dementia with Lewy bodies (DLB) is complex and also neuropathology studies demonstrate a high rate of misdiagnosis.

SPECT with 123 I-Ioflupane, commonly used for differential diagnosis between Parkinson disease(PD) and Essential Tremor, is a new and interesting technique used in identifying patients with DLB versus AD.

AUTOPHAGY PROMOTES SURVIVAL OF DOPAMINERGIC NEURONS

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L'AUTOFAGIA PROMUOVE LA SOPRAVVIVENZA DEI NEURONI DOPAMINERGICI

Dopaminergic (DA) containing neurons *in vivo* and *in vitro* possess a marked tendency to promote autophagy following a variety of toxic stimuli. On the other hand it is well known during Parkinson's disease DA neurons of the substantia nigra pars compacta (SNpc) undergo increased autophagy. Among the various DA neurotoxin/methamphetamine (METH) is probably the strongest autophagy inducer and a variety of *in vitro* studies demonstrated a massive autophagic induction following METH exposure. Despite such a clear evidence no study was designed to evaluate if autophagy produces either a protection or a deleterious effect under stress conditions. The few evidence available suggests that autophagy is part of a neurotoxic pathway contributing to neurotoxicity. In the present study we provide evidence *in vitro* showing that blockade of autophagy is instead deleterious for DA containing cells and under autophagic inhibition neuronal inclusions produced by METH are converted into frank cell death. The evidence for such protective role for autophagy was extended *in vivo* to C57black mice treated with METH. While METH alone is able to produce only the loss of striatal DA terminals with intact cell body, in the presence of autophagic inhibition, METH administration results in the loss of DA neurons including the cells body in SNpc and VTA. The present data indicate that autophagy is a powerful neuroprotective mechanism own by DA neurons to counteract the extension of the damage to the cell body. These findings call for the use of autophagic inducers to prevent DA cell death in a variety of experimental and clinical conditions.

ISOLATION OF SINGLE PURE INCLUSIONS IN NEURODEGENERATION

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ANALISI DI SINGOLE INCLUSIONI CARATTERISTICHE DI MALATTIE NEURODEGENERATIVE

Analysis of neuronal inclusions represent a novel strategy to understand the pathophisiology of a variety of neurodegenerative conditions. In fact, beside representing a milestone in the pathology of a variety of degenerative disorders, these pathological organelles maintain enzymatic activity and possess selective proteins which witness for the onset of the disease and the ongoing disease mechanisms. One of the major problem in studying neuronal inclusions derives from a technical difficulty in identifying specific classes of inclusions and isolating them from the cells. In fact, laser microssection may allow to isolate a neuronal inclusion visualized at light microscopy. However light microscopy does not really provide specific information on the fine structure of the intracellular bodies despite of the stain procedures. For instance, in keeping with the frequent alpha-synuclein (α-syn) immunostaining, which characterized intracellular bodies, there is a wide variety of structures which may stain for α-syn. It is necessary to go behind light microscopy in order to dissect the bulk of α-syn positive structures. To follow this approach we used a transmission electron microscopy which was applied to specifics cells fractions which were exposed to α-syn primary antibody bounded to biotyne. In order to isolate α-syn positive inclusions e magnetic beads (covered by a monolayer of streptavidine) were added to the solutions and removed by a magnet. Following ultrastructure observation we were able to analyze single purified α-syn structures to describe the colocalization of other proteins and, for the first time, to analyze the proteomic of purified inclusions independently by the altered proteomic within inclusion containing cells. These experiments were carried out in dopamine containing cells which provide the reliable sources filled with neuronal inclusions following specific neurotoxic treatments.

AUTOPHAGY AND MITOCHONDRIOGENESIS

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AUTOFAGIA E MITOCONDRIOGENESI

One of the major clearing system which characterized the eukariotic cells is the autophagic pathway further classified into microautophagy, chaperon mediated autophagy and microautophagy, for now on simple referred as autophagy . Differing from other protein clearing system (i.e. Ubiquitin protein system) the autophagy pathway is not very much limited by substrate and is able to metabolize entire organelles as mitochondria. Since recently literature shows that neuroprotection may be achieved by promoting the autophagic pathway, a major dilemma rose up concerning the risk of rendering the cells pure of mitochondria reservoir. In fact if the rate of mitochondria clearance is accelerate one would expect the removal of damaged organelles. On the contrary, the lack of the very same organelles to provide baseline energy store, in our recent study, we provide unexpectedly that autophagy inducers (lithium and rapamicine) beside promoting the clearance of mitochondria are also powerful inducers of mitochondriogenesis. This was shown by increase mitDNA and mitRNA citofluorimetry, mitotrack staining, mitochondria count and others methods. Such unexpected findings are fascinating since they provide a physiological compensation which keep the neuroprotective effect of autophagy and save level concerning mitochondria depletion. It is presently unknown which is the signaling pathway which bound autophagy and mitochondriogenesis. The ability to dissect these processes may help to differentially modulate this phenomenon.

BBB ALTERATIONS IN THE TMT TREATED RATS: NEUROIMAGING AND NEUROMORPHOLOGY

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ALTERAIONI DELLA BBB NEI RATTI TRATTATI CON TMT: NEUROIMAGING E NEUROMORFOLOGIA

TMT is well known to produce a distinct pattern of selective neuronal degeneration in the rodent CNS. Usually, damage visualization is done by immunohistochemical study. MRI is a non-invasive and dynamic technique, which is used in vivo for studies of biological systems. TMT intoxication is an important factor linked to induction of brain edema. Aquaporin-4 (AQP4), a water transporting protein, is thought to be the primary route by which water moves in and out of astrocytes, is over-expressed in some pathological conditions, and is a marker of the vascular permeability. The aim of our study was to investigate the integrity of the blood brain barrier (BBB), using a fluorescent tracer (rhodamine isothiocyanate), and the possible correlations between brain morphological changes at MRI and the expression of AQP4 by astrocytes, after TMT intoxication. Adult female Wistar rats were treated with TMT. The controls received an equivalent amount of saline solution. Three control rats and 8 treated rats were given an intraperitoneal injection of RhIC dissolved in normal saline. The brains were isolated; consecutive coronal sections were prepared on a vibratome and than incubated with primary fluorescent antibodies AQP4-Ig rabbit and GFAP-Ig rabbit. Imaging of fluorescence was performed on a confocal microscope. The brains of the rats injected i.p. with RhIC were isolated, fixed in PFA 4% and sections were cut for fluorescence. Although data in literature document absence of alterations of the BBB in TMT treated rats, the results of the MRI investigation with Gd-DTPA suggest the presence of such alterations that concur with the passage of contrast into the damaged tissue. We didn't observe the leakage of RhIC from the blood vessels into the parenchyma in treated rats, but we observed an increase of the vascularization. Preliminary data of the immunofluorescence showed an upregulation of the AQP4 in astrocytes after TMT intoxication, suggesting that the cells are involved in edema formation and a possible alteration of the vascular permeability.

INNERVATION OF PALATINE TONSILS IN SHEEP (*Ovis aries*)

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INNERVAZIONE DELLE TONSILLE PALATINE DI PECORA (*Ovis aries*)

The palatine tonsils (PTs), one of the components of the Waldeyer's ring, seem to play an important role in prions infection and neuroinvasion (Andreoletti et al., 2000; Jeffrey et al., 2001; van Keulen et al., 2002). Considering that in the process of neuroinvasion prions probably reach the central nervous system (CNS) by means of nervous fibres, knowledge of PTs innervation could provide a contribute to understand some aspect of sheep scrapie, the prototype of transmissible spongiform encephalopathies.

