Peroxisome proliferator-activated receptor (PPAR) agonists protect human podocytes against cell death induced by oxygen/glucose deprivation-reoxygenation

DIPARTIMENTO DI ANATOMIA, FARMACOLOGIA E MEDICINA LEGALE

II^ Giornata della Ricerca

6 Maggio 2010

Presso: Sezione di Anatomia, corso Massimo D'Azeglio 52, Aula C

PROGRAMMA E RELAZIONI

A cura della
Commissione Ricerca del Dipartimento

Patrizia PANZANELLI (Coordinatore, Sez. Anatomia**)**
Roberto CANAPARO (Sez. Farmacologia e Terapia Sperimentale**)**
Cristina CILLI (Sez. Musei**)**
Corrado GHE' (Funzionario Tecnico EP**)**
Sarah GINO (Sez. Medicina Legale**)**
Gianluca MIGLIO (Sez. Farmacologia e Farmacognosia**)**
Alessandra OBERTO (Sez. Farmacologia e Terapia Sperimentale**)**

GianCarlo PANZICA (Direttore, Sez. Anatomia)

Dipartimento di Anatomia, Farmacologia e Medicina Legale

II^ GIORNATA DELLA RICERCA

6 Maggio 2010

C.so Massimo D'Azeglio, 52

Aula C e Sala Settoria

PROGRAMMA

Mattina 9.30-12,00

Ore 9,00 Montaggio Poster

Ore 9,00-10,00 Sessione Poster (Prima parte) - **SALA SETTORIA**

Ore 10,00 Saluto ai partecipanti e apertura dei lavori da parte del Direttore, Prof. Giancarlo Panzica

Presentazioni Orali (Prima Parte) – **AULA C**

Ore 10,00 *Graft of embryonic neural precursors and adult mesenchymal stem cells in an experimental model of spinal cord hemisection.*
Boido M., Rupa R., Garbossa D., Vercelli A. (Sez. Anatomia)

Ore 10,15 *Hormonal restitutive therapy: cognitive functions and neural plasticity*.
Dallorto D., Mauro M., Ghi P., Orsetti M. (Sez. Farmacologia e Farmacognosia)

Ore 10.30 *Testosterone regulates adult neurogenesis in rat SVZ.*
Farinetti A., Vercelli A., Peretto P., Panzica G.C. (Sez. Anatomia)

Ore 10,45 *Synaptical maturation of adult born neurons.*
Pallotto M. (Sez. Anatomia)

Ore 11,00 *Molecular and functional heterogeneity of GABA synapses.*
Patrizi A., Briatore F., Frola E., Pregno G., Jetti S.K., Sassoè-Pognetto M. (Sez. Anatomia)

Ore 11,15 *Obestatin affords cardioprotection to the ischemic/reperfused isolated rat heart and inhibits apoptosis in cultures of similarly stressed cardiomyocytes.*
Arnoletti E., Bassino E., Penna C., Perrelli M.G., Ghè C., Alloatti G., Muccioli G. (Sez. Farmacologia e Terapia Sperimentale)

Ore 11,30 *Peroxisome proliferator-activated receptor (PPAR) agonists protect human podocytes against cell death induced by oxygen/glucose deprivation-reoxygenation.*
Rattazzi L., Miglio G. (Sez. Farmacologia e Farmacognosia)

Ore 11,45 *Subtyping of Y-chromosomal haplogroup E-M78 (E1b1b1a) by SNP assay and its forensic application.*
Caratti S. (Sez. Medicina Legale)

Ore 12,00-13,30 ***Pausa Pranzo e Discussione Poster*** **(SALA SETTORIA)**

Pomeriggio 13.30-17,30

Presentazioni Orali (Seconda Parte) – **AULA C**

Ore 13,30 *3D morphometry: quantitative and statistical analysis of face models.*
Verzé L., Ramieri G. (Sez. Anatomia)

Ore 13,45 *Three-dimensional sonographic approach in the study of fetal thymus.*
Olearo E., Oberto M., Gaglioti P., Panattoni G.L., Todros T.

Ore 14,00 *MyomiR 1/206 as differentiating agent for the treatment of Rhabdomyosarcoma.*
Bersani F. (Sez. Anatomia)

Ore 14,15 *Neural-restricted expression of Met delays disease onset but not progression of ALS mice.*
Caricati E., Genestine M., Richelme S., Raoul C., Pettmann B., Lamballe F., Panzica G., Maina F., Dono R. (Sez. Anatomia)

Ore 14,30 *Evidences for aberrant Met signalling involvement in the pathogenesis of heart disease.*
Sala V. (Sez. Anatomia)

Ore 14,45 *Synaptic determinants of Rett syndrome.*
Boggio E.M., Tomassy G.S., Morando L., Pizzorusso T., **Giustetto M**. (Sez. Anatomia)

Ore 15,00 *B7h triggering inhibits umbilical vascular endothelial cell adhesiveness to tumor cell lines and polymorphonuclear cells.*
Minelli R. (Sez. Farmacologia e Farmacognosia)

Ore 15,30-17,30 **Sessione Poster** (Seconda Parte) - **SALA SETTORIA**

Ore 17,30 ***Fine lavori***

RELAZIONI

- AULA C -

Boido M., Rupa R., Garbossa D., Vercelli A.

