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SOME MORPHOLOGICAL ASPECTS OF BRAIN MICROCIRCULATION

C. Cavallotti, F. M. Tranquilli Leali, M. Cameroni, P. Sagnelli
Department of Cardiovascular Respiratory and Morphological Sciences, University "La Sapienza" Rome, Italy cavallotti@uniroma1.it

Certain morphologic and physiologic data are essential for an understanding of the pathology of cerebral vascular disease. The most important consideration is the enormous oxygen requirement of the brain. It has been pointed out that the brain represents only 2 per cent of the body weight, yet in the resting state receives one-sixth of cardiac output, and is responsible for 20 per cent of the body's oxygen consumption. The responsibility for this disproportionately high requirement of the brain oxygen belongs principally to the nerve cells, by which is meant not only the nucleus and cytoplasm seen under ordinary magnification with traditional staining, but also the axonal and dendritic surfaces and connections.

Deprivation of oxygen results first in a loss of function followed by nerve cells destruction. The survival time of a nerve cell depends upon the duration and intensity of hypoxia under different circumstances, but experimentally it would appear that the nerve cell can resist total deprivation of oxygen for no longer than a few minutes.

All the major arteries at the base of the brain have a similar plan of branching. From each vessel there arise perforating branches, which provide blood to the midline structures. Short circumferential arteries supply structures out from the midline and long circumferential arteries extend out to the more distant parts of cerebrum and cerebellum.

The finer angio-architecture of the brain is like that elsewhere, a network of arteries, arterioles, capillaries, venules and veins, with generous anastomoses. This is in contrast in some of the older views, which held that the density of the capillary bed varies from place in place, and is presumed to be directly proportional to the metabolic activity of the area. As general rule, although not without its exceptions, capillary density corresponds to density of synaptic connections.

AGING OF THE RETINAL VESSELS

C. Cavallotti, F. M. Tranquilli Leali, M. Cameroni, P. Sagnelli
Department of Cardiovascular Respiratory and Morphological Sciences, University "La Sapienza" Rome, Italy cavallotti@uniroma1.it

The retina and the optic nerve head are the only parts of the central nervous system that can be inspected during life with an ophthalmoscopic examination.
Moreover, the ophthalmoscope allow us to inspect also the superficial retinal vessels.
The vascular supply of the retina comes from the ophthalmic branch of the internal carotid artery, which in turn gives origin to the central retina artery. The latter, upon issuing from the optic disk, divides into four arterioles, which supply the four quadrants of the retina.
The ganglion cells and bipolar cells receive their blood supply from these arterioles and their capillaries, whereas photoreceptor elements receive nourishment from the underlying choroidal vascular bed. These small vessels react in diseases like vessels of corresponding sizes in the brain.
Our previous results indicated that the aging and/or the deterioration of the capillary micro-circle can be estimated through the demonstration and the quantification both of the proteoglycans (important substances in the periendothelium tissue) and of proteins bound to the basal lamina (laminina and collagen IV).
The present results evidenced that: 1) the micro-angio-architecture of the eye vessels changes with the age; 2) the basal lamina of the capillaries and of all others vessels of the eye posses specific proteins, that are bound to the membrane (MBP= membrane bound protein). These proteins change with the age and pathological events 3) the sub-endothelial tissue of the eye vessels is rich of proteoglycans and glycosamino-glycans. Both these substances changes with the age; 4) the oxidative stress and the pigments, accumulated with the age also in the wall of the eye capillaries, modify the functional morphology of the capillaries of the eye with age.

POLYMORPHISMS IN DOPAMINE REGULATING GENES AND PREFRONTAL GRAY MATTER VOLUME

A. Di Giorgio[1,2,3], A. Papazacharias[1], G. Caforio[1], S. Aquilino[1], G. Blasi[1], T. Popolizio[3], M. Nardini[1], G. Pero[2], A. Bertolino[1, 3]

[1]Psychiatric Neuroscience Group, Section on Mental Disorders, Department of Psychiatric and Neurological Sciences, University of Bari, Bari, Italy; [2]Department of Chemical Sciences, Section of Biochemistry and Molecular Biology, University of Catania, Catania, Italy; [3]Section of Neuroradiology, IRCCSS "Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG), Italy.

Background: Functional polymorphisms in the Catechol-O-MethylTransferase (COMT) and the Dopamine Transporter (DAT) genes modulate dopamine *signaling*, which is crucial for normal function of prefrontal cortex (PFC) [1]. Recent evidence suggests also that dopamine may play an important role in brain development [2] and in ongoing local neural plasticity[3,4].

Objective and Hypothesis: The present study was designed to investigate the potential association between the COMT Val158Met and the DAT 3'VNTR polymorphisms as well as their interaction with brain morphometry. We hypothesized that genetically determined levels of dopamine may affect PFC grey matter volume.

Methods: 79 healthy individuals were genotyped for COMT and DAT polymorphisms. T1-weighted images were acquired on a 3T GE scanner (TR/TE/NEX=25/3/1; flip angle: 6°; matrix: 256 x 256; FOV: 25 x 25 cm; 124 sagittal slices, 1.3mm thickness). VBM was performed using a unified segmentation VBM protocol [5] implemented in SPM5 [6]. A multiple regression analysis was used to test for the association between gray matter volume and COMT, DAT polymorphisms as well as their interaction. Age, IQ, sex and total gray matter volume were used as covariates ($p<0.005$).

Results: We found an effect of both genotypes with the number of the COMT Met alleles and DAT 10-repeat alleles positively correlating with gray matter volume in PFC (MNI coordinates: 44, 30, 9). An inverse correlation was found with the COMT-DAT interaction term. Finally, a factorial ANOVA on gray volume extracted from the VOI in PFC demonstrated that subjects Met/Met and 10/10-repeat or with Val/Val and 9-carrier repeat genotypes had lower PFC gray matter compared with the other groups ($p =0.009$).

Conclusions: Consistent with our initial hypothesis, we demonstrated that COMT and DAT genotypes significantly contribute to modulate gray matter volume in PFC and that their interaction may explain up to 14% of variance.

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EFFECTS OF ENVIRONMENTAL DIOXIN CONTAMINATION ON THE EXPRESSION OF CALCIUM-BINDING PROTEINS IN THE SHEEP CEREBELLUM .

V. Maharajan[1], P.M. Mithbaokar[2], F.Fiorito[3], L.M. Pavone[2], L.Avallone and G. Paino[2]
[1]CNR Institute of Cybernetics, Pozzuoli, Naples, Italy; [2]Department of Biological Structures, Functions and Technologies and [3]Department of Pathology and Animal Health, University of Naples Federico II, Naples, Italy.

Environmental dioxin contamination in several industrialized parts of the world, including Italy, has demanded investigations to understand the cellular and molecular mechanisms of dioxin actions. The sheep from these contaminated areas displayed motor and ambulatory deficits suggesting us to investigate cellular alterations in cerebellum of animals exposed to dioxin. Herein we report immunohistochemical expression of calcium binding proteins, calbindin–D28k (CB) and Parvalbumin (PV) in cerebellum of neonatal and adult sheep from a dioxin contaminated area in the (Acerra, Naples Province) Campania Region of Italy in comparison with sheep of similar ages from an uncontaminated area in the same Region.
In control animals CB and PV showed specific and different staining patterns in adult and neonatal cerebellum but those in neonates were uniformly more intense than those in adults. While CB expression decreased in dioxin exposed cerebellum of adult and neonatal sheep, that of PV increased strikingly and uniformly in most cerebellar regions of both stages. Significantly, the fibers of white matter in adult and neonate showed uniformly increased calbindin and parvalbumin expression in animals from dioxin contaminated area. These results indicate an alteration induced by dioxin in the synthesis of these calcium binding proteins rather than a modification of their protein structures. The present study shows that alterations in the levels of calcium binding proteins could be a consequence of environmental dioxin on cerebellum of sheep or one of the mechanisms by which dioxin might induce motor and ambulatory deficits in exposed neonate and adult animals.

MATERNAL THYROID HORMONES ARE TRANSCRIPTIONALLY ACTIVE DURING EMBRYONIC DEVELOPMENT

C Nucera [1,4,6], P. Muzzi [2], C Tiveron [3], A Farsetti [4,5], F Moretti [4,5], L Della Pietra [4], F Mancini [4], A Sacchi [4], L Tatangelo [3], A Giaccari [6], F Trimarchi [1], F Vermiglio [1], A Vercelli [2], A Pontecorvi [4,6]

[1]Dip Clinico-Sperim di Med e Farmacol, Messina, [2]Dip Anat, Farmacol e Med Legale, Torino, [3]Centro Telethon, Ist Regina Elena, Roma, [4]Dip Oncol Sperim, Ist Regina Elena, Roma, [5]Ist Neurobiol e Med Molecol, C.N.R. Roma, [6]Ist Endocrinol, Univ Cattolica del Sacro Cuore "A. Gemelli", Roma.

Notwithstanding their role during development, direct evidence of the interaction of maternal thyroid hormones (TH) with embryonic thyroid receptors (TRs) is still lacking. Threfore, we generated a transient transgenic mouse (TTM) ubiquitously expressing a reporter gene (LacZ) tracing TH action. We engineered a construct TRE2× containing two TH-responsive elements controlling the expression of the LacZ which encodes for β-gal. TRE2× specificity was evaluated in NIH3T3 cells by cotransfecting TRE2× along with TRs and retinoic or estrogen receptors with their specific ligands. TRE2× transactivation was observed only upon physiological, TR mediated T3 stimulation. β-gal staining, absent up to embryonic day 9.5 (E9.5), was observed at E11.5 until the onset of fetal thyroid function (FTF) in several tissue primordia, overlapping with immunohistochemical TRs localization. Interestingly, no β-gal staining was detected in hypothyroid TTM. Our results provide the first in vivo direct evidence that during development and before the onset of FTF, maternal TH are transcriptionally functional through the action of TRs.