To study the connections between PTs and CNS, the fluorescent retrograde tracer Fast Blue (FB) was injected in the PTs. A large number of FB-labelled neurons was observed in the sympathetic cervical cranial ganglia (CCG). FB-labelled neurons were also found in the proximal (PGG) and distal (DGG) glossopharingeal ganglia, in the proximal vagal ganglia (PVG), and in the trigeminal ganglia (TG). Only few FB-labelled neurons were found in the pterygopalatine parasympathetic ganglia and in the cervicothoracic sympathetic ganglia.

Immunohistochemistry was performed on PTs, CCG, PGG, PVG and TG cryosections. Immunoreactivity (IR) for tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (DBH), nitric oxide synthase (NOS), calcitonin gene-related peptide (CGRP), and substance P (SP) was observed in fibres surrounding the PTs lymphatic nodules. Furthermore, TH- and DBH-IR fibres were seen also within the lymphatic nodules. In the PGG/PVG and TG, FB-labelled neurons expressed NOS-, CGRP-, and SP-IR. In the CCG the greater number of FB-labelled neurons were also TH- and DBH-IR.

The present results allow to attribute PTs innervation to vagal, glossopharyngeal and trigeminal cranial nerves and to postganglionic fibres of the sympathetic CCG and parasympathetic PPG. It is to note that the great involvement of sympathethic CCG in PTs innervation could be attributable to the involvement of such nervous division in the immune regulation of primary and secondary lymphoid organs (Bellinger et al., 2008).

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SESSIONE II

TROPHIC FACTORS, NEUROMEDIATORS AND RECEPTORS

FATTORI TROFICI, NEUROMEDIATORI E RECETTORI

CEREBRAL EFFECTS OF OBESOGENS

EFFETTI CEREBRALI DEGLI OBESOGENI

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Many substances commonly present in environment and food, known as "*endocrine disrupters*" are able to interfere with hormonal pathways in vertebrates. One of this substances is tributyltin (TBT), employed as antifouling agent in paint for marine shipping and for a variety of other uses. TBT may acts as obesogen, activating RXR and PPAR receptors and inducing adipogenetic genes synthesis for adipocites diffrentiation (1).

Aim of this work was studing if early exposition to TBT may not reversibly alter circuits controlling food intake and predispose organisms to obesity.

In this study we used mice from Y1/LacZ strain, transgenic mice with Y1 receptor promoter linked to reporter gene LacZ (2). TBT, diluited in olive oil, was orally administred at dose of 0,025µg/g/day/body weight to adult animals for 4 weeks and to pregnant females from gestational day 8 (G8) to pups postnatal day 21 (P21), day of pups' sacrifice.

Adult of both sexes show statistically significant reduction of food intake in comparison to controls, but no differences were find in body weight. On the contrary, in treated animals we observed a great reduction of blood leptin levels comparing to controls. This may be considered an indirect obesogenic effect because in treated animals food intake is reduct but body weight doesn't change and blood leptin levels collapse in comparison to controls, suggesting for a possible alteration of brain circuits controlling food intake.

In pups treated during perinatal life, we observed a significant reduction in body weight in comparison to controls and high leptin levels on P21. This is agree with recent studies which demonstrated that newborn male rats treated with leptin may develop leptin resistance and increase body weight in adult life (3).

Moreover, we analized brain sections cut with cryostate and processed for immunohystochemistry anti-NPY. These data show a significant reduction of peptide expression in adult male mice in comparison to controls, in PVN, DM, ArC. This is very interesting considering low blood leptin levels that normally are associated to high NPY expression.

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

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Dopamine receptors in bronchus associated lymphoid tissue (BALT)
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XVIII Convegno Nazionale GISN Accademia delle Scienze Bologna 21-22 Novembre 2008.
I recettori dopaminergici nel BALT

This study investigates age-related changes of the dopamine receptors in bronchus associated lymphoid tissue (BALT). Morsels of bronchial tissues were drawn from lungs, during autopsies, of 30 years (young), and 74 years (old/aged) humans.
Fluorescence techniques were used, associated with image analysis for the detection of dopamine receptors. Dopamine D1a and D1b receptors were stained using specific fluorescent monoclonal antibodies. The BALT of young men possesses a higher number of D1a and D1b receptors, while, in old men, these receptors are strongly reduced.

Fig. 1 — Scanning Electron Microscopy (SEM) of a sample of a bronchiole of a young man (30 years of age). One islet of bronchus associated lymphoid tissue is completely surrounded by the epithelial cells of the bronchiole. 100X; linear size scale 25 μm.

Fig. 2 — Light microscopy (LM) of a sample of the lung of a young man (30 years of age) stained
with Eosin-orange. B=Bronchiole (calibre ~ 200μm). Boxed area: bronchus associated lymphoid tissue (BALT). 400X; linear size scale 100 μm.

Fig. 3 — Fluorescent microscopy (FM) of same sample as in Fig. 2 stained for D1 dopamine receptors. B = bronchiole (calibre ~ 200 μm). Numerous fluorescent sites corresponding to the D1dopamine receptors are located in bronchiolar and /or in BALT tissues (Boxed area). 400X; linear size scale 100 μm.

Fig. 4 — Fluorescent microscopy (FM) of a sample as in Fig 3, but harvested from a old man (74 years of age), stained for D1 dopamine receptors. B= Bronchiole (calibre ~ 200μm). The fluorescent sites are strongly decreased as consequence of age-related changes. 400X; linear size scale 100 μm.

Fig. 5 — Fluorescent microscopy (FM) of a sample of bronchiolar tissue of a young man (30 years of age) treated for the demonstration of D2 dopamine receptors. Numerous fluorescent structures indicate the presence of D2 receptors in BALT. 100X; linear size scale 25 μm.

Fig. 6 — Same microscopic field and same treatment as in Fig.5 but in a bronchiolar sample coming from an old man (74 years of age). The fluorescent structures (D2 dopamine receptors) are localized around the BALT while in the core of the BALT the fluorescent structures are strongly decreased. 100X; linear size scale 25 μm.

The possible significance of reduced Da1 and Da2 dopamine receptors in BALT of aged men is discussed.

DOPAMINERGIC RECEPTORS IN THE NATIVE HUMAN HEARTS

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XVIII Convegno Nazionale GISN Accademia delle Scienze Bologna 21-22 Novembre 2008.

I recettori dopaminergici nel cuore umano

1) Morsels of human heart were obtained from native normal hearts in case of transplants , avoiding of course any potential risk for the patients. Till now four hearts were studied, coming from four young brain-dead donors (mean age 27 years±2). For technical reasons these hearts were not used for transplants. On this precious material we performed our experiments.
2) The present study investigated the presence of D1-D5 dopaminergic receptors sub-types in the human heart. Dopaminergic nerve fibres, running in the human heart, justify the presence of dopaminergic receptors in this organ.
3) Our results demonstrate that only four subtypes of dopaminergic receptors can be found in cardiac tissues (D1,D2, D4, D5, but not D3). Moreover, immunoblot analysis shows the same four subtypes of dopaminergic receptors migrating in polyacrilamide gel electrophoresis and/or in nitrocellulose membranes between 60 and 45 KDA (Kilodalton) corresponding to a molecular weight of about 60/62 KDa as Bovine Serum Albumin and/or 42/45.000 KDa as ovoalbumin.
4) D1-D5 dopamine receptors are distributed in the wall of both atria and/or ventricles of the human heart . A trans-mural gradient of D1-D5 dopamine receptors can be described in the wall of the human heart . Sections of atria and/or ventricles exposed to antidopamine D1-D5 or D2-D4 receptor antibodies showed fluorescent positive reaction in the epicardium and/or in the myocardium. D4 receptor immune-reactivity was remarkably less intense than D2 receptor immune-reactivity.
5) All the subtypes of dopamine receptors are in close relationships with all cardiac structures, controlling many cardiac functions.
6) Our morphometrical data confirm that all the layers and all the structures of the human heart express D1,D2,D4 and D5 receptor subtypes, but no D3.
7) D1 receptors were stored primarily in the epi-cardial layer and/or in the myocardium.
8) These findings suggest the possible role of dopamine receptors in controlling many functions of the human heart.