Dept. of Anatomy, Pharmacology and Forensic Medicine, University of Turin, Italy
Section: Anatomy

Graft of embryonic neural precursors and adult mesenchymal stem cells in an experimental model of spinal cord hemisection

Spinal cord injury (SCI) can determinate neurological deficits below the injury site, producing a functional damage to local neurons and axons fibres. Serotonergic raphespinal projections promote functional recovery after SCI, but spontaneous regeneration of most severed axons is limited by the glial cyst and scar, at the lesion site. In the present study we have examined whether stem cell transplantation could offer a promising approach for inducing regeneration through the damaged area, comparing the effects of transplantation of embryonic neural precursors (NPs) and adult mesenchymal stem cells (MSCs). Spinal cord hemisection was performed at the L2 neuromer in adult mice. Two weeks post-injury, we transplanted NPs or MSCs into the cord, at the L3 neuromer. Injured mice without a graft served as controls. In order to value the functional recovery, mice underwent a battery of motor tasks. Twenty-eight days after transplantation, animals were sacrificed and analyzed for survival of grafted cells, for effects of engraftment on the local cellular environment and for the sprouting of serotonergic axons.
Both types of stem cells survived several weeks and were integrated into the injured spinal cord; moreover NPs were able to express neuronal markers. All transplanted animals displayed an increased number of 5-HT-positive fibres caudal to the hemisection, compared to untreated mice. Finally stem cell transplantation significantly improved functional recovery in animals with SCI. These results point to a therapeutic potential for such cell grafting: both cell types probably could deliver trophic and immunomodulatory factors in proximity of lesion site, inducing axonal regeneration.
Supported by Girotondo Onlus and AIM Foundations and by Regione Piemonte grants to AV.

Dallorto D., Mauro M., Ghi P., Orsetti M.

Dept. of Anatomy, Pharmacology and Forensic Medicine, University of Turin, Italy
Section: Pharmacology and Pharmacognosy

Hormonal restitutive therapy: cognitive functions and neural plasticity

Neuroscience research has provided biological plausibility for the hypothesis that estrogen replacement therapy (ERT) would protect against cognitive aging in healthy women. Nevertheless the disparity between the basic and clinical science findings of estrogen regulation of cognitive functions has been the topic of much debate. On this point an important caveat is that hormones, including estradiol, do not enhance all aspects of cognition. The data from basic science analyses would indicate that estrogen regulates cognitive functions that require the following: (i) efficient transfer of information, (ii) distribution of information to multiple neural circuits, (iii) association of information across time and (iv) a higher order of information complexity (i.e. information across multiple sensory modalities or from multiple experiences to generate a higher executive function). (Brinton 2009).In this perspective we tested the ovariectomized rats in timing behavior test, a modified temporal response differentiation (TRD) task (Ferguson 1994). The TRD schedule is notably different from other temporal tasks in that it requires subjects to initiate and maintain a lever press for a fixed time (0,2,4 sec). Our preliminary cognitive data indicate that testosterone (TXT) and 17 β estradiol (E2) counteract the TRD deficit ovex-induced. Moreover changes detected in the hippocampal (CA1, CA3 and dentate girus) expression of synaptophysin, a marker of synaptic plasticity , supports the hypothesis that the HRT improve, in different manner, the simple or higher cognitive functions. Timing Behavior task could be a suitable test to understand estrogen action in the brain and to evaluate SERMs and HRT effects on cognitive functions.

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Farinetti A., Vercelli A., Peretto P., Panzica G.C.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Testosterone regulates adult neurogenesis in rat SVZ

Adult neurogenesis is a process occurring within the Central Nervous System in the olfactory bulbs, in the subventricular zone (SVZ), and in the dentate gyrus. Steroid hormones are supposed to play a role in regulating this phenomenon, therefore we studied here the activational effects of Testosterone (T) on the proliferation of neuroblasts in the SVZ. To this aim, we analyzed three groups of 5 adult male rats: castrated rats (CX), castrated and treated with T (CX+T), and controls rats (CON). All the animals received two intraperitoneal injection of BrdU and were sacrificed one day after the last BrdU administration to investigate the proliferation within the SVZ. The rate of neurogenesis was evaluated through the counting of immunocytochemically stained BrdU-positive cells. For the quantitative evaluation, we analysed three sections per animal, taken at rostral (Bregma 2.20 mm), medial (Bregma 1.60 mm) and caudal (Bregma 1.20 mm) levels. Our data revealed a significant decrease in the area covered by BrdU-positive elements in CX animals in comparison to both CX+T and CON. This effects is however limited to the medial level of SVZ.
In conclusion, present data suggest that the decrease of circulating levels of T caused by castration induces a decrease of the rate of neurogenesis in specific parts of the SVZ.
Supported by Regione Piemonte, University of Torino and Fondazione San Paolo.