SOMATOSTATIN DEPLETION BY CYSTEAMINE IMPAIRS THE DEVELOPMENT OF TYROSINE HYDROXYLASE IMMUNOREACTIVE CHICK EMBRYO PONTINE NEUROBLASTS

Lofrumento D.D., Romano M.L., Casciaro M., Barba F., Delle Side A. and Nicolardi G.
Lab. Human Anatomy - DiSTeBA - University of Salento, Lecce.

Somatostatin (Som) is a neuropeptide widely distributed throughout the central nervous system (CNS) of vertebrates and also transiently expressed in many areas of developing brain, suggesting a possible regulative role. Moreover cysteamine (2-mercaptoethylamine, CSH) depletes Som in a dose- and time-dependent manner [1].

To study the possible role of Som in the development of tyrosine hydroxylase (TH) immunoreactive (ir) neuroblasts of the chick embryo nucleus of the locus coeruleus (LC) and subcoeruleus (SC), Som was depleted by 100 mg/kg CSH dropped twice a day on chorioallantoic membrane. 25 μm paraffin serial sections from embryo brains at embryonic day (E) 13, E15, E17 and E19 were incubated with an anti-TH antibody according to ABC technique. Digital images of TH-ir neuroblasts of both LC and SC were analysed to measure area, perimeter, minor and major axis, diameters ratio and shape factor.

Following CSH administration, neuroblasts area values resulted significantly reduced in chick embryo brains at E13 and E15 (treated vs controls: $p<0.01$). At E17 higher values were shown by treated specimens, while at E19 no difference was observed. Also perimeter values were reduced in neuroblasts belonging to E13-E15 CSH treated embryos ($p<0.01$), but no significant difference at E17 was observed. Cell diameters values resulted significantly lowered by CSH treatment: minor axis showed lower values at all embryonic day considered, while major axis only at E13-E15 ($p<0.01$). Diameters ratio and shape factor resulted higher in neuroblasts from treated specimens, mostly at E13-E15 ($p<0.01$); since higher values indicates less lengthened cells. Our data suggests that Som depletion by CSH impairs the development of the LC and SC TH-ir neuroblasts in the chick embryo according to preliminary data showing that Som depletion also blocked the migration of those neuroblasts [2].

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PSA-NCAM IN THE HUMAN NERVOUS SYSTEM AT PERINATAL AND ADULT AGE.

M. Quartu, M. P. Serra, M. Bo, V. Ibba, M. Del Fiacco
Dipartimento di Citomorfologia, Cittadella Universitaria di Monserrato, Università di Cagliari.

Polysialylated neuronal cell adhesion molecule (PSA-NCAM) is considered a marker of developing and migrating neurons and of synaptogenesis in the immature vertebrate nervous system. However, it persists in the mature normal brain in some regions which retain a capability for morphofunctional reorganization throughout life. Its reappearance or upregulation, which occurs in situations such as peripheral nerve regeneration or sprouting within the hippocampus, is suggestive of plastic changes typical of a developmental state. Interestingly, as shown in embryonic mouse brain, it is also likely to contribute to neurotrophic factor signalling, either acting as alternative trophic factor receptor or modulating the interaction of neurotrophins with specific receptors. With the aim of providing information relevant to the potential for dynamic changes of specific neuronal populations in man, this study reports on the immunohistochemical occurrence of PSA-NCAM in the human hippocampal formation, brainstem, and trigeminal ganglion at prenatal and adult age. PSA-NCAM-like immunoreactivity (LI) occurs in these regions from prenatal life to adulthood and the aspect, amount, and localization of neuronal labelling may change with age. As a general rule, PSA-NCAM-LI appears as surface staining on neuronal perikarya and proximal processes and as filamentous and dot-like elements in the neuropil. In the hippocampus, neuronal structures occur in the Ammon's horn and fascia dentata. At prenatal age, positive neurons are rare and intense labelling can be observed in discrete nerve fiber systems distributed in the pyramidal and molecular layers of the CA3 field of the Ammon's horn and in the alveus. By contrast, in the adult brain, neuronal perikarya are more frequently stained and nerve fibers are arranged in loose plexuses within the pyramidal layer of the Ammon's horn and the granule cell layer and hilus of the fascia dentata. In the brainstem, at all examined ages, labelling is virtually restricted to the medulla oblongata, where all sensory nuclei contain positive structures represented by neuronal perikarya and nerve fiber networks. Thus, PSA-NCAM occurs in the spinal trigeminal nucleus substantia gelatinosa, dorsal column nuclei, solitary nuclear complex and vestibular and cochlear nuclei. Labelling in the spinal trigeminal nucleus is consistent with the observations in the trigeminal ganglion, where PSA-NCAM labels a subpopulation of small- to medium-sized primary sensory neurons. In the ganglion, immunolabelling also occurs in nerve fibers and pericellular networks, and in satellite cells. Preliminary fluorescence double labelling carried out for PSA-NCAM and BDNF shows both codistribution and colocalization of the two markers, suggesting that interactions between PSA-NCAM and neurotrophic factors may be also envisaged in the adult human brain. The results obtained suggest that selective populations of central and peripheral neurons may retain the capacity to structural and functional plasticity throughout life. Localization of PSA-NCAM in primary sensory neurons and central nuclei also suggests that this molecule may participate in the processing of the relevant sensory neurotransmission.

NEUROMORPHOLOGY OF BRAIN PROTECTION ELICITED BY CHOLINERGIC NEUROTRANSMISSION ENHANCING DRUGS.

D. Tomassoni, S. K. Tayebati, E. Traini, F. Mignini and F. Amenta.
Department of Experimental Medicine and Public Health, University of Camerino, Camerino, Italy

Long lasting arterial hypertension affects cerebrovascular tree and may cause brain damage. Cognitive impairment of vascular origin, which characterizes vascular dementia (VaD) is accompanied by cholinergic dysfunction. Enhancement of deficient cholinergic neurotransmission is an important strategy for treating adult-onset dementia. Cholinesterase inhibitors (ChE-Is) are the first class of drugs licensed for symptomatic treatment of cognitive impairment of mild-moderate staged of Alzheimer's disease (AD).

The present study was designed to assess neuroprotective effects of treatment with the ChE-I galantamine (GAL) and glyceryl phosphoryl choline (GPC) and their association on frontal cortex and hippocampus microanatomy of spontaneously hypertensive rats (SHR) used as an animal model of VaD. Normotensive Wistar-Kyoto (WKY) rats were used as a reference group.

Male SHR of 32 weeks of age were treated for 4 weeks with 3 mg/kg/day galantamine or 100 mg/kg/day GFC alone or in association, to study the possible its neuroprotective effect. Nerve cell number, phosphorylated 200-kDa neurofilament immunoreactivity and glial fibrillary acidic protein (GFAP) as a marker of astroglial reaction were assessed by neuroanatomical, and immunohistochemical techniques associated with quantitative analysis.

In SHR the number of neurons in zones II, III and IV of frontal cortex and in the CA1 subfield of hippocampus and dentate gyrus was decreased compared to WKY rats. An astrogliosis consisting in GFAP-immunoreactive astrocytes hyperplasia and hypertrophy was also observed primarily in the hippocampus. Treatment with galantamine or GFC countered nerve cell loss. GFC but not galantamine reduced also astrogliosis. Galantamine and GFC in association displayed a more remarkable effect than single drugs alone.

These data collectively indicate that association of a ChEI with the cholinergic precursor GFC has a neuroprotective effect superior than that elicited by the two drugs alone. These findings suggest to assess the activity of this interesting cholinergic association in clinical trials.

RESEARCH ACTIVITIES OF THE INSTITUTE OF NEUROLOGICAL SCIENCES OF THE NATIONAL RESEARCH COUNCIL IN CATANIA

Maria Vincenza Catania
Institute of Neurological. Sciences (ISN-CNR), Catania, Italy.

Understanding the molecular mechanisms underlying neurological diseases is one of the most ambitious and relevant challenges of life science during present times. The increased prevalence of neurodegenerative disorders that is associated with the increasing life span has not been followed by a sufficient development of new therapeutic choices. On the other hand, rare neurological diseases are more diagnosed nowadays than in the past, but do not received financial support and are not sufficiently studied. From a modern point of view, the chances of unravelling the molecular basis of both common and rare neurological disorders strongly rely on a multidisciplinary approach. In our Institute, researchers specialized in different disciplines of Neuroscience work with the common aim of understanding the physiopathology of several neurological disorders. The research group of Functional Genomics is currently working on developing advanced technologies to be used for genomic screening in neurological diseases. By whole-genome expression profiling and stringent significance tests, genes and gene groups de-regulated in the motor cortex of patients with sporadic amyotrophic lateral sclerosis (ALS) have been identified. The role of individual candidate genes has been interpreted in a framework of differentially expressed pathways. The group of Cellular and Molecular Neurobiology is currently focused on understanding the role of glial cells in the mechanisms of neuron differentiation and survival as well as neurodegeneration. We are particularly interested in understanding the role of reactive glial cells in the onset and progression of motor neuron death, which is the hallmark of ALS. Another project is focused on Fragile X syndrome, a common form of inherited mental retardation, autism and epilepsy. We have characterized the epileptic phenotype of the FMR1 knockout mouse (KO), an accredited animal model of this disorder and have also reported a reduced association between metabotropic glutamate receptor type 5 and Homer protein, in the synapses of FMR1 KO mice . The possible functional consequences of this disrupted interaction are currently under investigation. The biochemical characterization of glioma cell cultures is presently undertaken, with particular attention to the expression of Notch receptors, which are involved in cell differentiation and apoptosis. Clinical research is also represented in our Institute with personnel working in the field of Nuclear Medicine and Pediatric Neurology.

NORADRENERGIC MODULATION OF N-METHYL-D-ASPARTATE RECEPTORS IN THE VESTIBULAR COMPLEX

Barresi M[1], Grasso C[1], Pellitteri R[2], Russo A[1], Ciranna L[1], Li Volsi G[1], Licata F.[1] and Santangelo F[1].