DISTRIBUTION OF VGLUT-1 AND VGLUT-2 IMMUNOREACTIVITY WITHIN PUTATIVE GLUTAMATERGIC AXON TERMINALS IN ADULT RAT CEREBELLAR CORTEX

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DISTRIBUZIONE DI TERMINALI PUTATIVI GLUTAMATERGICI IMMUNOREATTIVI PER VGLUT-1 E VGLUT-2 NELLA CORTECCIA CEREBELLARE DI RATTO ADULTO

Three systems of glutamatergic fibres exist in the cerebellar cortex. Two are extrinsic, represented by the systems of afferent fibres to the cortex (mossy and climbing fibres); one is intrinsic, represented by the system of parallel fibres. These systems end in the cortex according to specific patterns: the mossy fibres end in the granular layer at the level of synaptic glomerular complexes; the climbing fibres end mainly in the inner zone of the molecular layer, on the proximal dendrite tree of the Purkinje neurons; the parallel fibres end diffusely in the molecular layer, on dendrite three of the Purkinje neurons. In this study we analyzed the distribution of two markers of glutamatergic axon terminals, the Vesicular Glutamate Transporter-1 (VGLUT-1) and the Vesicular Glutamate Transporter-2 (VGLUT-2) (1) and correlated data on their distribution with those on the distribution of terminals of the above glutamatergic fibre systems. The aim was to evaluate whether the systems of glutamatergic fibres of the cerebellar cortex express specifically VGLUT-1 or VGLUT-2 or both. Moreover, with the aim of studying molecular mechanisms of exocytosis of glutamate-containing synaptic vesicles, we investigated whether VGLUT-1 and/or VGLUT-2 co-localize/co-localizes with the synaptosomal associated protein of 25 kDa (SNAP-25) (2). All studies were carried out on adult rat cerebellar cortex using LM immunofluorescence techniques for single or double labelling. Results. VGLUT-1 was observed in punctate elements diffusely distributed in the neuropil of the granular layer (terminals of mossy fibres) and molecular layer (terminals of parallel fibres). VGLUT-2 was observed in punctate elements distributed mainly in the inner zone of the molecular layer (terminals of climbing fibres) and punctate elements in the granular layer (terminals of mossy fibres). Techniques of double labelling revealed co-localization of VGLUT-1 and SNAP-25 in the molecular and granular layers (axon terminals of parallel fibres and, respectively, mossy fibres), and of VGLUT-2 and SNAP-25 only in the granular layer (axon terminals of mossy fibres), whereas no co-localization was observed in the molecular layer (where terminals of climbing fibres lie).

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EXPERIMENTAL STUDY ON THE EFFICACY OF METHYLPREDNISOLONE SODIUM SUCCINATE ADMINISTRATION AFTER ACUTE SPINAL CORD CONTUSION INJURY IN RATS

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STUDIO SPERIMENTALE SULL'EFFICACIA DEL TRATTAMENTO CON MTEILPREDNISOLONE SUCCINATO DOPO LESIONE MIDOLLARE CONTUSIVA ACUTA NEL RATTO

The beneficial neuroprotective effects of high-dose methylprednisolone sodium succinate (MPSS) in the treatment of acute spinal cord injury (SCI) have been recently questioned. In this study we evaluated, in a standardized rat experimental model, whether MPSS reduces the size of lesion area if systemically administered after traumatic SCI as recommended by NASCIS-2 and NASCIS-3 international protocols.

A contusion injury of 200 kdyn was produced in adult female rats at T10 vertebral level using the PSI Infinite Horizon impactor®. The rats were divided in two groups: MPSS-treated group and saline-treated group. Animals were behaviourally tested using BBB locomotor rating scale, inclined plane test, horizontal ladder, beam walk and 3D analysis of hindlimb motion. At week-7 postoperative, 2-cm-long spinal cord samples at the level corresponding to the impact site were withdrawn, fixed in 4% paraformaldehyde and embedded in paraffin. Specimens were serially sectioned and stained with luxol fast blue/cresyl violet and Cajal's silver method. The spatial extent and volume of injured spinal cord tissue was estimated using the Cavalieri stereological method.

Results showed that injured animals showed a progressive increase of functional recovery along the 7-weeks post-injury observation period. Functional recovery was not significantly different in MPSS-treated group compared to saline-treated group. Stereological analysis showed a large variability in the volume of injured tissue among animals and did not disclose a positive effect on the lesion volume and lesion length in the MPSS-treated group. Taken together results of this study suggest that MPSS failed to exert a protective effect after acute SCI.

ORGANIZATIONAL EFFECTS OF BISPHENOL-A ON KISSPEPTIN EXPRESSION IN THE HYPOTHALAMUS OF CD1 MOUSE

EFFETTI ORGANIZZAZIONALI DEL BISFENOLO-A SULL'ESPRESSIONE DELLA KISSPEPTINA NELL'IPOTALAMO DI TOPI CD-1

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The GnRH neurons of the hypothalamus play a pivotal role in the central regulation of fertility, however they lack estrogen receptor alpha and androgen receptor, therefore sex hormones should indirectly regulate GnRH secretion through other steroid-sensitive circuits. Recently, among these circuits the one characterized by the production of kisspeptin (a 54-amino acid protein encoded by the gene *Kiss-1*) has been considered to have a prominent role.

Estrogenic endocrine disruptors (EEDs) are naturally occurring or man-made compounds present in the environment that are able to bind to estrogen receptors and interfere with normal cellular development in target organs and tissues. Due to this ability, EEDs can interfere with the processes of sexual differentiation of brain and behavior.

In the present experiment, different doses of bisphenol-A (10, 20 or 40 μg/kg/day) were orally administered to pregnant CD1 female mice from prenatal day 10 to postnatal day 8. Puppies of both sexes were sacrified at the age of 2 months by intracardiac perfusion.

By immunohistochemical techniques we studied the kisspeptin system in the arcuate, periventricular, and anteroventral periventricular nuclei. Quantitative analysis of kisspeptin expression demonstrated a high sexual dimorphism in control animals: all considered nuclei have a higher cell number and fibers' density in females. Bisphenol-A significantly increased kisspeptin expression in males, inducing the disappearance of sexual dimorphism in all nuclei.

These results suggest that alterations of sexually dimorphic behaviors and of reproduction due to early exposure to Bisphenol-A may be linked to modifications of Kisspeptin-GnRH circuits.

Supported by Regione Piemonte.

III SESSIONE
ENTERIC AND PERIPHERAL NERVOSU SYSTEM
SISTEMA NERVOSO ENTERICO E PERIFERICO

INTRINSIC INNERVATION OF THE ILEOCAECAL JUNCTION IN THE HORSE

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INNERVAZIONE INTRINSECA DELLA GIUNZIONE ILEO-CIECALE NEL CAVALLO

Abstract

REASON FOR PERFORMING STUDY: In horses functional studies related to the enteric nervous system (ENS) controlling the sphincters are lacking.

OBJECTIVES: We investigated immunohistochemically the morphology, distribution, density, phenotypes, and projections of neurons controlling the ileocaecal junction (ICJ).

METHODS: Two young horses were anesthetized and underwent laparotomy. Neuronal retrograde fluorescent tracer fast blue (FB) was injected into the wall of the ICJ. A postsurgical survival time of 30 days was used. Following euthanasia, the ileum and a small portion of caecum were removed. Cryostat sections were used to investigate the immunoreactivity (IR) of the neurons innervating the ICJ for choline acetyltransferase (ChAT), neuronal nitric oxide synthase (nNOS), substance P (SP), calcitonin gene-related peptide (CGRP), and neurofilament NF200kDa (NF).