Pallotto M.
Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Synaptical maturation of adult born neurons

Neuronal progenitors are produced in the Subventricolar Zone. They migrate tangentially along the rostral migratory stream and reach the olfactory bulb (OB), where they differentiate into granule cells (GC) and periglomerular cells. While it is known that newborn GCs become functionally integrated into the OB network, the underlying mechanisms are unclear. The aim of this study was to investigate the spatial and temporal patterns of synaptical maturation of newborn granule cells in the adult mouse OB. The first GABAergic and Glutamatergic synaptic inputs onto GFP-positive cells were detected in the GC layer at 3 dpi (days post injection). Until 5 dpi, inhibitory contacts largely exceeded excitatory ones, suggesting a prevalent GABAergic innervation in the early stages of development. These results were supported by patch-clamp recordings, that revealed the presence of both EPSCs and IPSCs in maturing GCs. Glutamatergic synapses on spines were characterized by a complex reorganization of PSD geometry, as documented by serial electron microscopy. Thus, while most synapses at 7dpi had a simple shape, synapses impinging onto older cells exhibited complex postsynaptic profiles, with the PSD forming an almost complete ring around a central perforation. In the external plexiform layer, GCs first received glutamatergic innervation at 4 dpi, and subsequently developed reciprocal dendrodendritic synapses, as confirmed by electron microscopy at 7 dpi.

These findings demonstrate that adult-generated GCs start to establish synapses as soon as they reach their final destination in the OB, while their maturation is a longer process. The early development of GABAergic inputs suggests that GABA may play an important role in the synaptic integration, maturation and survival of newborn GCs. To test this hypothesis, we are studying the effects of a selective ablation of GABA-A receptors on GC development.

Patrizi A., Briatore F., Frola E., Pregno G., Jetti S.K., Sassoè-Pognetto M.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Molecular and functional heterogeneity of GABA synapses

GABAergic inhibition may be “the real essence” in neuronal computation. Practically all neurons in the brain express GABA receptors, which occur in several different subunit combinations and control many crucial aspects of neural function. Changes in GABAergic transmission contribute to the etiology of prominent brain disorders, including epilepsy, anxiety, and schizophrenia. Therefore, the mechanisms that regulate the development and maturation of GABA synapses have important consequences for brain function and disease. Research over the last two decades has revealed an extraordinary diversity in the molecular organization of GABA synapses. We are just starting to appreciate the physiological importance of this diversity, and how it is determined during brain development. In this perspective, I will focus on the evidence that has become available from the analysis of genetic loss-of-function models, i.e. mutant mice lacking crucial molecular constituents of synaptic specializations. These studies have revealed that signalling GABAA receptors shapes inhibitory synaptogenesis by coordinating the precise matching of pre- and postsynaptic sites. Of particular relevance are the possible interactions of GABAA receptors with postsynaptic scaffolds and synaptic cell-adhesion molecules, such as the neurexin-neuroligin adhesion system. An exciting concept emerging from these studies is that GABAergic synaptogenesis depends on multiple parallel pathways, which involve distinct cellular and molecular events. It will be important to understand whether these pathways are largely independent or whether they are linked into signaling complexes that act synergistically to coordinate synapse development.

Arnoletti E., Bassino E., Penna C., Perrelli M.G., Ghè C., Alloatti G., Muccioli G

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Pharmacology and Experimental Therapy

Obestatin affords cardioprotection to the ischemic/reperfused isolated rat heart and inhibits apoptosis in cultures of similarly stressed cardiomyocytes

Obestatin 1-23 (OB), a newly discovered peptide encoded by the ghrelin gene, induces expression of genes regulating pancreatic β-cell differentiation, insulin biosynthesis and glucose metabolism. It also activates anti-apoptotic signalling pathways such as PI3K and ERK1/2 in pancreatic β-cells and human islets. Since these kinases have been shown to protect against myocardial injury, we sought to investigate whether OB would exert cardioprotective effects. Both isolated perfused rat heart and cultured cardiomyocyte models of ischemia/reperfusion (I/R) were used, measuring infarct size and cell apoptosis as end points of injury. The presence of specific OB receptors on cardiac cells, as well as the signalling pathways underlying the OB effect were also studied. In isolated heart, the addition before ischemia of rat OB, reduced infarct size and contractile dysfunction in a concentration-dependent manner, whereas OB-(23-1), a synthetic analogue with inverse aminoacid sequence, was ineffective. The cardioprotective effect of OB was observed at concentrations of 10-50 nM and was abolished by inhibiting PI3K or PKC by addition of wortmannin (100 nM) or chelerythrine, (5 μM), respectively. In rat H9c2 cardiac cells or isolated ventricular myocytes subjected to I/R, 50 nM OB reduced cardiomyocyte apoptosis and reduced caspase-3 activation; the anti-apoptotic effect was blocked by inhibition of PKC, PI3K or ERK1/2 pathways. In keeping with these functional findings, binding data revealed the presence of specific high-affinity OB binding sites, mainly localized on membranes of cardiomyocytes. Our data suggest that, acting on specific receptors, OB activates PKC, PI3K and ERK1/2 signalling and protects cardiac cells against myocardial injury and apoptosis induced by I/R.