[1]Dept. Physiological Sciences, University of Catania, Italy; [2]Institute of Neurological Sciences, National Research Council, Section of Catania, Italy

In our previous studies we had demonstrated that noradrenaline (NA) modulates glutamate elicited excitations of vestibular secondary neurons (VSN). The aim of this study was to ascertain whether a component of this modulation involves N-methyl-D-aspartate (NMDA) receptors.

Electrophysiological recordings of single neuronal activities, performed in vivo during microiontophoretic application of drugs (NMDA, NA and its agonists and antagonists), showed that NA enhanced NMDA-evoked responses in 12 out of 17 (71%) VSN studied and depressed them in 3 (18%) neurons. In the remaining 2 (11%) neurons, the amine was ineffective on NMDA-evoked responses. All effects were reversible and dose-dependent. Applications of α_1 and α_2 receptor agonist, cirazoline and clonidine respectively, mimicked NA-induced enhancement, while β receptor agonist isoprenaline, mimicked NA-induced depression. The action of noradrenergic agonists, was antagonized respectively by α_1 receptor antagonist, prazosine, by α_2 receptor antagonist, yohimbine and by β receptor antagonist, timolol.

Immunohistochemical experiments, carried on by using antibodies against both noradrenergic (α_1, α_2, β_1, β_2) and ionotropic (NR1, NR2A) glutamate receptors, demonstrated that in single VSN neurons there is co-expression of immunoreactivity for NMDA glutamate receptors and for a noradrenergic receptors.

These findings indicate that noradrenergic and NMDA receptors co-localized in single VSN neurons can be the morphological support of functional interaction of these receptors. The expression of this interaction is the modulation exerted by NA on NMDA-evoked responses and therefore on the vestibular function.

PRENATAL EXPOSURES TO THE CB-1 RECEPTOR AGONIST WIN 55212-2 AND/OR CO: LONG TERM EFFECTS ON THE GABA NEURONAL SYSTEMS OF THE RAT CEREBELLAR CORTEX

V. Benagiano[a], L. Lorusso[a], P. Flace[a], A. Rizzi[a], L. Bosco[b], R. Cagiano[c], G. Ambrosi[a]
[a]Dept. of Human Anatomy & Histology, [b]Dept. of Bioethics, [c]Dept. of Pharmacology and Human Physiology - University of Bari - Italy

Three groups of rats were subjected to the following experimental conditions: exposure to cannabinoids by maternal treatment during pregnancy with the CB-1 receptor agonist WIN 55212-2 (WIN) (0.5 mg/Kg/day); exposure to CO by maternal exposure during pregnancy to CO (75 ppm); exposure to WIN+CO at the above doses; a fourth group was used as control. The body weight of dams, length of pregnancy, litter size at birth, body weight and pup postnatal mortality were monitored in order to evaluate possible effects of the exposures on reproduction and prenatal and postnatal development. In the different groups, the long term effects of the exposures were studied in adult rats by LM analyses of the structure of the cerebellar cortex and of the distribution in the cortex of markers of GABAergic neurons such as GAD-65/67 and GABA itself.

Exposures to WIN or/and CO did not affect reproduction nor development. Moreover, the exposed rats showed no structural alterations of the cerebellar cortex and displayed qualitative distribution patterns of GAD-65/67 and GABA immunoreactivities (IRs) similar to those of the controls. However, quantitative analyses indicated significant changes of both these IRs: in comparison with the controls, they were significantly increased in WIN-exposed rats, reduced in CO-exposed rats, but not significantly different in WIN+CO-exposed rats. The changes were detected in the molecular and Purkinje neuron layers, but not in the granular layer.

Prenatal exposures of rats to WIN and CO at doses which do not affect reproduction, general processes of development and histomorphogenesis of the cerebellar cortex cause changes of GAD-65/67 and GABA IRs in some GABAergic neuronal systems of the adult rat cerebellar cortex, indicating selective up-regulation of GABA-mediated neurotransmission as a long term consequence of chronic prenatal exposures to cannabinoids or CO. Since the changes consist of overexpression or vice versa underexpression of these IRs, functional alterations of opposite types in the GABAergic systems of the cerebellum following exposure to WIN or to CO can be postulated, in agreement with results of behavioural and clinical studies. No changes of the IRs were detected after prenatal exposure to WIN and CO in association.

NEUROPROTECTIVE EFFECTS OF ACETYL-L-CARNITINE ON NEUROPATHIC PAIN AND APOPTOSIS: NICOTINIC RECEPTOR ROLE

[*]Di Cesare Mannelli L., [§]Pacini A., [§]Toscano A., [*]Ghelardini C., and [*]Bartolini A.

[*]*University of Florence, Dept. of Preclinical and Clinical Pharmacology, Viale Pieraccini 6, 50139, Florence, Italy.* [§]*University of Florence, Dept. of Anatomy Hystology and Forensic Medicine, Viale Morgagni 85, 50139, Florence, Italy.*

Pathologies related to nervous tissue alterations are characterized by a chronic pain syndrome defined by persistent or paroxysmal pain independent or dependent of a stimulus. Pathophysiological mechanisms related to neuropathic disease are associated with mitochondrial dysfunctions that lead to an activation of the apoptotic cascade. In a model of peripheral neuropathy, obtained by the loose ligation of the rat sciatic nerve (chronic constriction injury; CCI), Acetyl-L-Carnitine (ALCAR; 100 mg/Kg i.p twice daily for 14 days) was able to reduce hyperalgesia. In the same conditions, ALCAR prevented regulated cell death improving a nerve apoptotic state that encompass cytochrome C cytosolic release, activation of the cysteine protease caspase 3 up to genome fragmentation. The muscarinic blocker atropine, injected to the dose of 5 mg/Kg i.p. simultaneously to ALCAR, did not antagonize the ALCAR anti-hyperalgesic effect in the paw-pressure test; at the same dose atropine reduced the analgesic effect of ALCAR in a significative manner. On the contrary anti-neuropathic effect of ALCAR is prevented by the nicotinic antagonist mecamylamine (2 mg/Kg i.p. twice daily for 14 days). Moreover, pharmacological silencing of the nicotinic receptor significantly reduced the XIAP-related protective effect of ALCAR on the apoptotic state of the ligated nerve. Taken together these data highlight the relevance of nicotinic modulation in neuropathy treatment.

DISTRIBUTION OF METABOTROPIC GLUTAMATE RECEPTORS IN THE SPINAL CORD OF TRANSGENIC MICE G93A

Laureanti F. [4], D'Antoni S. [1-2], Berretta A. [1-2], Seminara G. [1-2], Giuffrida-Stella A.M. [2], Longone P. [3], Spalloni A [3], Giuffrida R. [4] and Catania M.V. [1]

[1]Inst. of Neurol. Sciences (ISN-CNR), Catania, Italy; [2]Dept Chem Sciences, Univ Catania, Catania, Italy; [3]Mol Neurobiology, S. Lucia Foundation, Rome, Italy; [4]Dept Physiol Sciences, Univ Catania, Catania, Italy

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by a progressive muscle atrophy, weakness and/or spasticity, due to selective loss of motor neurons (MNs). Approximately 3% of ALS cases are inherited in an autosomal dominant manner and are caused by mutations in the gene encoding the free radical-scavenging enzyme, copper-zinc superoxide dismutase (SOD-1). Glutamate excitoxicity has been proposed as a mechanism in the pathogenesis of ALS. The role of ionotropic AMPA receptors in MN excitotoxicity has been extensively studied, whereas the role of metabotropic glutamate receptors (mGluRs) is still controversial. mGlu receptors are G-protein coupled receptors subdivided into three groups on the basis of sequence similarities, pharmacological profile, and transduction pathways. Group-I mGluRs (mGlu1 and mGlu5) are coupled to phosphoinositide hydrolysis, whereas group II (mGlu2 and mGlu3) and group-III (mGlu6, mGlu7, mGlu8) are negatively coupled to cAMP formation. We used immunohistochemistry to study the distribution of mGluRs in G93A mice, a mouse model of ALS, at different ages with the aim of clarifying their preclinical and postclinical expression. In addition, we studied the expression of mGluRs in glial cells and MNs in controls and G93A mice by using double-labelling immunofluorescence. When compared to controls, GFAP staining was already stronger in G93A mice at one month and increased during the development of the disease. In wild type mice, immunostaining for mGluR1α, mGluR5 and mGluR2-3 was mostly localized in the dorsal horn of spinal cord, whereas mGluR4 and mGluR6/7 was present mainly in neuron cells of ventral horn. The expression of group I and II mGluRs was increased in the ventral horn of G93A mice at four months of age, corresponding to late stages of disease (near complete hindlimb paralysis), but not at earlier developmental stages (1, 2 or 3 months of age). In four month G93A mice, mGluR1α, mGluR5 and mGluR2-3 immunoreactivity was present in both white and grey matter. However, mGlu1 and mGlu2/3 immunoreactivity was more evident in reactive astrocytes than neurons, whereas mGluR5 immunoreactivity was more clearly detected in motoneurons than reactive astrocytes. mGluR4 and mGluR6-7 immunoreactivity was present in both white and gray matter but was not increased in G93A mice compared to control.

These data suggest a possible role of group-I and –II mGluRs in reactive gliosis in ALS.