RESULTS: Ileal FB-labeled neurons innervating the ICJ were located in the myenteric (MP) and submucosal plexus (SMP) up to 48 cm and 28 cm, respectively, from the point of the FB injections. Descending MP and SMP neurons were nitrergic (54±11% and 68±4%, respectively), cholinergic (60±19% and 82±11%, respectively), NF-IR (54±9% and 78±21%, respectively), and SP-IR (about 20% in both the plexuses). CGRP-IR was expressed only by SMP descending neurons (45±21%). In both the plexuses descending neurons co-expressing nNOS- and ChAT-IR were also observed (25±11% and 61±27%, respectively).

CONCLUSIONS: The presence of long projecting neurons controlling the ICJ this far from the sphincter indicates that, during small intestine resection, it is preferable, whenever is possible, to conserve at least the last portion of the ileum in order to avoid problems of food transit between the ileum and the caecum, and to prevent gaseous reflux from the caecum into the ileum.

POTENTIAL RELEVANCE: The knowledge of the phenotype of ENS neurons of the ileum might be helpful for developing pharmaceutical treatment of the ICJ motility disorders.

FIRST EVIDENCE FOR THE INNERVATION OF THE PHEROMONAL GLAND IN THE GYPSY MOTH *LYMANTRIA DISPAR*

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PRIMA PROVA DELL'INNERVAZIONE DELLA GHIANDOLA A FEROMONE NELLA FALENA EUROPEA *LYMANTRIA DISPAR*

The gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Limantriidae) is a polyphagous pest that can exploit a variety of species of deciduous and coniferous hosts, causing severe ecological and economic costs. Several physical, chemical and biological fight means remain of partial effectiveness, show undesired side effects and are rather expensive. The mating success of *L. dispar* depends on the production and release of the sex pheromone (+)disparlure by calling female moths and understanding of the biological basis of the pheromone production and release may result crucial for the successful design and application of new control strategies. In this insect, no clear-cut evidence is available in support of either a neural or an hormonal mechanism of regulation of the pheromonal gland activity. With the aim of contributing to the comprehension of the biological aspects of the pheromonal secretion and release, we further examined the organization and structure of the *L. dispar* pheromonal gland. The monolayered gland cells are covered by the folded cuticle of the intersegmental membrane between the 8^{th} and 9^{th} abdominal segments. The cells bear a large, often irregularly shaped nucleus, granules of variable amount and electron-density and mostly located in the basal compartment of the cytoplasm, a rich labyrinthine zone laying on a basement membrane, different junctional structures, and an apical membrane packed with microvilli. Nerve fibres enwrapped in glia approach the glandular epithelium and occur beneath the basement membrane, in close contact with the secretory cells. This finding represents the first evidence for the innervation of the pheromonal gland in *L. dispar* and definitely supports the relevance of a neural mechanism in the control of its pheromone production. Junctional systems with the aspect of gap junctions connecting adjacent gland cells were also detected. Their occurrence indicates the possibility of functional diffusion of a local nerve imput to neighbouring cells, as well as the metabolic coupling of adjacent cells. Some lines of evidence suggest that the biogenic monoamine octopamine may be involved in the neural control of the gland.

QUANTITATIVE EVALUATION OF NEURONS AND GLIAL CELLS IN MYENTERIC GANGLIA OF HUMAN COLON

Chiara Ippolito[1], Cristina Segnani[1], Corrado Blandizzi[2], Roberto De Giorgio[3], Nunzia Bernardini[1]
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VALUTAZIONE QUANTITATIVA DELLA COMPONENTE NEURO-GLIALE NEI GANGLI MIENTERICI DI COLON UMANO

Background. An important requirement in pathological diagnostics in the human enteric nervous system is the estimation of the total numbers of myenteric neurons and glial cells. While myenteric ganglia wholemounts are the best preparations for total cell counting, archivial wax-embedded samples are often the only available material. In order to obtain valid counting of myeteric ganglia cells on paraffin sections, detailed morphological studies are required taking into account some technical notes.
Aim. To count myenteric neurons and glial cells in paraffin samples of human colon.
Patients and Methods. Full thickness distal colonic samples were collected from patients undergoing surgery for uncomplicated colon cancer. The colonic neuromuscular compartment, with particular regard to myenteric ganglia, was evaluated in paraffin serial cross-sections. CB, HuC/D and NFL or S100β and GFAP stainings were employed to examine body numbers of neural and glial cells, respectively.
Results and Conclusions. HuC/D and S100β antigens were found to be the best markers for detection of neurons and glial cells, respectively. There were significant correlations between numbers of neurons/glial cells and the respective myenteric ganglion areas. The number of estimated myenteric cells is comparable to that reported in wholemount preparations thus confirming the validity of these evaluations on sections from archivial colon paraffin-wax blocks. These findings suggest that HuC/D-S100β immunostained paraffin cross-sections of human colon may be regarded as valuable tools for quantitative estimation of myenteric neurons and glial cells.

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DOPAMINE, TRANSPORTERS AND RECEPTORS: MOLECULAR BIOLOGY AND MORPHOLOGICAL ANALYSIS IN IMMUNE PRIMARY AND SECONDARY ORGANS

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Dopamine plays a role in neuroimmune modulation and induces selective effects on immune cell subsets. Peripheral blood lymphocytes contain and synthesize dopamine and express dopamine receptor subtypes. The presence of a thymic and splenic dopaminergic system was first suggested by studies identifying the expression of dopamine receptors in these organs.
Dopamine localization and the expression and localization of vesicular monoamine transporter (VMAT) type-1 and 2 and of dopamine D1-like and D2-like receptor subtypes were investigated by immunohistochemical, immunochemical techniques and by RT-PCR. Thymus, spleen and fycoll-isolated thymocites and splenocites of 1-month-old male Wistar rats were used. In the thymus and spleen, dopamine was developed in the cortico-medullary junction and medullary and white pulp border respectively.
Both thymus and spleen expressed VMAT-1 and VMAT-2 immunoreactivity as well as dopamine D1 and D2-like receptors. Immunohistochemistry revealed VMAT-1, VMAT-2 and dopamine receptor immunoreactivity primarily in the thymic cortical-medulla transitional zone and to a lesser extent in the medulla but not in the cortex. In the spleen, VMAT-1, VMAT-2 and receptor immunoreactivity was located primarily in the white pulp border and to a lesser extent in the white pulp. These findings indicate that both thymus and spleen express a dopaminergic system characterized by the presence of dopamine, VMAT-1 and -2 and the D1- and D2-like receptors. The presence of these dopaminergic markers suggests that dopamine likely originating from immune cells and from sympathetic neuroeffector plexus is released in the lymphoid microenvironment. Based on the microanatomical localization of dopaminergic markers investigated will play a role in maturation, selection and activation of immunocompetent cells.

HUMAN AND MOUSE ENS NEUROSPHERE TRANSPLANTS REGULATE THE FUNCTION OF AGANGLIONIC EMBRYONIC DISTAL COLON.

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NEUROSFERE UMANE E DI TOPO TRAPIANTATE NEL COLON DISTALE AGANGLIARE DI EMBRIONI DI TOPO SONO IN GRADO DI REGOLARNE LA FUNZIONE.