Rattazzi L., Miglio G.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Pharmacology and Pharmacognosy

Peroxisome proliferator-activated receptor (PPAR) agonists protect human podocytes against cell death induced by oxygen/glucose deprivation-reoxygenation

PPAR are ligand-activated transcription factors and three subtypes (α, β/δ and γ) have been identified. PPAR agonists have been shown to decrease glomerular injury in *in vivo* models of renal ischema/reperfusion (I/R) [1,2]. However, whether a direct effect on cellular components of the glomerular filtration barrier contributes to the observed protection remains to be established. By using oxygen/glucose deprivation (OGD)-reoxygenation as an *in vitro* model that mimics *in vivo* I/R, the effects of selective PPAR agonists on podocyte death have been compared. Human immortalized podocytes have been pre-treated with gemfibrozil (PPARα), PPARGW0742 (PPARβ/δ), pioglitazone or rosiglitazone (PPARγ), either as acute (single dose) or repeated (3 days exposure) challenge. Cell death was measured as decrease in cell number, necrosis and apoptosis. Only the repeated pre-treatment with each agonist significantly prevented cell death, mainly by decreasing apoptosis. In comparison, in a model of serum-deprivation (48 h)-induced apoptosis both pre-treatments were effective, although the repeated one achieving the highest maximal effects. These results suggest that PPAR agonists protect podocytes mainly by decreasing apoptotic cell death. These findings contribute to clarify the pathophysiological role of PPARs in the kidney and indicate them as pharmacological targets for I/R-induced glomerular diseases.

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Caratti S.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Forensic Medicine**,** Laboratorio di Scienze Criminalistiche – Sezione di Genetica Forense

Subtyping of Y-chromosomal haplogroup E-M78 (E1b1b1a) by SNP assay and its forensic pplication

The study of Y-chromosomal binary polymorphisms (Single Nucleotide Polymorphisms, SNPs), fundamental for reconstructing the genetic history of human populations, may also prove useful in the forensic field as a tool for the prediction of population-of-origin of unknown casework samples. Several papers describing the rapid and simultaneous typing of major human Y-chromosomal haplogroups have been recently published and the continual discovery of new SNPs is leading to an increased resolution of the Y chromosome phylogeny. Haplogroup E is the most polymorphic in the Y chromosome, with 83 polymorphisms defining 56 different haplogroups; in particular, the haplogroup E-M78 (E1b1b1a) is very common in Europe (with a frequency in Italy ranging from 4 to 10%), in northern and eastern Africa and in western Asia. The recent discovery of new binary polymorphisms included in E-M78, defined several sub-haplogroups with very different evolutionary history and geographic distributions limited to specific subcontinents, which study could lead to the refining of methods for prediction of population-of-origin of unknown samples. Here we describe a system for the molecular dissection of haplogroup E-M78 (E1b1b1a), consisting of multiplex PCR and minisequencing of M78 and nine population-informative Y-SNPs (M148, M224, V12, V13, V19, V22, V27, V32, V65) in a single reaction. Sensitivity and admixture studies demonstrated that the SNP protocol allows robust genotyping from as little as 50 pg of male DNA, even in the presence of 500-fold amounts of female DNA. In order to evaluate the suitability of E1b1b1a subhaplogrouping for population-of-origin prediction, the distribution of E-M78 and its derived variants was determined in an Italian population sample (n=326).

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Verzé L., Ramieri G.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

A major 3D morphometry: quantitative and statistical analysis of face models

A major problem in analysing facial models has been the inability to capture 3-D facial anatomical coordinates. Digital in vivo scanning now gives access to a huge amount of accurate, high-resolution, reproducible and easily obtainable anatomical data. A new conceptual approach to the study of facial morphology is being developed, which allows description of the complex 3D form of the face in a way that is adapted to statistical evaluation and comparison. Quantitative and statistical analysis of the mean face in adults was achieved. We are using laser scanning to create a mean 3D morphology or model of the normal human face and to investigate the soft tissue effects of surgical treatment in adult subjects and to correlate the surface changes with the underlying bone displacements. After laser scanning of the face, different analyses were performed: Anatomical Landmarks Dislocation, Clearance Vector Mapping, and Geometry Morphometrics, to assess applicability, informative value and the ease of performing statistical analysis. The following conclusions were drawn from our study. 3D optical scanning of the face is able to detect even small post-treatment changes and can provide valuable information for quantitative and statistical analysis of face models. Among the more sophisticated approaches, new techniques of facial 3D morphometrics promise to permit new and interesting investigations in the analysis of 3D facial morphology and clinical models. Further development, careful validation, and collection of normative reference data are required.