A CHRONIC BLOCKADE OF METABOTROPIC GLUTAMATE RECEPTOR SUBTYPE 5 EXERTS A PROTECTIVE EFFECT AGAINST MOTOR NEURON DEGENERATION

S. D'Antoni[1,2], A. Berretta[1,2], G. Seminara[1,2], A. Spalloni[5], P. Longone[5], G. Battaglia[6], F. Laureanti[3], R. Giuffrida[3], F. Nicoletti[7], M. A. Sortino[4], A. M. Giuffrida-Stella[2], M. V. Catania[1]

[1]Inst. of Neurological Sci., CNR, Catania 95123, Italy; [2]Dept. of Chem. Sciences, Section of Biochem. and Mol. Biol., [3]Dept. of Physiological Sci., [4]Dept. of Exptl. and Clin. Pharmacol., Univ. of Catania, Catania, Italy; [5]Unit of Mol. Neurobiology, S. Lucia Fndn., Roma, Italy; [6]Dept. Neurosci., Neuromed, Pozzilli (IS), Italy; [7]Dept. Pharmacol., Univ. of Rome "La Sapienza", Rome, Italy

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder, characterized by progressive loss of motor neurons (MNs) and significant astrogliosis. ALS pathogenesis is thought to be multifactorial and likely involves AMPA/kainate receptor-mediated calcium influx and excitotoxicity. An altered cross-talk between glial and neuronal cells might also be crucial for the pathogenesis of ALS. mGluRs modulate excitotoxicity in several experimental paradigms, but their role in MN degeneration has not been extensively investigated. To this aim, we carried out excitoxicity experiments in mixed spinal cord cultures pre-treated from 11 to 14 days in vitro (DIV) with group-I (mGluR1 and mGluR5) and group-II (mGluR2 and mGluR3) antagonists. Exposure of cultures to AMPA (50 uM) for 15 minutes at 14-15 DIV resulted in about 50% MN death, as assessed on the following day by counting surviving MNs. A pretreatment for three days with MPEP 3 uM (mGluR5 antagonist), but not with CPCCOOEt 10 uM (mGluR1 antagonist) or LY341495 10 nM (mGluR2/3 antagonist) significantly reduced AMPA-toxicity. In contrast, no protective effect is observed when MPEP is added either during or after the AMPA toxic pulse. In addition, extracellular glutamate levels were not affected by MPEP treatment, as assessed by HPLC analysis in the culture medium. Considering that calcium is the main effector of AMPA toxicity, we tested whether MPEP can affect calcium influx in MNs by studying cobalt uptake, an indicator of calcium influx, and we found that the MPEP pre-treatment reduces the percentage of cobalt-positive MNs. Double-labelling immunocytochemistry and Western blotting analysis revealed the presence of mGluR5 in MNs and pure cultured spinal cord astrocytes, respectively. Thus, a chronic treatment with MPEP can have a direct effect on MNs and/or modulate the astrocytic release of factors indirectly affecting AMPA toxicity. Interestingly, a pretreatment with an antibody that inhibits the biological activity of brain-derived neurotrophic factor (BDNF) reduced AMPA toxicity, suggesting that endogenous BDNF heightens the sensitivity of MNs to excitotoxic insults. The protective effect of the anti-BDNF blocking antibody is not further increased by MPEP co-administration.

Our data suggest that a chronic blockade of mGluR5 exerts a protective effect against MN degeneration, which might be mediated by receptors located on both MNs and astrocytes and might involve BDNF biochemical cascade.

INNERVATION OF PALATINE TONSILS IN SHEEP: A TRACING STUDY

Russo D.[1], Mongardi Fantaguzzi C.[2], Lucherini M.C.[2], Di Guardo G.[3], Clavenzani P.[2], Lalatta-Costerbosa G.[2], Chiocchetti R.[2]

[1]Department of Biological Structures Functions and Technologies, Faculty of Veterinary Medicine, University of Naples Federico II, via Veterinaria 1, 80137 Naples, Italy; [2]Department of Veterinary Morphophysiology and Animal Productions, Faculty of Veterinary Medicine, University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy.; [3]Department of Comparative Biomedical Sciences, Faculty of Veterinary Medicine, University of Teramo, Piazza Aldo Moro 45, 64100 Teramo, Italy.

Palatine tonsils (PTs), together with Peyer's patches (PPs) of the ileum, seem to be the first colonization and replication sites for prions, the agents causing transmissible spongiform encephalopathies (TSE), as it has been reported following oral infection with both sheep scrapie (the TSE "prototype") and bovine spongiform encephalopathy (BSE) (Andreoletti et al., 2000; Jeffrey et al., 2001; van Keulen et al., 2002). From the above entry sites infectious prions take part to a very complex biological process termed "neuroinvasion", which likely involves at its turn peripheral nerves connecting these gut-associated lymphoid tissues (GALT) with central nervous system (CNS) (Press et al., 2004; Beekes and McBride, 2007).

In the classic treatise of Veterinary Anatomy, PT innervation is explicitly ascribed only to the tonsillar branches of the glossopharyngeal nerve (IX cranial nerve) (Barone, 1981; Berg, 1982; Habel, 1982). In human Anatomy there is also an explicit reference to the TP innervation by trigeminal nerve (V cranial nerve) (Shankland, 2000, 2001).

To study the connections between PTs and CNS, we injected in the PT the fluorescent retrograde tracer Fast Blue (FB). We found FB-labelled neurons in the sympathetic cervical cranial ganglia (CCG), in the trigeminal ganglia (TG) and in the proximal glossopharingeal ganglia. In the CCG and TG FB-labelled neurons were found in both ipsilateral and controlateral ganglia. Nevertheless, the greatest number of FB-labelled neurons were found in the ipsilateral ganglia.

Immunohistochemistry was performed on PT and TG cryosections. Immunoreactivity (IR) for neurofilaments 200 kDa (NF), 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 PT follicles and a large number of TH-IR fibres were seen in their peripheral zone. In the TG, it was possible to notice NF-, NOS-, CGRP-, and SP-IR cell bodies and TH- and DBH-IR fibres.

The present results allow to attribute PT innervation to glossopharyngeal and trigeminal cranial nerves and to postganglionic fibres of the CCG. Furthermore, they provide a plausible anatomic basis through which infectious prions may gain access to the CNS by travelling along several cranial and sympathetic nerves.

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CHANGES IN BRAIN LIPID FRACTIONS INDUCED BY CHOLINE CONTAINING PHOSPHOLIPIDS.

E. Traini, D. Tomassoni, S. K. Tayebati, and F. Amenta.
Department of Experimental Medicine and Public Health, University of Camerino, Camerino, Italy,

The crucial role of lipids in cell signalling and tissue pathophysiology is demonstrated by the large number of diseases and neurological disorders in which lipid metabolism is altered.
Cytidine-5-diphosphocholine (CDP-choline or Citicoline) is an essential intermediate in the synthesis of phosphatidylcholine (PC), a major brain phospholipids. Choline alphoscerate (L-α-glycerylphosphorylcholine α-GPC) is a semi-synthetic derivative of PC, involved in brain phospholipids metabolism being transformed by the enzyme glyceryl-phosphorylcholine diesterase into a molecule of choline and another of glycerol-1-phosphate. As the intermediate in PC biosynthesis, it is thought that CDP-choline α-GPC could counter membrane damage and provide benefit in central nervous system disorders and injury. CDP-choline and α-GPC also serve as choline donors in the biosynthesis of the neurotransmitter acetylcholine so there were used as a cholinergic drugs in different deficient cholinergic neurotransmission disease.
The present study was designed to establish if treatment with the CDP-Choline and α-GPC affect brain lipid fraction on Wistar rats, treated with a choline-equivalent dose of the two drugs. Lipid fractions, extracted from brain areas (frontal cortex, striatum, hippocampus and cerebellum) were evaluated by thin-layer-chromatography (TLC) followed by densitometric analysis. Furthermore the microanatomical localization and pattern of these lipids were also studied by histochemical techniques (Luxol Fast-Blue, PAS, Sudan Black) in brain section.
Comparative analysis of chromatographs revealed an increase of phospholipids fractions, PC and phosphatidylethanolamine, without significant changes of other polar lipid fractions in the different brain areas of treated rats. This increase was higher in α-GPC treated rats compared to those treated with CDP-choline.
These data demonstrate that cholinergic precursors drugs, used to enhance cognitive function in cerebrovascular disorders, may have cerebroprotective effects increasing the membrane phospholipids trophism. Furthermore these preclinical data confirm clinical evidence that, among the two drugs, α-GPC is more effective than CDP-choline.

GROUP II METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION INCREASES BRAIN DERIVED NEUROTROPHIC FACTOR AND GLIAL-DERIVED NEUROTROPHIC FACTOR EXPRESSION IN THE MOUSE BRAIN

Di Liberto V.[1], Bonomo A.[1], Battaglia G.[2] Molinaro G.[2], Riozzi B.[2], Spinsanti P.[3], De Vita T.[3], Belluardo N.[1], Nicoletti F.[2,3], Bruno V.[2,3], Mudò G.[1]

[1]DIMES, Human Physiology Section, University of Palermo, Italy. [2]Istituto Neurologico Mediterraneo Neuromed, Pozzilli, Italy; [3]Department of Human Physiology and Pharmacology, University of Rome "La Sapienza", Rome, Italy.

Metabotropic glutamate receptors (mGluRs) have been implicated in mechanisms of neuroprotection and are considered as targets for neuroprotective drugs. In this context an interplay among neurotrophic factor and mGluRs signalling system has been suggested in view of neurotrophic factors key role on neuroprotection. This study was designed in order to explore a potential regulatory role of selective mGluR2/3 agonist LY379268 treatment on brain derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) expression in the mouse brain. Using in situ hybridization, a dose effects study has evidenced that LY379268 at dose of 0.25 mg/kg i.p. significantly increases mRNA expression of GDNF in the striatum and of BDNF in the cerebral cortex and the hippocampal formation. A time-course analysis of this regulation showed that both BDNF and GDNF expression increases 3h after LY379268 treatment and back to basal levels 6h after treatment. This up-regulation of BDNF and GDNF mRNA levels was followed by protein increase 24h following LY379268 treatment. The observed regulation of both BDNF and GDNF expression was blocked by pre-treatment with mGluR2/3 antagonist LY341495. A seven day treatment with LY379268 (0.25 mg/kg, i.p.,) showed a significant protection against MPTP toxicity, as assessed by immunoblotting analysis of tyrosine hydroxylase in the striatum and neuronal counts in the substantia nigra pars compacta. Taken together these findings suggest that neuroprotective actions displayed by mGluR2/3 agonist treatment may be mediated by up-regulation of BDNF and/or GDNF expression.