Enteric nervous system progenitor cells (ENSPC) differentiate after transplantation, but their functional effects have not yet been demonstrated. We presently investigated whether transplanted embryonic mouse and neonatal human ENSPC can regulate the contractility of an aganglionic bowel. These two types of ENSPC were grown as neurospheres, transplantated into aganglionic embryonic mouse hindgut explants taken at E11.5 and maintained for 10 days in culture, to reach term equivalence. Engraftment and neural differentiation were confirmed using immunofluorescence and TEM. Contraction frequency and calcium movement of transplanted bowel was measured and compared to that of E11.5 ganglionic and aganglionic bowel cultured for the same period. TTX was used to assess neural modulation of bowel contractility. Anti c-Kit antibodies were added at the start of some explant cultures to block ICC differentiation. At E11.5 the colon exhibited no immunoreactivity for neurons, ICC or muscle cells, but ganglionic samples cultured for 8 days had a staining pattern comparable to that of a P0 bowel; aganglionic untransplanted colon lacked neurons; ICC were present in both transplanted and untransplanted aganglionic bowel but not in the specimens treated with anti-c-Kit antibodies. Both mouse and human ENSPC were seen migrating and differentiating after neurosphere transplantation and TEM demonstrated neuronal differentiation and synapses formation. Transplantation restored intracellular free calcium levels and contraction frequency of the aganglionic bowel to that of ganglionic one. TTX reversed both these effects, indicating neurosphere-derived neurons are functioning. c-Kit blocking antibody inhibited the contraction of ganglionic and aganglionic colon explants, indicating ICC are essential in generation of contractions. Briefly: neonatal human gut is a source of ENSPC that can be transplanted in an aganglionic bowel, grafted mouse and human ENSPC neurospheres regulate aganglionic colon motility by a neurally-mediated mechanism, ICC are necessary for bowel contractility.

IV SESSIONE

NEURODEGENERATION, NEUROREGENERATION AND PHARMACOLOGYCAL THERAPY

NEURODEGENERAZIONE, NEURORIGENERAZIONE E TERAPIA FARMACOLOGIA

DEXAMETASONE AND ESTRADIOL UP AND DOWN MODULATE NEURAL BIOMARKERS EXPRESSION IN RAT BONE MARROW MESENCHYMAL STEM CELL CULTURES

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IL DESAMETASONE E L'ESTRADIOLO MODULANO L'ESPRESSIONE DI ALCUNI BIOMARKERS NEURALI IN COLTURE DI CELLULE STAMINALI MESENCHIMALI DI MIDOLLO OSSEO DI RATTO

MSCs are multipotent cells in the bone marrow stromal line, known for their potential to differentiate into several types of cells.
It is well known that growth factors are mitogenic polypeptides playing a crucial role during astroglial and neuronal cell proliferation and differentiation in culture. In addition, dexamethasone (DEX) and estradiol (E_2) are neurosteroids acting in neural cell line and in particular in astroglial compartment.
The aim of the investigation was to study the effects of dexamethasone (DEX) or estradiol (E_2) on proliferation and differentiation of rat bone marrow mesenchymal stem cells (MSCs) in culture, evaluating by western blot and immunocitochemical analysis of some specific neural proliferative and differentiative markers.
MSCs were harvested from bone marrow of femurs of 4-8 month-old rats. Cytofluorimetric analysis revealed that MSCs were negative for CD45, CD34 and positive for CD90, CD105.
After 24h starvation period, MSC cultures were treated for 48h with DEX 10^{-9} M or E_2 5 x 10^{-9} M. Qualitative and quantitative analysis were performed by immunocytochemical and western blot analysis respectively for nestin, neurofilament, β-tubulin and MAP-kinase.
Our results show an enhancement of the above mentioned neural markers and MAP-Kinase in MSCs cultures treated with DEX. E_2-treatment increased MAP-Kinase and β-tubulin expression, but it decreased nestin and neurofilament expression.
In conclusion, our results indicate that DEX induces an up regulation of some neural protein expression indicating an its possible role played on differentiation of these MSCs to neural line. In addition, it shows an involvement on signal transduction patways. On the contrary, E_2 treatment induces up and down modulation of nestin, neurofilament, β-tubulin and MAP-kinase expression. Moreover, are in progress experiments on DEX-growth factors crosstalk on GFAP, vimentin, nestin, PARP and MAP-Kinase expression in astroglial cell cultures at 15 DIV, in order to compare the different effects evaluated in both in vitro models. This to better clarify the molecular mechanisms underlying biochemical and immunocytochemical characterization of neural and stem cell line induced by neurosteroids and/or growth factors.

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ALPHA7 nAChR AGONIST: ANTI-HYPERALGESIC AND NEUROPROTECTIVE ROLE ON NEUROPATHY

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STIMOLAZIONE DEL RECETTORE NICOTINICO ALPHA7: RUOLO ANTIPERALGICO E NEUROPROTETTIVO IN CORSO DI NEUROPATIA

Lesions or diseases of the peripheral or central nervous system arise for neuropathic syndromes. Spontaneous and evoked types of pain are the main clinical characteristic of this widespread disorder. Aimed to enquire the anti-neuropathic role of the alpha7 nicotinic receptor stimulation, we evaluated the pharmacological profile of the alpha7 agonist PNU-282987 in a model of peripheral neuropathy, obtained by the loose ligation of the rat sciatic nerve (CCI).

Acute administration of PNU-282987, 10 and 30 mg kg^{-1} p.o. (15 days after ligation), was able to reduce hyperalgesia in the paw pressure test in a more long-lasting manner in respect to nicotine treatment (1,5 mg kg^{-1} i.p.). The alpha7 antagonist methyllicaconitine (1 mg kg^{-1} e.v.) reverted this effect. No analgesic effects were detected. Chronic PNU-282987 treatments, 30 mg kg^{-1} once a day for 7 days and 10 mg kg^{-1} for 28 days were performed to evaluate the synergistic effects on pain threshold and neuroprotection. These treatments were both able to decrease pain perception and, in the meantime, to ameliorate the morphological features of the injured nerve. The histological studies performed on Azan-Mallory stained samples described that CCI model is able to induce oedema among the fibers and a macrophagic infiltrate, mainly distal to the ligation site and more evidently at six days after ligation than at 28. Moreover, osmicated preparations allow to observe a significant degeneration of axons, particularly of the large fibers: the axons were less compact and showed decreased diameter, the myelin sheath was significantly thinned. Repeated treatment with PNU-282987 is able to protect the nerve tissue, reducing the presence of oedema and macrophagic infiltrate. On the coronal sections of the nerve a significant higher myelin sheath and axonal diameter were observable. These results strongly suggest the pivotal role of alpha7 nAChR in the neuroprotection during neuropathy.

TISSUE ENGINEERING OF DAMAGED PERIPHERAL NERVES: NEGATIVE RESULTS

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INGEGNERIA TISSUTALE DEI NERVI PERIFERICI LESIONATI: RISULTATI NEGATIVI

Tissue engineering for the reconstruction of peripheral nerves has raised great interest over the last years. A number of experimental studies showing the potential effectiveness of both cell and tissue transplantation in promoting nerve regeneration has been published and negative results have almost never been reported so far. Here we report on the results of two experimental studies which demonstrated that both cell and tissue transplantation can, in fact, hinder the nerve regeneration process.

In the first study, 1-cm-long sciatic nerve defects in adult male rats were repaired either PLGA conduit alone or enriched with cultured neural stem cells (NSC-PLGA). Nerve fiber regeneration was assessed by stereology and functional recovery was assessed by SFI calculation and ankle kinematics. In the second study, 1-cm-long median nerve defects in adult female rats were repaired either skeletal-muscle-vein-combined (SMVC) conduits or adipose-tissue-vein-combined (ATVC) conduit. Nerve fiber regeneration was assessed by stereology and functional recovery was assessed by grasping test.

Results showed that both PLGA and SMVC conduits, in agreement with previous studies, led to successful nerve fiber regeneration and functional recovery. By contrast, in rats where NSC-PLGA and ATVC conduits were used, nerve fiber regeneration was worse as shown by significantly lower number and smaller diameter of axons. Yet, in case of nerve repair by ATVC conduits also functional recovery was significantly reduced in comparison to SMVC.

Taken together, these negative results on cell and tissue transplantation for nerve reconstruction suggest that tissue engineering of damaged nerves should be dealt carefully, especially in the perspective of a clinical application, since the use of inadequate cells or tissues, both heterologous and autologous, can significantly hinder the axon regeneration process and eventually functional recovery.