Olearo E., Oberto M., Gaglioti P., Panattoni G.L., Todros T.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Three-dimensional sonographic approach in the study of fetal thymus

Fetal thymus was studied in term of presence/absence, as an expression of some congenital deseases (cono-truncal anomalies) and as a marker of feto-placental inflammatory states. However no studies comparing thymic size between healthy fetuses and fetuses affected by fetal growth restriction, are available in literature. The trasverse diameter and perimeter of thymus in healthy fetuses were evaluated by ultrasound, using the "three vessel view". It is now possible to evaluate fetal thymic size and growth through different gestational ages by three-dimensional sonography: this technique was not previously used for evaluation of fetal thymus. By a dedicated software (VOCAL) we were able to reconstruct the fetal thymic volume, that was obtained automatically from different ultrasound scan sections originated by the 3D sweep of the ultrasound beam and we were able to draw a contourn line along the thymus perimeter of different planes. The aim of our study is to determinate possible differences between healthy fetuses and the ones affected by developmental anomalies concerning growth, morphology and function of fetal thymus on different gestational ages. Ultrasound measurements will be compared with anatomic findings on fetuses from abortions and intrauterine demises to evaluate the accuracy of ultrasound volumes in order to reproduce the "real" organ.

Bersani F.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

MyomiR 1/206 as differentiating agent for the treatment of Rhabdomyosarcoma

Many microRNAs (miRNAs), posttranscriptional regulators of numerous cellular processes and developmental events, are downregulated in tumors. However, their role in tumorigenesis remains largely unknown. In this work, we examined the role of the muscle-specific miRNAs miR-1 and miR-206 in human rhabdomyosarcoma (RMS), a soft tissue sarcoma thought to arise from skeletal muscle progenitors. We have shown that miR-1 was barely detectable in primary RMS of both the embryonal and alveolar subtypes and that both miR-1 and miR-206 failed to be induced in RMS cell lines upon serum deprivation. Moreover, reexpression of miR-206 in RMS cells promoted myogenic differentiation and blocked tumor growth in xenografted mice by switching the global mRNA expression profile to one that resembled mature muscle. Finally, we showed that the product of the MET proto-oncogene, the Met tyrosine-kinase receptor, which is overexpressed in RMS and has been implicated in RMS pathogenesis, was downregulated in murine satellite cells by miR-206 at the onset of normal myogenesis. Thus, failure of posttranscriptional modulation may underlie Met overexpression in RMS and other types of cancer. We propose that tissue-specific miRNAs such as miR-1 and miR-206, given their ability to modulate hundreds of transcripts and to act as nontoxic differentiating agents, may override the genomic heterogeneity of solid tumors and ultimately hold greater therapeutic potential than single gene-directed drugs.

Caricati E., Genestine M., Richelme S., Raoul C., Pettmann B., Lamballe F., Panzica G., Maina F., R. Dono R.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Neural-restricted expression of Met delays disease onset but not progression of ALS mice

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that affects motoneurons (MNs) in the spinal cord, brainstem and cerebral cortex. ALS initiates with muscle weakness and evolves rapidly into generalized paralysis and death of the patient for respiratory failure. The discovery that approximately 20% of familial ALS is caused by mutations in the *sod1* gene has been followed by the generation of transgenic mice and rats that develop age-dependent degeneration of MNs. These animal models, by mimicking human pathology, have been essential to study ALS and develop preclinical studies. Neurotrophic factors are of a crucial importance for MNs survival and function. The receptor tyrosine kinase Met and its ligand HGF trigger MNs survival during development and after axotomy. Interestingly, it has been shown that over-expression of HGF delays disease progression and prolongs life span of the SOD1 mutant transgenic mice (SOD1-G93A). In this work, we evaluate the impact of Met signaling during onset and progression of the MNs disease through a genetic approach. Towards this goal, we have generated conditional transgenic mice that express Met in a neural-restricted manner (met-tg; nestinCre). We have next crossed met-tg; nestinCre with SOD1-G93A mice (met-tg; nestinCre; SOD1-G93A), and followed their lifespan and motor function using behavioral tests (swimming tank and hind path foot printing). Our analysis shows that enhanced Met signaling in SOD1 mutant mice significantly increases motor capacity of the SOD1 mice and prolongs their lifespan. Our studies also show that the functional recovery observed in met-tg; nestinCre; SOD1-G93A mice is linked to a delay in the onset of the disease rather than its progression.

Sala V.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Evidences for aberrant Met signalling involvement in the pathogenesis of heart disease

The hepatocyte growth factor (HGF) is a pleiotrophic cytokine with paracrine action involved in many physiological processes, including development of skeletal muscle and liver. Literature offers only few information about its role and that of its tyrosine-kinase receptor c-Met in the normal development of myocardium and in the pathogenesis of heart disease.
In order to examine the only autocrine effects of a sustained activation of Met signalling in the cardiomyocyte, we have generated a transgenic mouse model with cardiac-specific and tetracicline-inducible expression of Tpr-Met. Tpr-Met is a truncated form of c-Met receptor, with constitutively active kinase activity and no negative feedback regulatory domains. In our transgenic model, Tpr-Met is expressed specifically in cardiomyocytes together with the reporter gene GFP.
Prenatal expression of Tpr-Met is lethal soon after birth, with no living mice at post-natal day 4.
In order to avoid early lethality and to examine effects of Met activation in the already-formed cardiomyocyte, we have induced Tpr-Met transgene starting from postnatal life. We thus generated a model of heart failure with early exordium, characterized by reduced levels of Connexin 43 protein, upregulation of foetal genes, concentric hypertrophy, second (pseudo-normal) and third (restrictive) grade diastolic dysfunction, with only mild alterations in ECG parameters.
In the whole, our results demonstrate that excessive activation of HGF / Met system can lead to heart damages and suggest that Met downstream pathways can be implicated in the pathogenesis of heart disease.