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GIOVANNI MARIA LANCISI AND THE VIEW OF NERVOUS SYSTEM ORGANIZATION BETWEEN 17TH AND 18TH CENTURIES.

Francesco Amenta
Dipartimento di Medicina Sperimentale e Sanità Pubblica, Università di Camerino, 62032 Camerino, Italy.

Giovanni Maria Lancisi was born in Rome on 26 Oct. 1654 and passed on 20 Jan. 1720. Following preparatory studies, he took courses in philosophy at the Collegio Romano, but soon he abandoned theology and entered the Sapienza to study medicine. He obtained his M.D. in 1672 when he was still a month shy of 18. He continued to study medicine independently. After graduation he become a famous physician who attended three Popes and served as Archiater of the Papal State and Canon of the Church of San Lorenzo, Professor of Surgery, Anatomy and Theoretical and Practical Medicine at Rome University from 1684 to 1719 (8), a member of the Collegio Romano from 1689, and a representative of the Repubblica Letteraria Italiana.

He contribute to Medicine, Physiology and Anatomy research. Having examined the causes of sudden deaths, in 1706 he published De Motu Cordis Mortibus, in which he dealt with the problems of cardiac pathology. He extended his study of the subject in his second book, De motu cordis et Aneuysmatibus, published in 1728. He also did important epidemiology studies on malaria, influenza and cattle plague. And he carried out extensive anatomical and physiological studies.

The main neuroanatomical contributions of Lancisi, were Dissertatio Physiognomica (1710), in which he described the relationship between the mimicry of the frontal muscle and the fibers of the meninges and brain and Dissertatio De Sede Cogitantis Animae (1712), dedicated to the physician Giovanni Fantoni (1675– 1758), in which he considered the problem of the cerebral localizations and specificity of the cortical functions. In this publication agreed with Descartes that the seat of the soul and superior psychic functions should be a median and unpaired organ, but favoured the corpus callosum over the pineal gland. He thought the epiphysis played an important part, as it was connected to the thalami through the habenulae. The Dissertatio De Sede Cogitantis Animae continues:". . . In the superior part of the corpus callosum, . . . I observed one thing completely disregarded or unobserved until now: each medullar transversal fiber is intersected at a right angle to two nerves, which are medullary, round, and have a changeable diameter. These ones are not covered with dura mater, but with the only arachnoidal membrane run along the corpus callosum forwards and backwards, being almost in contact". Moreover, he described the "Nervali longitudinales ab anterioribus ad posteriora excurrentes," which are still called the "medial longitudinal striae of corpus callosum," or nerves of Lancisi . In his dissections, he also noticed the presence of striae longitudinales laterales, although he thought that "it was more probable those were not proper nerves, but edges of the corpus callosum which were raised slightly from the medullary plane".

The description of the dorsal area of the corpus callosum in Gray's Anatomy reads, "Along the middle line, is a linear depression, the raphe, bounded laterally by two or more slightly elevated longitudinal bands, called the striæ longitudinales, and, still more externally, other longitudinal striæ are seen, beneath the convolution, which rests on the corpus callosum. These are the striæ longitudinales laterales" called also nerves of Lancisi.

EXPRESSION AND CHARACTERIZATION OF HUNTINGTIN GENE IN AMPHIOXUS

S. Candiani[1], M. Pestarino[1],D. Zaccheo[1], E. Cattaneo[2] and M. Tartari[2]
[1]*Department of Biology, University of Genoa, viale Benedetto XV 5, 16132, Genoa, Italy*
[2]*Centre for Stem Cell Research and Department of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133, Milan, Italy*

Huntington's disease is an inherited neurodegenerative disorder resulting in neuronal cell death in discrete brain regions caused by mutation of a protein called huntingtin. Huntingtin, is a soluble protein of 3144 aa, ubiquitously expressed in moderate amounts in and outside the nervous system. The disease-causing gene mutation consists of an expanded CAG tract (>35 repeats) at the 5' end, which is translated into a polyglutamine stretch (polyQ). Considerable progress has been made toward understanding the role of the mutant huntingtin protein. Nevertheless, little is known about the normal biological function of huntingtin and no detectable sequence similarity of huntingtin to other proteins has been found. In order to identify important functional domains of huntingtin, we isolated and characterized the amphioxus huntingtin (AmphiHtt) gene, coming from an invertebrate chordate whose phylogenetic node of divergence is thought to go back 540 million years. Along the deuterostome branch, the cloned ascidian huntingtin suggests a more recent evolution of the 5' end of the gene, which is characterised by the lack of a polyQ tract. The AmphiHtt sequence has two glutamines at the corresponding position to polyQ in vertebrates, thus indicating that the common ancestor of amphioxus and vertebrates already possessed this characteristic. Our findings demonstrate that during amphioxus development AmphiHtt mRNA is detected in the neural plate at early neurula stage, suggesting that amphioxus huntingtin could be involved in neurogenesis process. The expression of AmphiHtt was found in endodermal and mesodermal structures but during restricted temporal window (late neural stage), whereas follows an antero-posterior gradient, and is enriched in the anterior neural tube at larval stage. Finally, we found that the sequence of amphioxus huntingtin is not critically different from that of vertebrates, and that its expression is particularly enriched in the nervous system. In this view, it can be inferred that an ancestral neuronal function of huntingtin was present 540 millions years ago.

PERIPHERAL INFLAMMATORY PRIMING INCREASES DEGENERATION OF SOD1-MUTANT MOTONEURONS AND AFFECTS T CELL RECRUITMENT AFTER DAMAGE

C.Cupidi[1,2], R. M. Kassa[1], R. Mariotti[1], M. Bentivoglio[1]

Depts. of [1]Morphological and Biomedical Sciences, University of Verona, and [2]Clinical Neurosciences, University of Palermo.

Inflammatory stimuli and immune responses have been involved in the still unknown pathogenesis of amyotrophic lateral sclerosis (ALS). Retrograde axonal signalling may also be implicated in selective motoneuron damage in ALS, mainly in the transfer of harmful peripheral signals from muscular target. The aim of the study was to determine the role of retrograde inflammatory signals in motoneuron degeneration in the presence of a disease-inducing genetic background. We applied a double-hit paradigm, in which facial nerve transection was preceded or not by inflammation of target muscle by LPS injection, in SOD1(G93A) transgenic (tg) mice and in wild-type (wt) littermates. In both wt and tg mice, LPS elicited mild microglial activation in the facial nucleus without motoneuron loss. At 14 days post-axotomy, marked glial activation and neurodegeneration were evident in the facial nucleus. Cell counts showed a greater degree of loss of axotomized motoneurons in both tg and wt mice after LPS pretreatment, but such loss was greater in the former group. Both microglial and astrocytic cells showed a more marked activation in tg mice respect to wt ones, mainly when LPS injection preceded facial nerve transection. Moreover, recruitment of T lymphocytes in the axotomized facial nucleus was affected by inflammatory priming in a different manner in SOD1-mutant and wt mice, since the former mice showed a lower degree or no T cell infiltration in the axotomized facial nucleus after LPS pretreatment. The selectivity of this event was indicated by the occurrence of T cells throughout the brainstem of the same tg mice, at sites of origin of descending spinal pathways. This abnormal lymphocytic response is not due to an impairment of cellular adhesion mechanism or to an alternative recruitment of neutrophils. Altogether the data indicate that exposure to peripheral inflammatory signals greatly increases the vulnerability of SOD1-mutant motoneurons to subsequent peripheral damage, and point out dysregulation of the immune response to such signals. (Supported by FIRB, COFIN).

THYROSINE PHOSPHORYLATION AND DISSOCIATION OF ZONULA OCCLUDENS-1 IS COUPLED WITH HYPOXIA-INDUCIBLE FACTOR ACTIVATION IN THE BRAIN OF DYSTROPHIC MDX MOUSE.

D. Mangieri[*], V. Longo[*], M. De Giorgis[*], C. Capobianco[§], M. Scivetti[**], V. Benagiano[*], L. Roncali[*], D. Ribatti[*] B. Nico[*].

[*]*Department of Human Anatomy and Histology, University of Bari Medical School, Bari, Italy,* [§] *Department of Emergency and Transplantation, University of Bari Medical School, Italy,* [**]*Department of Odontostomatoloy and Surgery, University of Bari Medical School, Italy*.

In Duchenne muscular dystrophy (DMD), an X-linked genetic disease characterized by a lack of dystrophin associated with damage to the muscle fibers, metabolic and structural alterations of the Central Nervous System (CNS) are described. In the brain of 12 month old dystrophic mdx mice, an experimental animal model of DMD, we have previously demonstrated an increment in angiogenesis together with glial alterations, overexpression of vascular endothelial growth factor (VEGF) in neurons and its receptor -2 (VEGFR-2) in endothelial cells and damage to the blood-brain barrier (BBB). Here, we investigated in the brain of younger mdx mice (five months) and in control ones, the expression of hypoxia-inducible factor-1α (HIF-1α), a transcription factor activated in hypoxic condition and involved in angiogenesis, and we correlated it with the expression of VEGF and VEGFR-2 and of the endothelial tight junction proteins zonula occludens-1 (ZO-1) and claudin-1.

Results showed an activation of mRNA HIF-1α by RT-PCR and a strong HIF1-α labeling of perivascular glial cells and cortical neurons by immunohistochemistry, in mdx mouse as compared to controls. Moreover, overexpression of VEGF and VEGFR-2, respectively, in neurons and in endothelial cells coupled with changes to endothelial ZO-1 and claudin-1 expression in the latter were detected by immunoblotting and immunohistochemistry, in the mdx brain as compared with controls. Furthermore, by immunoprecipitation, an up-phosphorylation of ZO-1 was demonstrated in mdx endothelial cells in parallel with the reduction in ZO-1 protein content.