JNK IN PERIPHERAL NERVE REGENERATION

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JNK NELLA RIGENERAZIONE DEI NERVI PERIFERICI

Sciatic nerve ligation (SNL) and axotomy is a useful animal model to investigate neuropathic pain. Previous studies demonstrate that the proinflammatory cytokine interleukin-1(IL-1)-β modulates both the generation and the maintenance of inflammatory and chronic neuropathic pain. IL-1β can also activate JNK via MKK and then phosphorylate c-Jun to form an activator protein 1 (AP-1) complex to mediate many cellular events, including cell proliferation, transformation, survival, death, and immune response. This signal pathway is involved in IL-1β-induced neuropeptide Calcitonin Gene-Related Peptide (CGRP) secretion. CGRP is a 37-amino acid peptide that is widely distributed in the mammalian nervous system and may play an important biological role in physiological and pathological states.

The nature of peripheral nerve injury has an impact on CGRP expression dynamics: SNL, which is associated with complete blockade of axonal transport, induces a sustained decrease in CGRP-immunoreactivity (IR) in rat primary sensory neurons of L4-L5 dorsal root ganglia (DRGs).

The aim of this study was to investigate the changes, following SNL and axotomy, in CGRP-IR in DRG neurons of ko mice for single JNK isoforms compared to wild type. We intend to correlate the obtained data with the functional activation of p-c-Jun after axotomy to clarify if it has a role in peripheral axonal regeneration and also which JNK isoform is responsible for CGRP-IR.

Before performing the experiment we assessed that there were no differences in the footprint patterns between wt and ko animals. At different time intervals from lesion, ipsi- and contralateral L3-L6 DRGs were studied by IHC against CGRP, p-c-Jun and substance P. In separate sets of experiments, JNK and p-JNK expression was analyzed by WB.

Preliminary WB results show similar JNK activation in the untreated Ko mice for single JNK isoforms compared to wild type. The quantitative analysis of the CGRP and p-c-Jun immunopositive profiles in the DRGs 72h following SLN was assessed with the Neurolucida software. We find a marked decrease in the densities of CGRP-IR neurons both in wild type and JNK3 ko mice compared to the controls. In contrast p-c-Jun profiles were significantly increased in the ipsilateral DRGs of KO3 mice respect to wt and untouched animals.

On the ground of these observation we propose that JNK3 is not directly responsible for the regulation of CGRP expression. c-Jun phosphorylation my be elicited by JNK1/JNK2 isoforms, which are mostly involved in neuron survival and axonal regeneration.

Supported by EC Stressprotect and Regione Piemonte grants.

PERIPHERAL NERVE REGENERATION ALONG MUSCLE-VEIN-COMBINED BIOENGINEERED SCAFFOLDS.

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RIGENERAZIONE NERVOSA PERIFERICA LUNGO INNESTI BIOINGEGNERIZZATI DI MUSCOLO-IN-VENA.

Peripheral nerve reconstruction is a fascinating scientific field which brings together basic and clinical neuroscience. The aim of this study was to investigate the efficacy of a tissue engineered repair strategy for peripheral nerve lesions with substance loss based on the employment of a vein segment filled with skeletal muscle to bridge the nerve gap.
10-mm-long defects of rat median nerve were repaired using fresh muscle-vein-combined bioengineered scaffolds and posttraumatic functional nerve recovery was monthly assessed by grasping test until 6 months after surgery. In a subgroup of animals, repaired nerves were collected at 5, 15, 30 days postoperatively. All nerve samples were analysed by light, confocal and electron microscopy and by RT-PCR.
Behavioural analysis showed that functional recovery started at month-2 postoperatively and, at month-6 was not significant different than controls. Morphological analysis showed the early presence and proliferation of Schwann cells along the conduits. Yet, it was shown that fresh skeletal muscle fibers which were used to enrich vein tubes support axon regrowth and Schwann cell migration by means of their basal lamina. Stereological quantitative analysis showed that nerve maturation along bioengineered scaffolds was still not complete at the 6-month postoperative time point.
On the other hand, RT-PCR permitted to investigate the expression of mRNAs coding for glial markers as well as the glial growth factor (NRG1) and its receptors (erbB2 and erbB3). We could demonstrate the presence of an early overexpression of NRG1, erbB2 and erbB3 during nerve regeneration. These results suggest the existence of a NRG1's autocrine/paracrine trophic loop shared by both glial and muscle fibers which could be responsible for the effectiveness of muscle-vein-combined conduits for repairing nerve defects.

NEUROGENESIS IN DORSAL ROOT GANGLIA OF ADULT RATS AFTER TRAUMATIC LESION OF BRACHIAL PLEXUS NERVES

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NEUROGENESI NEI GANGLI SENSITIVI DI RATTI ADULTI IN SEGUITO A LESIONE TRAUMATICA DEI NERVI DEL PLESSO BRACHIALE

One of the consequences of a crush injury applied to the median, ulnar and radial nerves at their point of origin from the brachial plexus, is damage to dorsal root ganglia (DRGs) neurons, specifically belonging to the cervical (C5-8) and thoracic (T1) levels. The aim of this study was to define with morphological and quantitative methods the effect of a peripheral nerve lesion in the corresponding DRG neurons in adult rats. Results from *in vivo* experiments were further supported by an *in vitro* study using a DRG explant model.
Under operative microscope, each nerve was carefully exposed from its origin at brachial plexus and then the crush lesion was applied using a non-serrated clamp that could guarantee standardized and reproducible lesion, in terms of force and pressure exerted, as well as, duration of the compression. Animals were then sacrificed at 1, 5 and 10 days after the injury and DRG corresponding only to the level C5-T1 were extracted. The ultrastructural analysis showed that, already 5 days after surgery, most of neurons appear extremely rich in subcellular organelles and neurofilaments, thus suggesting that those cells positively reacted to the nerve damage. Only few apoptotic cells were detected. Moreover, an unusual number of small size cells morphologically different from the glial satellite cells were detected. Immunohistochemical analysis in laser confocal and electron microscopy performed in crush-injured animals pre-treated with BrDU, revealed a relevant number of BrDU-positive neurons suggesting that neurogenesis occurs in adult DRGs that undergo peripheral nerve injury. Stereological quantification confirmed that the total number of sensory neurons significantly increased in DRGs after nerve-crush compared to control. Moreover, at different post-operative time points, we investigated the different pattern of protein expressed during axonal regeneration of the sensory neurons both *in vivo* and *in vitro*. Results of the proteomic analysis revealed a group of proteins, mostly recognized as cytoskeleton proteins, differently expressed in crushed DRG compared to control.

PROTECTIVE EFFECTS OF ACETYL-L-CARNITINE IN SCIATIC NERVE FOLLOWING LOOSE LIGATION: A FUNCTIONAL AND MORPHOLOGICAL STUDY.

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Chronic pain syndromes with allodynia and hyperalgesia, altered functionalities and sensitivity, are defined as peripheral neuropathies. Histologically they are characterized by tissue degeneration and nerve damage with cell loss due to apoptosis or necrosis. In a model of peripheral neuropathy, obtained by the loose ligation of the rat sciatic nerve, hyperalgic effects and morphological changes of nerve structure in the portion encompassing approximately 1 cm upstream (proximal) and 1 cm downstream (distal) from the ligation were investigated. Analysis included control animals, acetyl-L-carnitine-treated (ALCAR; 100 mg/Kg i.p. twice daily for 14 days) or gapapentin-treated (70 mg/Kg i.p. twice daily for 14 days) rats.