Boggio E.M., Tomassy G.S., Morando L., Pizzorusso T., **Giustetto M**,

Dept. of Anatomy, Pharmacology and Forensic Medicine, University of Turin, Italy and National Institute of Neuroscience-Italy.

Synaptic determinants of Rett syndrome

There is mounting evidence showing that the structural and molecular organization of synaptic connections are affected both in human patients and in animal models of neurological and psychiatric diseases. As a consequence of these experimental observations, it has been introduced the concept of synapsopathies, a notion describing brain disorders of synaptic function and plasticity. A close correlation between neurological diseases and synaptic abnormalities is especially relevant for those syndromes including also mental retardation in their symptomatology, such as Rett Syndrome (RS). RS (MIM312750) is an X-linked dominant neurological disorder that is caused, in the majority of cases by mutations in methyl-CpG-binding protein 2 (MeCP2). In the past years I focused my research interests on the synaptic alterations produced by mutations of the gene MeCP2 in mouse models of RS and experimental therapies aimed to reverse the neuropathological signs of the disease. Different experimental approaches have revealed that RS could be the consequence of an impairment in the homeostasis of synaptic transmission in specific brain regions. Indeed, we found that the growth of GABAergic synapses induced by environmental enrichment is impaired in the absence of MeCP2. Based on the results produced by our and other research groups, it is reasonable to propose that understanding how the brain is affected by diseases such as RS is at reach. This effort will bring us closer to identify the neurobiological bases of human cognition.

Supported by Telethon; Fondazione San Paolo

Minelli R.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Pharmacology and Pharmacognosy

B7h triggering inhibits umbilical vascular endothelial cell adhesiveness to tumor cell lines and polymorphonuclear cells

Vascular endothelial cells (EC) are key players in leukocyte recruitment into tissues and metastatic dissemination of tumor cells. EC express B7h that is the ligand of the ICOS T cell costimulatory molecule. The aim of this work was to assess the effect of B7h triggering by a soluble form of ICOS (ICOS-Fc) on the adhesion of colon carcinoma cell lines to human umbilical EC (HUVEC). We found that B7h triggering inhibited HUVEC adhesiveness to HT29 and DLD1 cells (by 50 and 35% respectively), but not to HCT116 cells. The effect was dependent on the ICOS-Fc dose, detectable as soon as 30 min after treatment and still present after 24 hrs; it was inhibited by soluble anti-ICOS reagents (mAb and B7h-Fc) and silencing of B7h on HUVEC, and it was not displayed by a F119S mutated form of ICOS-Fc that does not bind B7h. HUVEC treatment with ICOS-Fc did not modulate expression of adhesion molecules and cytokines, but substantially down-modulated phosphorylation of p38 and ERKs induced by E-selectin triggering, which has been shown to increase HUVEC adhesiveness to colon carcinoma cell lines. Moreover, HUVEC treatment with ICOS-Fc also inhibited adhesion of polymorphonuclear cells (PMNs) and several tumor cell lines from different origins. Therefore, the B7h:ICOS interaction may modulate spreading of cancer metastases and recruitment of PMNs in inflammatory sites, which opens a novel view on the use of ICOS-Fc as an immunomodulatory drug.

SESSIONE POSTER

- SALA SETTORIA -

Bertini G., Ferro M.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Pharmacology and Experimental Therapy

Thiopurine S-methyltransferase pharmacogenetics: a prospective study of the effect of oncomitant medications on thiopurines metabolism in Inflammatory Bowel Disease (IBD) and geno-phenotype differences between healthy volunteers and IBD patients.

Introduction: Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyses the S-methylation of aromatic and heterocyclic sulphydril compounds. The function of this protein in human biology is uncertain, but TPMT is an important enzyme in the metabolism of anti-leukemic and immunosuppressive drugs (thiopurine drugs), therefore thiopurines are used in the treatment of Inflammatory Bowel Disease (IBD). Moreover this enzyme is an examples of the potential pharmacogenetics has to contribute in the tailoring of drug therapy. **Aim:** To explore the interactions between thiopurines and concomitant medications on TPMT activity in IBD patients and to evaluate the impact of TPMT genotype and phenotype in healthy volunteers and IBD patients. **Methods:** 183 IBD patients and 571 healthy volunteers were enrolled in this study. Blood samples were collected in two 3 ml test tubes containing EDTA for genotype and phenotype determination. In IBD patients clinical characteristics and concomitant medications were recorded. **Results:** Based on TPMT genotype, 95% of IBD patients were wild-type homozygous, the remaining being heterozygous whereas, in healthy population, 93% were homozygous, 6.6% individuals were heterozygous and one subjects had the rare *3B/*3C genotype. TPMT phenotype showed a difference in TPMT activity comparing IBD cases and healthy volunteers. In IBD patients no difference in TPMT activity was noted according to medications exposure and patients on thiopurines had higher TPMT activity levels, but no dose-effect. No difference in TPMT activity was observed in 41 patients co-treated with 5-ASA. **Conclusion:** Our data suggest a difference in TPMT phenotype between healthy population and IBD patients and absence of interactions between thiopurines and other medications.