Overall, these data suggest that the activation of HIF-1α in the brain of dystrophic mice coupled with VEGF and VEGFR-2 up-regulation and ZO-1 and claudin-1 rearrangement might contribute to both BBB opening and increased angiogenesis.

TISSUE TRANSGLUTAMINASE DYSREGULATION AND MITOCHONDRIAL ACTIVITY IN EPILEPTIC MOUSE.

M. F. Serapide[2], A. Campisi[1], M. Maritati[1] ,F. Laureanti[2], V. Battaglia[3], M. Mancon[3], C. Paz[4] and A.Toninello[3].

[1]Dipartimento di Chimica Biologica, Chimica Medica e Biologia Molecolare, Università di Catania, [2]Dipartimento di Scienze Fisiologiche, Università di Catania, [3]Dipartimento di Chimica Biologica, Università degli Studi di Padova, [4]Departamento de Neurofisiología, Instituto Nacional de Neurología y Neurocirugía M.V.S., Insurgentes Mexico.

The neural loss associated with some acute and chronic degenerative processes of Central Nervous System might be caused by excitotoxicity for prolonged activation of excitatory amino acid receptors, i.e. the glutamate receptors. In the same disorders an dysregulation of neuronal tissue transglutaminasi (TG-2) may play a role in their pathogenesis because of extremely insoluble protein complexes present in the brain after their crosslinking. There are only a few evidences about the implications of TG-2 in the epileptogenesis and they are related to kainic acid-induced epileptic seizures. In order to verify if a dysregulation of TG-2 can be evidenced at cellular and/or subcellular level in the human status epilepticus we used the kindling, an animal model of epilepsia, resembling the human disease, whereby the application of repeated (8 times a day for 1 and 3 days at 1-h intervals) low-level electrical stimulations (0,5 ms rectangular pulses at a rate of 60 Hz ; duration = 1 sec; intensity=100-150 μA) to the left basolateral amygdaloid nucleus leads to permanent increases in seizure susceptibility. The immunolocalization and the expression of the TG-2 were studied in various regions of the mouse brain with immunohistochemical technique and Western Blot analyses. Respect to the controls, in mice 1 days-kindled an increased activity of TG-2 was found in the bilateral cerebral cortex and hippocampus with a prevalence ipsilaterally to the stimulated side. Only a few TG-2 positive cells were found in the cerebellum and brainstem. In the mice 3 days-kindled in the studied four structures a glial reactivity was found. The Western Blot analyses confirmed these data; moreover the study of beta-tubulin expression as well as p53 and caspase-3 cleavage revealed neuronal injury and activation of the apoptotic pathway. At subcellular level the TG-2 activity was correlated to modifications of mitochondrial transmembrane potential and to the activity of two ionophores, nigericin and valinomycin which form ion channels on mitochondrial membrane. An activation of the K+/H+ exchanger was shown. Probably, the kindling induces an activation of mitochondrial TG-2, responsible of the transient uptake of large Ca^{2+} concentrations. This might promote the efflux of Mg^{2+} by the transient opening of the transition pore. The loss of Mg^{2+}, which operates as "carrier brake" for the activity of the K^+/H^+ exchanger, would activate it.

Our data shownan important implication of TG-2 in the epileptogenesis.

DEGENERATION OF THE NIGROSTRIATAL DOPAMINERGIC NEURONAL PATHWAY INDUCED BY MPTP IN p66$^{ShcA-/-}$ MICE.

[1]De Nuccio F., [1]Martignago R., [2]Pelicci PG., [2]Migliaccio E. Delle Side A., and [1]Nicolardi G.
[1]Human Anatomy Laboratory,Dept. Biol. Environm. Technol. Sci., University of Salento, Lecce, Italy, [2]Experimental Oncology Department, European Institute of Oncology, Milan, Italy

p66^{ShcA} isoforms are a stress-regulated protein acting as sensor for ROS and other kinds of genotoxic stress, and p66^{ShcA} is considered to be a longevity gene (Migliaccio et al., 1999). Three Shc genes have been found in the mammalian system: ShcA, ShcB (Sli) and ShcC(Rai). ShcA gene encodes for three isoform proteins, p46, p52 and p66. p66^{ShcA} is present throughout various tissues in humans and mice with the exception of brain and neurons, where p46 and p52 are expressed. At first we reported the presence of p66shc mRNA and protein in the substantia nigra (SN) and striatum wild-type (WT) mice. We then examined whether 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) applied to p66$^{ShcA-/-}$ mice affects the density of dopamine transporter (DAT) immunoreactivity in the SN and striatum. In this study, we used mice WT and p66$^{ShcA-/-}$, provided from the Institute of Oncology "Pelicci G.", of both sexes and weighing between 25-35 gr. Mice were processed to a single intraperitoneal injection of 30mg/Kg MPTP and killed after 70 days. As control (ctr), an equal number of mice were processed to a single intraperitoneal injection of phisiologic solution, in the same dose.
Our data showed that p66$^{ShcA-/-}$ mice are protected against MPTP-induced neuronal damage in the striatum and substantia nigra. In particular, mean+/- S.E.M. intensity of DAT-immunoreactivity for striatum and SN values were respectively: 0.23 ± 0.012/0.23 ± 0.024 for WT ctr, 0.16 ± 0.012/0.13 ± 0.019 for WT MPTP-treated, 0.22 ± 0.012/0.17 ± 0.018 for p66$^{ShcA-/-}$ ctr and 0.20 ± 0.010/0.18 ± 0.016 for p66$^{ShcA-/-}$ MPTP-treated. These data suggest that p66(Shc) is involved in the pathogenesis of the degeneration of the nigrostriatal dopaminergic neuronal pathway induced by MPTP.

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AUTOPHAGY IN THE DORSAL ROOT GANGLIA FOLLOWING SCIATIC NERVE INJURY

E. Tonoli, G. Manassero, A. Vercelli
Dipartimento di Anatomia,Farmacologia e Medicina Legale, Università degli Studi di Torino

Neuropathic pain is caused by nervous system lesion or inflammation: symptoms may include allodynia and hyperalgesia. A useful animal model to study neuropathic pain is the sciatic nerve ligation. Axonal injury elicits changes in macromolecule synthesis in the corresponding cell bodies of the dorsal root ganglia (DRG): nerve injury activates several signalling pathways that may lead to both cell death and regenerative response. In this study we analyze the role of autophagy in sciatic nerve ligation: as reported by Rubinsztein *et al*. (2005) autophagy could be one of the mechanisms that lead to neuronal regeneration or degeneration.

C56BL/6 mice underwent sciatic nerve ligation (Malmberg *et al*., 1998) and were perfused at different times after surgery (24h, 5days); untouched animals were used as control. L5 dorsal root ganglia ipsi- and contralateral to ligation were rapidly dissected for acid phosphatase (AP) reaction (Gomori method). The Gomori staining allowed to indentify the diffuse and granular AP reaction products characteristic of lysosomal system including autophagic vacuoles. The numbers of neurons in ipsilateral, contralateral and uninjured L5 DRGs were estimated using ImageJ software, then the percentage of Gomori positive cells was calculated on the total neuronal cell number.

At 24h we noted no differences between the ipsi- and contralateral ganglia of ligated animal (40,19% vs.39,60%). After 5 days, in the ipsilateral ganglia 44% cells were positive vs. 33,8 % on the opposite side. In the untouched animal the percentage was the 36,48%.

These preliminary results show a time dependent increasing of the AP activity in the injured side compared to the contralateral and untouched DRGs. We are performing more specific analysis on the expression of the autophagosomal marker microtubule associated protein 1 light-chain 3 (mLC3), to assess the change in the autophagic activity and to understand its involvement in this model.

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NEW INSIGHTS ON THE MECHANISM OF ACTION OF CHOLINE- CONTAINING PHOSPHOLIPIDS IN RAT BRAIN

S. K. Tayebati, M.A. Di Tullio, D. Tomassoni and F. Amenta.
Dipartimento di Medicina Sperimentale e Sanità Pubblica, Università di Camerino

The crucial role of lipids in cell signalling and tissue physiology is demonstrated by the involvement of lipid dysregulation in several neurological disorders, including bipolar disorders, schizophrenia, Alzheimer's (AD), Parkinson's (PD), and Niemann-Pick diseases. Altered lipid metabolism is also thought to contribute to cerebral ischemic (stroke) injury.

Phospholipids are important components of all mammalian cells and have a variety of biological functions: (1) they form lipid bilayers that provide structural integrity necessary
for protein function, (2) they function as an energy reservoir (eg, triglycerides), and (3) they serve as precursors for various second messengers such as arachidonic acid, docosahexaenoic acid, ceramide, 1,2-diacylglycerol, phosphatidic acid, and lyso-phosphatidic acid.

Neurotransmitter systems are also involved in the regulation of the majority of brain functions. The dopaminergic and cholinergic ones are involved in the pathophysiology of several neurodegenerative disorders such as PD, Huntington's disease, tardive dyskinesia and AD. Altered dopamine/acetylcholine synthesis, receptor densities and status have a relevant role in the pathophysiology and treatment approach for these diseases.

Neurotransmitter transporters represent a class of rather recently discovered proteins playing a relevant role in synaptic machinery. These include the cell surface re-uptake mechanisms for monoamine and amino acid neurotransmitters and vesicular transporter mechanisms involved in neurotransmitter storage.

This study was designed to detect the effect of treatment with choline-containing phospholipids [CDP-choline (325mg/Kg/day) and alpha glyceril phosphoril choline (α-GFC) (150mg/Kg/day)] on vesicular acetylcholine transporter (VAChT) and dopamine transporter (DAT) levels in different cerebral areas of adult Wistar rats (250g). Morphological, Western blot analysis and an ELISA assay were used to evaluate neurotransmitter transporters changing in rat brain.