Morphologically, Masson's trichromic technique was used for evaluating sciatic nerve microanatomy and Kluver Barrera's staining for assessing myelin deposition. Phosphorylated 200-kDa neurofilament (NFP) and myelin basic like-protein (MBP) immunoreactivity were assessed by immunohistochemistry associated with quantitative analysis.

Reduction of myelin deposition, axonal injury and accumulation of inflammatory cells were detected in the distal zone of ligated sciatic nerve. The proximal part of the ligated nerve displayed the typical normal pattern of nerve fibre organization, with a morphology similar to that of the controlateral non-ligated nerve. A decreased NFP immunoreaction and a redistribution of MBP immunoreactivity, with a small immunostained clusters, were observed in the distal part of ligated nerves. Treatment with ALCAR and gabapentin induced anti-hyperalgesic effects. Treatment with ALCAR but not with gabapentin prevented neuromorphological changed and increased axonal NFP immunoreactivity.

The occurrence of relevant microanatomical changes in the sciatic nerve after loose ligation, further supports the view that this type of intervention may represent an useful model to evaluate neuropathy-related functional and morphology changes. ALCAR described previously as regulator of cell death protector, exerted anti-hyperlagesic effects and prevented myelin and axonal alterations in damaged sciatic nerve.

MOLECULAR MECHANISMS INVOLVED IN NEURONAL CELL DEATH BY DEAFFERENTATION AND TARGET ABLATION IN THE DEVELOPING VISUAL SYSTEM.

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MECCANISMI MOLECOLARI COINVOLTI NELLA MORTE CELLULARE NEURONALE IN SEGUITO A DEAFFERENTAZIONE E PRIVAZIONE DEL BERSAGLIO DURANTE LO SVILUPPO DEL SISTEMA VISIVO.

The developing rodent visual system represents a stimulating experimental model for studying developmentally-regulated neuronal death. Retinal ganglion cells project to the superficial layers of the superior colliculus (SC) and to the dorsal lateral geniculate nucleus (dLGN), which in turn has its major projection to the visual cortex. In rodents, the mature distribution of retinal axons to the primary visual centers is achieved following postnatal refinement, consisting of pruning of ipsilateral projections. At the end of development only a small fraction (around 10%) of the ipsilateral projection is maintained. In the same early postnatal period, primary visual centers undergo developmentally regulated neuronal death, and are more sensitive than in the adult to visual system manipulation. In fact, neonatal eye enucleation by depriving the contralateral primary visual centers of 90% retinal projections increases significantly developmental neuronal death due to deafferentation. This is particularly consistent in the superficial layers of the SC. On the other hand, also the loss of the axonal target (target deprivation) has dramatic effects on neuronal death, such as it happens in the dLGN following neonatal visual cortex ablation. We have studied in the past the molecular pathways involved in neuronal death in the developing visual system, identifying a key role for caspase 3 and caspase 8 activation and poly (ADP-ribose) polymerase (PARP), and apoptotic cell death as the main cell mechanism. More recently, we have being considering the role of c-Jun N-terminal kinase (JNK), as a molecule involved in neuronal death upstream of capsase-3 activation. We have shown that both deafferentation and target deprivation upregulate caspase-3 activation in the superficial layers of the SC and in the dLGN. The administration of the inhibitory peptide D-JNKI-1 or (XG-102) significantly prevents the increase in caspase 3 activation in both primary visual centers, thus inferring that JNK activity blockade can improve neuronal survival and JNK plays a key role in neonatal neuronal death. In fact, stereological counts show that neuronal survival following JNK blockade is increased in primary visual centers. Neuronal tract tracing experiments are actually performed to elucidate the projection of the geniculate neurons which have lost their visual cortical target, and probably project elsewhere. Therefore, JNK could be a promising target in case of perinatal lesions in the central nervous system in order to prevent neuronal death and promote brain repair.

Support: Stressprotect 6FP Grant and Regione Piemonte

V SESSIONE
ANIMAL MODELS OF NEUROPATHOLOGIES
MODELLI ANIMALI DI PATOLOGIE DEL SISTEMA NERVOSO

BRAIN MICROANATOMICAL CHANGES IN AN ANIMAL MODEL OF HYPERALDOSTERONISM.

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Renin-angiotensin-aldosterone (RAA) system is responsible for blood pressure and volume homeostasis. Inappropriate activation of RAA system is associated with increased cardiovascular damage and deleterious clinical outcomes. Both clinical and preclinical studies have suggested additional, direct roles for aldosterone on cardiovascular and cerebrovascular systems. Elevated plasma aldosterone levels represent a risk factor for stroke and cerebral disorders, although mechanism(s) underlying this role remain(s) to be elucidated. The present study was designed to asses the effects of treatment with aldosterone alone or with salt load on the morphology of the central nervous system in an animal model of hyperaldosteronism.

Male Wistar rats of 8 weeks of age were treated for 28 days with aldosterone (40 μg/Kg/day) via osmotic mini-pumps with or without addition of 1% NaCl to drinking water. In control animals, mini-pumps were filled with vehicle and no salt was added to drinking water. The morphology of intracerebral and pial arteries, nerve cell number, aquaporin-4 (AQP-4), glial fibrillary acidic protein (GFAP) and phosphorylated 200-kDa neurofilament immunoreactivity were assessed by quantitative microanatomical and immunohistochemical techniques.

Rats treated with aldosterone alone or plus salt load developed arterial hypertension. In small intracerebral arteries (diameter range 25-10 μm) narrowing of arterial lumen and a consequent increase of the wall-to-lumen ratio were observed . An increased intensity of AQP-4 immunostaining was noticeable in intracerebral small vessels of rats treated with aldosterone plus salt load. Nerve cell loss and cytoskeletal breakdown occurred primarily in the frontal cortex and hippocampus of rats treated with aldosterone alone or plus salt load.

The above data indicate that administration of aldosterone is associated with central nervous system injury characterized both by microvascular changes, nerve cell loss and neurofilament breakdown. Based on our data we are unable to establish if changes in brain microanatomy, observed after aldosterone administration, is related with blood pressure rise or with neurotoxicity linked to specific mineralcorticoid receptor activity.

Study supported by the grant MIUR-COFIN No. 2006060985_003.

NG2-EXPRESSING OLIGODENDROCYTE PRECURSOR CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Cellule NG2-positive progenitrici di oligodendrociti nell'encefalomielite allergica sperimentale.

Cells that express the Nerve-Glial Antigen-2 (NG2) proteoglycan are the largest proliferative progenitor population in the postnatal Central Nervous System (CNS).
Multiple Sclerosis (MS) is a chronic inflammatory disease of CNS characterized by focal destruction of myelin. The pathological hallmarks of MS include perivenular immune cell infiltration, oligodendrocyte death, demyelination and axonal damage. Experimental autoimmune encephalomyelitis (EAE), an animal model of MS, has been extensively used to study the pathogenesis of MS and treatment options.
In this study we evaluated the response of endogenous $NG2^+$ oligodendrocyte precursor cells (OPCs) in adult mouse brain to demyelinating lesions in myelin oligodendrocyte glycoprotein (MOG) induced EAE. We examined the relationship between $NG2^+$ OPCs and mature neuroglia cells, both astrocytes ($GFAP^+$) and oligodendrocytes ($CNPase^+$ MOG^+ MBP^+), in the normal and demylinated brain. In control animals NG2-expressing cells are closely intermingled with other glia but represent a distinct cell population. In animals injected with MOG_{35-55} large areas of demyelination appeared at 10-12 days post-injection (dpi), coinciding with severe clinical symptoms. At 18-20 dpi, the response of $NG2^+$ OPCs to demyelination was represented by increase in number and NG2-immunoreactivity of these cells and alteration of morphology, being their bodies enlarged and their processes more numerous and intensely stained. The results suggest that endogenous $NG2^+$ OPCs increase in response to signals triggered by demyelinating events to generate a large progenitor population. The evidence of remyelination in EAE models is successful. Further studies are needed to understand how remyelination occurs, why it fails in MS and how remyelination can be enhanced.