IBD Onlus e la Banca del Sangue dell'Ospedale San Giovanni Battista

Bertocchi I.[a], Oberto A.[a], Longo A.[a], Mele P.[a], Palanza P.[b], Sprengel R.[c], Eva C.[a]

[a] Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
[b] Università di Parma, Dip. di Biologia Evolutiva e Funzionale
[c] Max Planck Institute for Medical Research, Heidelberg, Germany

Limbic neuropeptide Y-Y1 receptor expressing neurons are mandatory for anxiolytic-related behaviour and body weight control

Neuropeptide Y (NPY) is one of the most abundant neuropeptides in the mammal central nervous system where it plays a pivotal role in the regulation of stress response, emotional and feeding behaviour via the activation of the Y1 receptor (Y_1R) subtype. Recent clinical data implicate NPY-Y_1R in the pathophysiology of human anxiety. Nevertheless, germinal Y_1R knockout has little impact on emotional and feeding behaviour in mice.
We generated a conditional knockout mouse line ($Y_1R^{fb-/-}$) in which Y_1R function is inactivated postnatally in forebrain by using the doxicycline-controlled *Cre/lox*P system.
$Y_1R^{loxP/loxP}$ animals showed the normal widespread brain expression pattern of Y_1R whereas in $Y_1R^{fb-/-}$ conditional mutants, Y_1R mRNA and Y_1R immunoreactivity were selectively decreased in the hippocampus and in the amygdala, but not in the hypothalamic nuclei.
Starting at P40, $Y_1R^{fb-/-}$ conditional mutants show a long lasting reduction of body weight which is associated with decreased leptin and glucose plasma levels. Moreover, selective ablation of Y_1R in adult mice forebrain increased anxiety-related behaviour in the elevated plus-maze test. There was a significant effect of genotype for percentage of time spent and percentage of entries into the open arms. The anxious phenotype of conditional $Y_1R^{fb-/-}$ mutants was associated with a significant decrease of CRH immunoreactive fibers in the central amygdala. General locomotor activity in the open field protocol as well as spontaneous locomotor activity in cages were similar in both groups.
Our data clearly show that deletion of limbic Y_1R increases anxious-related behaviour. Furthermore, we provide evidence for a role of limbic Y_1R in the control of energy homeostasis that is independent of hypothalamic functions.

Bo E., Sterchele, D., Viglietti-Panzica C., Panzica G.C.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Effect of endocrine disrupter TBT on mouse brain circuit controlling food intake

Many substances, such as the biocide tributyltin (TBT), are known as "*endocrine disrupters*" because of their ability to interfere with hormonal pathways in vertebrates. Aim of this work was studing if early exposition to TBT may not reversibly alter circuits controlling food intake and predispose organisms to obesity. TBT, diluited in olive oil, was orally administred to adult C57/BL6 mice at dose of 0,025 μg/g/day/body weight 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.

Briatore F., Patrizi A., Viltono L., Sassoè-Pognetto M., Wulff P.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Quantitative organization of GABAergic synapses in the molecular layer of the mouse cerebellar cortex

In the cerebellar cortex, interneurons of the molecular layer (stellate and basket cells) provide GABAergic input to Purkinje cells, as well as to each other and possibly to other interneurons. GABAergic inhibition in the molecular layer has mainly been investigated at the interneuron to Purkinje cell synapse. In this study, we used complementary subtractive strategies to quantitatively assess the ratio of GABAergic synapses on Purkinje cell dendrites *versus* those on interneurons. We generated a mouse model in which the GABAA receptor a1 subunit was selectively removed from Purkinje cells using the Cre/loxP system. Deletion of GABAARa1 resulted in a complete loss of postsynaptic GABAA receptor aggregates from Purkinje cells, allowing us to determine the density of residual GABAA receptor clusters in interneurons. In a complementary approach, we labeled GABAergic synapses in wild-type mice with an antibody against a-dystroglycan, a Purkinje cellspecific
marker of inhibitory postsynaptic sites, enabling us to determine the density of GABAergic synapses in Purkinje cells. Combining these inverse approaches, we found that synapses received by interneurons represent approximately 40% of the global population of GABAergic synapses in the molecular layer, with possibly higher values in the outermost part of this layer. Notably, this proportion was stable during postnatal development, indicating that synaptogenesis of Purkinje cells occurs in concert with that of interneurons. Considering the pure quantity of GABAergic synapses onto molecular layer interneurons these data suggest that mutual inhibition must play an important, yet largely neglected, computational role in the cerebellar cortex.