CDP-choline and α-GFC treatment increased DAT and VAChT concentrations in frontal cortex and cerebellum, but not in hippocampus and striatum. In general, morphological analysis confirmed these results.

These findings indicate that treatment with choline-containing phospholipids may interfere with dopaminergic/cholinergic neurotransmitter transporter systems in rat brain. This suggests that CDP-choline and α-GFC would merit further investigation as co-therapies in the treatment of both AD and PD.

PROUROKINASE MUTANT (M5) VS RT-PA IN THE ACUTE TREATMENT OF STROKE IN THE RAT: PRELIMINARY DATA.

Tomasi S.[1], Sarmientos P.[2], Gurewich V.[3], Vercelli A.[1]

[1]Department of Anatomy, Pharmacology Forensic Medicine, University of Torino, C.so D'Azeglio 52 10126 Torino, Italy; [2]Vascular Research Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston,MA,USA; [3]Primm s.r.l., Milan, Italy

Prourokinase (proUK) is a zymogenic plasminogen activator that induces fibrin-specific clot lysis without binding to fibrin; it has an unusually high intrinsic activity that at pharmacological dose is prone to nonspecific activation to urokinase; as a result, therapeutic thrombolysis with proUK is accompanied by important side effects like major bleeding. Several mutations of proUK were tested in order to reduce intrinsic activity and M5 mutant was selected. M5 fibrinolytic properties were tested in a rat model of permanent ischemia as described by Renolleau et al. (1998). Five groups of animals were infused four hours after ischemia onset with different drugs associations: group 1 (n=7) received alteplase (10 mg/kg,10% as bolus and remainder iv over 30'); group 2 (n=7) received alteplase infusion as group 1, preceded by iv bolus of C1-inhibitor; group 3 (n=5) received M5 (15 mg/kg over 30',10% as bolus) with C1-inhibitor; group 4 (n=5) received vehicle only; group 5 (n=5) C1-inhibitor only. Animals were perfused 24 hours later; clinical oucome and histological appearance of ischemic sections were evaluated. Rats infused with alteplase only showed tipically important bleeding soon after infusion with high mortality rate (57%) and important haemorrhagic infiltration. Group 2 showed a better clinical outcome (mortality rate 28%) and short lasting bleeding with thin blood epidural infiltration. No animal died in group 3; clinical conditions were not impaired by weak bleeding observed after infusion; important epidural hematome was observed in one rat. Rt-PA infusion seems to cause important bleeding with a higher frequency than M5; the haemorrhage is higher when rt-PA is given alone. This variation in mortality probably reflects different kind of bleeding observed: intense, long lasting, often lethal in group 1, very less intense in group 2. This different clinical behaviour didn't correlate clearly with histopathological appearance, but seems to be more related to different timings and liability of M5 and alteplase to cause bleeding. C1-inhibitor seemed to play a protective role both in association with rt-PA and M5. Further studies using embolic clot model as described by Zhang et al. (1997) are being performed in order to clarify the therapeutic potential of M5.

STUDY OF MARKERS EXPRESSION IN RAT OLFACTORY ENSHEATHING CELLS CULTURES GROWTH IN DIFFERENT CONDITIONS

Spatuzza M. [1], Pellitteri R. [2], Russo A. [1], Stanzani S. [1]

[1]Dep. Physiological Sciences, University of Catania, Catania (Italy); [2]Institute of Neurological Sciences, CNR, Section of Catania.

Olfactory Ensheathing Cells (OECs) are cells that ensheathe unmyelinated olfactory axons. They derive from precursor cells in the olfactory epithelium which is placodal in origin. OECs present many phenotypic properties with astrocytes and Schwann cells (SCs). They resemble astrocytes in that they express an astrocyte-specific marker (GFAP) and their similarities to SCs include immunostaining for the low-affinity p75 nerve growth factor receptor (p75 NGFR), Laminin, S-100 protein, and N-CAM. Immunocytochemical studies reveal that OECs are able to produce different growth and survival factors. The properties of these cells have inspired neuroscientists to explore their ability to support axonal regeneration as when transplanted, OECs remyelinate axons and improve functional recovery after spinal cord injury. In this study, we examined expression of different markers in rat OECs grown in different conditions. OECs were prepared from postnatal rat (P2) olfactory bulbs, grown both in serum containing medium and serum-free medium with added some growth factors (bFGF and GDNF). After ten days, OECs cultures were processed for immunostaining for calponin, nestin, , Protein Gene Product (PGP 9.5) and Microtubule Associate Protein-2 (MAP-2). Our results showed that when OECs cultures were grown in serum containing medium with bFGF, nestin and calponin showed higher labelling expression than grown with GDNF or without growth factors. When OECs were grown in serum-free medium growth factors-treated showed an increased expression of nestin. Moreover, the expression of neuronal markers (PGP 9.5 and MAP-2) was increased in OECs grown in serum-free medium both treated with growth factors and without them. In conclusion, this result suggests that there is a synergic action between serum and growth factors in the expression of calponin, in contrast the highest expression of neuronal markers in serum-free medium might be explained by serum contains molecules that inhibit the effect of growth factors on the PGP and MAP-2 expression. Therefore, as OECs express both neuronal and glial markers when grown in different conditions, these cells might be important in the therapeutic management of CNS injury and demyelinating diseases.

SLEEP DEPRIVATION INDUCES MICROGLIA ACTIVATION IN BRAIN REGIONS INVOLVED IN SLEEP AND WAKE REGULATION

Andrioli A., Xu Y.Z., ColavitoV., Bertini G., Grassi-Zucconi G., Bentivoglio M.
Dept. Morphological and Biomedical Sciences, University of Verona, Verona, Italy.

Sleep deprivation (SD) studies have suggested that sleep is centrally important for metabolism, immune response and cardiovascular function. There is also evidence that prolonged SD increases brain oxidative stress, inhibits NADPH-d and NOS expression, induces microglia and astroglia activation in the rat hippocampus and impairs cognitive mechanisms such as memory, visual attention and spatial learning. A central organization of sleep-wake control, based on the existence of sleep-promoting structures in the anterior hypothalamus and wake-promoting systems in the posterior hypothalamus, has been proposed. Selective activation of neurons during sleep has been documented in subregions of the preoptic area, especially in the ventrolateral and in the median preoptic (MPO) nuclei. Basal forebrain (BF) as well as orexinergic neurons (ORX) in the posterior lateral hypothalamic area have been implicated in wake regulation. Aim of the present study was to investigate in rats the effects of two paradigm of SD (12 h of total SD and 72 h of REM SD) on microglia activation in neuronal cell groups involved in sleep and wake regulation (BF, MPO and ORX). Twelve hours total SD was obtained by gentle handling the animals. Seventy two hours REM SD was obtained by the platform method, placing the animals in single cages on a small platform (6.5 cm) surrounded by water, while control rats were placed on larger platforms (14 cm) or kept in their home cage. Microglial cells were visualized by OX-42 immunocytochemistry and their activation was studied by morphological and immunosignal optical density analyses. The results showed that in both paradigms of SD, microglial cells exhibited more intense immunoreactivity and more marked hypertrophy than in control animals in brain regions involved in sleep and wake regulation (MPO, BF) compared to regions not selectively involved in these mechanisms. Densitometric analysis revealed significantly higher microglia immunosignal intensity in MPO of 12 h SD and in both MPO and BF in 72 h SD animals. ORX neurons did not show instead significant inter-group differences. The present finding that microglia, sensor of neuronal health and well-being, actively respond to SD by phenotypic changes suggests the interesting possibility of a role of this glial cell population in the production of signal molecules (neurotrophins, cytokines and NO) capable to affect neuronal functions, directly or through interaction with astrocytes. Therefore, the present findings strengthen previous data on a role of immune-related molecules in sleep regulation. *(Supported by EC grant LSHM-CT-2005-518189)*

MORPHOLOGICAL CHARACTERIZATION OF SCHWANN CELL BEHAVIOUR AFTER NERVE REPAIR BY MEANS OF TISSUE-ENGINEERED MUSCLE-VEIN COMBINED GUIDES

Geuna S.[1], Tos P.[2], Raimondo S.[1], Fornaro M.[1], Nicolino S.[3], Perroteau I.[3], Giacobini-Robecchi M.G.[1]

[1]Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Ospedale San Luigi Gonzaga, Orbassano (TO); [2]Divisione di Microchirurgia Rcostruttiva, Ospedale C.T.O., Torino, Italy; [3]Dipartimento di Biologia Animale e dell'Uomo, Università di Torino

The study of peripheral nerve repair and regeneration is seen an increasing interest from both clinical and basic researchers. In this scientific context, particular attention are attracting Schwann cells which have been shown to play a critical role in axon regrowth and maturation. When a non-nervous conduit is used to bridge a nerve defect, the conduit is soon colonized by a number of Schwann cells which make a pathway for regrowing axons. Over recent years we have investigated the behaviour of migratory glial cells along a particular type of autologous tissue-engineered conduit made of a vein filled with fresh skeletal muscle, using the rat sciatic and median nerve models.

With this particular type of autograft, our data shows that many Schwann cells soon take up a close relationship with grafted muscle fibers, and especially with their basal lamina which appear to serve as a migration pathway for them. The early and massive colonization of the conduit is sustained by both Schwann cell migration and proliferation, as demonstrated by PCNA immunostaining. Later, as they meet regenerating axons, Schwann cells become closely associated with them and eventually loose their connections with grafted muscle fibers because of the formation of the perineurial envelopes.

We have also investigated the expression of the glial growth factor neuregulin-1 (NRG1) and its two receptors, erbB2 and erbB3 showing that the NRG1/ErbB trophic system is upregulated during early nerve regeneration stages along the muscle-vein combined tubes.