EXPRESSION OF CAVEOLIN-1 PROTEIN IN BRAIN VASCULATURE OF MOUSE WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Espressione della proteina Caveolin-1 nell'encefalo di topo con encefalo-mielite sperimentale autoimmune.

The blood-brain barrier (BBB) is a complex unit primarily formed by endothelial cells sealed by tight junctions (TJs) which controls the exchanges of solutes between blood and brain and vice versa. Dysregulation of the BBB is involved in many CNS diseases, e.g. in multiple sclerosis (MS), where changes in the molecular composition of the TJs, with consequent increased permeability, are among the disease earliest events. It is known that also caveolin-1 (Cav-1), a major constituent of caveolae, plays different roles related to the unique feature of the BBB microvessels. Interactions between Cav-1 and several molecules, such as VEGF, MMP-2, glucose transporters, P-glycoprotein, and the TJ proteins, have been demonstrated and may have important implications in brain microvessel functions, including blood-brain barrier activity.

This study analyses expression and distribution of endothelial caveolin-1 in brain microvessels of mice with experimental autoimmune encephalomyelitis (EAE), employed as animal model of human MS. 20-μm vibratome sections from EAE brains and control samples were submitted to immunostaining by antibody against Cav-1 and viewed under a laser confocal microscope. The observations show that the endothelial cells of both large vessels and microvessels are Cav-1 immunoreactive and that the staining is markedly enhanced in EAE brains compared with controls ones. The increment of Cav-1 expression in EAE microvessels has been related to the reduced expression of the endothelial TJ protein Claudin-5 and to angiogenesis promotion occurring

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EXPRESSION OF INNATE IMMUNITY RECEPTORS IN AN EXPERIMENTAL MODEL OF PARKINSON'S - LIKE DISEASE

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ESPRESSIONE DEI RECETTORI DELL'IMMUNITA' INNATA IN UN MODELLO SPERIMENTALE DI PARKINSONISMO

The innate immunity receptors CD14 ant toll like receptor 4 (TLR4), that function as lipopolysaccharide (LPS) receptor and LPS signal transmitter into the cell respectively, have been recently related to neuroinflammation processes. Parkinson's disease (PD) is a progressive neurodegenerative disorder in which loss of dopaminergic neurons of substantia nigra (SN) occurs, leading to a drastic reduction of dopamine levels in the striatum. Recently it has been found that, both in patients and in experimental animal models of PD, neuroinflammation appears to be an ubiquitous finding. Upregulation of inflammatory response in the brain is associated with a number of neurodegenerative diseases; moreover recently have been found evidences that systemic infections and inflammation can cause exacerbation of symptoms and drive the progression of neurodegeneration in chronic neurodegenerative diseases. Therefore, the aim of this work was to study a possible role of LPS receptor complex in the pathogenesis of 1-methyl-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced parkinsonism.
In particular, we investigated the expression of CD14 and TLR4 in SN and striatum in brains obtained from MPTP treated mice, the most commonly used toxic model of PD in mouse. In particular, the analysis of the gene transcripts and protein expression of CD14 and TLR4 showed an augmented expression of both receptors in the SN of MPTP treated mice in comparison to untreated ones. No significant differences were found in the mRNA and protein levels of both CD14 and TLR4 in the striatum of MPTP treated animals if compared with controls.
Overall, these results show that innate immunity receptors CD14 and TLR4 are over expressed in distinct anatomical areas of the brain from mice with MPTP induced Parkinson's-like disease.

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EXPRESSION OF LAYER-SPECIFIC GENES IN THE CORTEX OF SOD1-MUTANT MICE, A MODEL OF FAMILIAL ALS

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ESPRESSIONE DI GENI STRATO-SPECIFICI NELLA CORTECCIA DI TOPI SOD1-MUTANTI, UN MODELLO DI SLA FAMILIARE.

Progressive loss of cortical motor cells and lower (cranial and spinal) motoneurons is the hallmark of the fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS). Mutations in the gene encoding superoxide dismutase (SOD)-1 have been discovered in familial ALS (FALS) cases, and mice overexpressing mutant human SOD1 are extensively used as murine models of FALS, as they develop a disorder resembling clinical and pathological features of the human disease. In these mice, spinal motoneuron pathology has been extensively studied, while information on corticospinal neurons, which reside in layer V, is limited, but we have recently documented progressive shrinkage of these cells and other cortical changes. A number of layer-specific transcription factors have been recently introduced as markers of layers during cortical development of several species, and expression of some of them persists in the adult cortex. We here investigated, by means of *in situ* hybridization, whether the expression of *ER81* was altered in the cortex of presymptomatic and end-stage SOD1(G93A) mice. *Er81*, a member of the Ets transcription factors, is expressed in most of layer V neurons projecting to the spinal cord or superior colliculus or contralateral cortex. The results we have hitherto obtained indicate a selective decrease of *Er81* transcript in neurons of the motor and somatosensory cortex of SOD1-mutant mice with respect to age-matched wild-type littermates, whereas other layer-specific genes (e.g. *RORbeta*, a marker of layer IV neurons) did not show overt differences between the two genotypes. The study of the visual cortex (which notably does not contain corticospinal neurons) is at present in progress as further assessment of the selectivity of the findings in SOD1-mutant mice. The data further emphasize the occurrence of changes in the cortex in murine FALS, pointing to the involvement of the entire motor circuit in the disease, and provide evidence that a layer-specific transcription factor is affected in a degenerative condition which involves the adult cortex.

DIENCEPHALIC CHANGES IN RODENT MODELS OF SLEEPING SICKNESS

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ALTERAZIONI DIENCEFALICHE IN MODELLI DI MALATTIA DEL SONNO IN RODITORI

Sleeping sickness or human African trypanosomiasis (HAT), a neglected re-emergent disease, is a severe infection caused by the parasite *Trypanosoma brucei* (*Tb*) transmitted by the vector tsetse fly (genus *Glossina*). The disease occurs in two stages: the first hemolymphatic stage follows the tsetse fly bite, and the onset of the second meningoencehalitic stage is marked by *Tb* passage across the blood-brain barrier. Stage 2 configurates a chronic neuroinflammatory condition which is fatal if untreated, and currently used drugs are very toxic. HAT, which occurs in several foci in sub-Saharan Africa, is hallmarked by disturbances of the sleep-wake cycle and sleep structure. Brain changes occurring during *Tb* neuroinvasion are still largely unknown. In a series of investigations, we have examined in mice and rats infected with *Tb brucei* (*Tbb*, a parasite strain which is not pathogenic to humans) the occurrence of changes in "diencephalic clocks", namely in the hypothalamic suprachiasmatic nucleus (SCN), which plays a role of master circadian pacemaker in the mammalian brain, and in neurons of the lateral hypothalamus containing the recently identified peptides orexin (Orx, which stimulates arousal) and melanin-concentrating hormone (MCH, which decreases arousal). In addition, we have also recently analyzed the same areas in another rodent model of the disease, represented by *Mastomys* infected with *Tb gambiense* (*Tbg*), which causes in humans the Gambian form of sleeping sickness. Altogether the data indicate that *Tbb* infection affects functionally SCN neurons eliciting also an activation of surrounding glial cells. *Tbg* infection, on the other hand, leads to surprisingly severe structural damage of SCN neurons. Both *Tbb* and *Tbg* affect the expression of hypothalamic neuropeptides which play a role in sleep-wake regulation, and we have evidence, in the *Tbb* infection model, also of a functional dysregulation of Orx neurons. Altogether the findings indicate that subsets of diencephalic neurons are markedly susceptible to the chronic neuroinflammatory pathology (which, during *Tb* infection, is caused by the host-parasite interplay), and this opens novel perspectives on neural-immune interactions.