Overall, our results describe the morphological basis for explaining the effectiveness of fresh-muscle-vein-combined technique for nerve repair (a technique already used with patients) and throw an interesting light on the possible role of NRG1 through the erbB2/erbB3 heterodimer receptor for nerve regeneration inside non-nervous conduits.

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MICROVESSEL DIVERSITIES IN HUMAN GLIOBLASTOMA MULTIFORME

F. Girolamo[1], D. Virgintino[1], M. Errede[1], K. Frei[2], L. Roncali[1]
[1]*Department of Human Anatomy and Histology, Bari University Medical School, Bari, Italy*.
[2]*Department of Neurosurgery, University Hospital Zurich, Zurich, Switzerland*.

The tumor vascular network is formed by multiple angiogenic events, sprouting, bridging, and intussusceptive growth, and vasculogenic mechanisms, de novo vessel formation by angioblasts and stem cells and vascular mimicry. The complexity of tumor vessel formation is reflected by the described various types of microvascular architectures, different for three-dimensional morphology, distribution, and cellular phenotypes. Two remarkable features of brain microvasculature are the presence of a barrier-specific endothelial profile (blood-brain barrier, BBB) and a high ratio (0,86%) of pericyte vessel investment. This special traits push us to further investigate and compare brain tumor vessel features with classical neoplasm vascularization. The research focused on human glioblastoma multiforme (GBM) using confocal microscopy analysis and a panel of antibodies against normal/tumoral markers of vascular cells. Single and multiple immunolabellings were carried out with CD31 for endothelial cells, α-smooth muscle actin (α-SMA) and NG2 proteoglycan for pericytes, GFAP for astroglia, and collagen IV for vascular basal lamina, together with the BBB transporter P-glycoprotein (P-gp) and with markers of vascular activation, such as matrix metalloproteinase-2 (MMP-2), CD105 (endoglin), platelet-derived growth factor receptor-β (PDGFR-β), and CD248 (endosialin). The observations have demonstrated that GBM samples are characterized by a high vascular heterogeneity in form of classical vascular sprouts and cord-like structures made of NG2 reactive pericytes and aspects of vessel 'cooption' and intussusceptive growth characterized by MMP2-reactive endothelial cells and NG2 pericytes. Tumor microvessels are also surrounded by a multilayered basal lamina and multiple layers of pericytes. A modified phenotype is observed for both endothelial cells and pericytes, in tumor endothelia P-gp is scarcely expressed, whereas MMP-2 and endoglin are a constant endothelial feature, α-SMA, NG2, and endosialin differently mark the pericyte layers. In conclusion, the understanding of the unique characteristics of GBM-associated microvasculature may be utilized as additional diagnostic and prognostic criteria and may contribute to the definition of antiangiogenic therapeutic designs.

GROWTH FACTORS-PRETREATED ASTROCYTE CULTURES PROLIFERATE AND DIFFERENTIATE DIFFERENTLY AFTER ASTROGLIAL CONDITIONED MEDIA TREATMENT

V. Bramanti[1], D. Bronzi[2], D. Tomassoni[3], G. Raciti[4], M. Avitabile[3], F. Amenta[3], R. Avola[1]

[1]Dept. Chemical Sciences, sec. Biochemistry and Molecular Biology, University Catania, Italy; [2]Dept. Physiological Sciences, University Catania, Italy; [3]Dept of Experimental Medicine and Public Healt, University of Camerino, Italy; [4]Dept. Biological Chemistry, Medical Chemistry and Molec. Biology, University Catania, Italy.

Astroglial conditioned media (ACM) influence development and maturation of cultured nerve cells and modulate neuron-glia interactions (1, 2). In particular, ACM released some endogenous trophic molecules that may interact with some growth factors added exogenously in neuronal on astroglial cell cultures.

To clarify mechanisms of astroglial cell proliferation/differentiation in culture, [methyl-^{3}H]-thymidine or [5,6 - ^{3}H]-uridine incorporation in cultured astrocytes , after pre-treatment with epidermal growth factor (EGF), insulin (INS), insulin-like growth factor-I (IGF-I), and basic fibroblast growth factor (bFGF) and subsequent treatment with ACM was studied. DNA labelling revealed a marked and significant stimulatory effect of ACM from 15 days in vitro (DIV) cultures in 30 DIV astrocytes after12h pre-treatment with growth factors. The main effects were found after INS or EGF pre-treatment in 30 DIV cultures. ACM collected from 15 or 60 or 90 DIV enhanced RNA labelling of 15 and 30 DIV astrocyte cultures, being the highest value that of 30 DIV cultures added with ACM from 90 DIV. Extra cellular signal-regulated kinase 1 (ERK1) immunoblotting suggests that increased DNA labelling after EGF or INS pre-treatment in 30 DIV cultures, followed by addition of ACM from 15 DIV cultures, may depend by ERK1 activation.

In conclusion, our results suggest that DNA and RNA labelling are differentially up and down regulated in astrocyte cultures pre-treated with exogenously added growth factors or treated with endogenous trophic molecules released in the conditioned media. In addition, the particular environment created by astroglial cultures can modulate their own proliferation and differentiation, through the release of soluble molecules actively interacting with their genomic program particularly stimulated under our experimental culture conditions.

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SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY (SPECT) WITH ^{123}I-IOFLUPANE: IN VIVO MOLECULAR IMAGING TECHNIQUE OF DOPAMINE TRANSPORTER (DAT)

A. Distefano.
Institute of Neurological Science (National Council of Research) Catania

Among the monoamine transporters, the dopamine transporter (DAT) has been extensively studied. DAT is present exclusively in dopamine-synthesising neurones (J.Neuroscence 1996; J Comp Neurol 1997) and it can be considered a specific marker of these neurons in the CNS. Because DAT distribution in the CNS coincides with dopaminergic innervation, DAT ligands have been developed for use in neuroimaging as markers of dopaminergic system in vivo.
SPECT (Single Photon Emission Computed Tomography) is a molecular imaging technique that uses radiolabelled molecules to image molecular interaction of biological processes in vivo.
To date, ^{123}I-Ioflupane, an analogue of cocaine, is the tracer used most widely for SPECT imaging of DAT density in the human brain.
The radiotracers for imaging DAT with SPECT must have particular properties. They should have: high affinity and selectivity for DAT sites, the ability to pass the blood-brain barrier, low non-specific binding and no or negligible blood-borne metabolites into the brain which could interfere with quantification of DAT binding.
^{123}I-Ioflupane SPECT is a marker of nigrostriatal neuronal integrity, allowing differentiation of parkinsonism with loss of dopaminergic terminals from parkinsonism without nigrostriatal degeneration.
Parkinson's disease (PD) is pathologically hallmarked by degeneration of neural connection, more specifically dopaminergic neurons between the subtantia nigra (SN) and the striatum.
Parkinson's disease is the second most common degenerative disorder, after Alzheimer's disease affecting nearly 2.0 % of adults over the age of 65, characterized by the cardinal signs, according to the "Parkinson's Disease Society Brain Bank" criteria: bradykinesia, rigidity, tremor at rest and abnormalities of balance, posture, gait and levodopa responsiveness. Population-based studies have shown that at least 15% of patients with a diagnosis of Parkinson's disease do not fulfill strict clinical criteria for the disease.
Because PD is associated with progressive neuronal loss of the substantia nigra and other brain structures, the Single Photon Emission Computed Tomographic studies with ^{123}I-Ioflupane (DaTSCAN®), is a sensitive early diagnostic marker and it can discriminate patients with PD from normal subjects, drug-induced parkinsonism, psychogenic parkinsonism, vascular parkinsonism or with essential tremor.

BRAIN MAGNETIC RESONANCE IMAGING IN TMT TREATED RATS

F. Pizzolante[1], P.R. Andjus[2], G. Bačić[2], S. Ceccariglia[1], A. D'Altocolle[1], A. Del Fà[1], C. Gangitano[1], F. Michetti[1].

[1]*Institute of Anatomy and Cell Biology. Catholic University of the "Sacred Heart", Rome-Italy.*
[2]*Institute of General and Physical Chemistry, Belgrade University, Belgrade-Serbia.*

Aim 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. MRI versatility can be improved by the introduction of external agents, like the paramagnetic metal complexes and ultrasmall paramagnetic iron oxide particles (USPIO). The aim of our study was to investigate possible morphological changes in the hippocampus area in a MRI after TMT intoxication. In addition, the state of the blood brain barrier (BBB) was checked with the application of Gd-DTPA contrast. Methods Ten female Wistar rats were treated with TMT at a single dose of 8 mg/kg ip. 3 control rats received an equivalent amount of saline solution. Imaging studies were performed under general anesthesia, on a 1.5 T MRI. In 6 treated rats MRI was also performed after Gd-DTPA injection i.v. The brains were than isolated and fixed in 4% PFA solution. Coronal sections were prepared on a cryostat microtome and than incubated with primary fluorescent antibodies Ox42-Ig mouse and S100-Ig rabbit. Imaging of fluorescence was performed on a confocal microscope. Results In 5 animals MRI investigation showed lateral ventricles dilation. MRI evaluation didn't show significant alterations on the hippocampus area. MRI after injection of Gd-DTPA showed in 3 animals a positive enhancement in the frontal sections, corresponding to max dilation of the lateral ventricles and the frontal sections of hippocampus. MRI signal abnormalities after MACS administration were not detected in the 3 treated animals. Sections of treated animals processed with antibodies against markers of reactive microglia and astroglia Ox42 and S100 showed intense glial reaction. Discussion MR imaging in TMT treated rats revealed the presence, in vivo, of important alterations such as the dilation of lateral ventricles. Although data in literature document absence of alterations of the BBB in hippocampus of 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. The effects of TMT intoxication on the permeability of BBB and the use of USPIO antibodies against microglial markers in MR imaging of this experimental model with stronger MRI should be further investigated. MRI analysis from the TMT-treated hyppocampus needs to be further studied in order to find right protocols for assessing fine tissue deterioration.

Indice degli Autori