Gatti S.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Gene profiling in HGF-stimulated early postnatal hearts

We investigated the role of HGF in cardiomyocytes in vivo, by using animals with cardiac-specific HGF overexpression and full genome DNA microarray analysis. We performed microarray analysis of mouse heart at two distinct developmental ages (postnatal days P2 and P7), which correspond to the transition between the plastic and post-plastic phase. When comparing HGF-expressing hearts versus wild-type hearts at P7 we found ~200 genes significantly upregulated more than 1.7-fold. Several of these genes play a role in cell cycle regulation, translation, signal transduction, transcriptional regulation, cytoskeleton/extracellular matrix and membrane vescicle traffic. The majority of these genes were downregulated from day 2 to day 7 in the wild-type heart. Thus, gene upregulation induced by HGF opposed at physiological downregulation. We confirmed that in vivo treatment with HGF increased Ki67 expression by 3-fold during day 7, indicating that HGF prolongs the plastic phase in neonatal mouse heart. As adults, the mice with cardiac-specific HGF overexpression develop a systolic contractile defect, even when HGF expression is suppressed after birth.
Conclusions: The analysis of global gene expression in the whole heart may be useful in understanding the orchestrated process of postnatal development or terminal differentiation in the cardiac environment. These data are likely to be helpful in studying the Developmental Origins of Cardiac Disease.

Imbalzano E.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Pharmacology and Experimental Therapy

Sonodynamic Therapy: high energy shock waves and 5aminolevulinic acid in a syngeneic model of colon cancer

In a previous study we demonstrated that high energy shock waves, generated by a piezoelectric generator, were able to activate a sonosensitizer, 5-aminolevulinic acid (ALA) and induce inhibition of cell growth in HT29 human colorectal cancer cell, therefore in this study we investigated the cytotoxic effect of sonodynamic therapy in a syngeneic colon cancer model. *In vitro*, DHD/K12/Trb rat colon cancer cells growth was determined by trypan blue exclusion assay and cell death was investigated by flow cytometry. ALA (50 mg/ml) and HESW (E1, EDF=0.22 mJ/mm2, 1000 shots or E2, EFD=0.88 mJ/mm2, 500 shots) showed a significant reduction of cancer cell proliferation at day 3 compared to cells exposed to ALA ($p<0.01$) or HESW ($p<0.001$) alone. An enhancement of necrotic and apoptotic cells was observed after combined treatment at day 1 with ALA and HESW E1 (a 3.1 and 6.4 fold increase vs. ALA alone) or E2 (a 3.4 and 5.3 fold increase vs. ALA alone). *In vivo*, apoptosis detection was carried out by TUNEL assay, mRNA expression of proapoptotic gene *Bad* and antiapoptotic gene *Bcl2* was evaluated by quantitative SYBR Green real time RTPCR and cleavage of poly(ADPribose) polymerase (PARP) was investigated by Western Blotting. An enhancement of apoptosis was observed in tumor tissues after the combined treatment at day 1 with ALA (375 mg/kg i.v.) and HESW (E2) compared to that ALA exposure alone with improved apoptotic index (a 2.0 fold increase), *Bad* enhanced mRNA expression ($p<0.01$), *Bcl2* decrease mRNA expression ($p<0.05$) and increased PARP cleavage. The interaction between HESW and ALA is then effective in inducing apoptosis on a syngeneic colon cancer model.

Inturri S.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Forensic Medicine**,** Laboratorio di Scienze Criminalistiche – Sezione di Genetica Forense

Linkage and linkage disequilibrium analysis of X-STRs in Italian informative families

X-chromosomal short tandem repeat (STR) loci provide an extremely useful tool in paternity testing, especially in deficiency cases with female offspring. Moreover, X-STR haplotype analysis allows to detect kinship between alleged relatives in large and incomplete pedigrees.Likelihood ratio calculations in relationship testing with X-STRs require a precise knowledge not only of allele and haplotype frequencies, but also of the genetic linkage and linkage disequilibrium (LD) status among markers. Twenty X-STRs were typed in 80 informative families of Italian descent, composed by mother and two or more sons, for a total of 93 meiosis. The analyzed X-STR panel included six clusters of closely linked markers (each spanning < 3 cM): DXS10135-DXS10148-DXS8378 (Xp22); DXS7132-DXS10074-DXS10079 (Xq12); DXS6801–DXS6809–DXS6789 (Xq21); DXS7424– DXS101 (Xq22); DXS10103-HPRTB-DXS10101 (Xq26); DXS8377-DXS10134-DXS7423- DXS10146 (Xq28). Recombination fractions between pairs of markers calculated by pedigree analysis were compared with those obtained converting physical distances by means of Haldane's mapping function. The observed differences confirm that the occurrence of recombination is not even along the X chromosome and that the conventional subdivision of X-STRs in four groups of completely unlinked markers cannot be regarded as true. Evidence of significant LD was found between markers DXS6801 and DXS6809. The effect on likelihood calculations of inferring haplotype frequencies from allele distributions rather than haplotype count in the relevant population was evaluated.

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Leo C.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

New mouse models to study pathological consequences of chronical activation of HGF/cMET axis specifically in the heart

Riassunto: The Hepatocyte Growth Factor (HGF) is a pleiotropic cytokine involved in many physiological processes, including skeletal muscle, placenta and liver development. In the adult heart, HGF has been shown to exert cardioprotective, anti-apoptotic and pro-regenerative effects in animal models of myocardial damage. Our study aims to explore effects of HGF/c-Met, especially in cardiac postnatal development and setting of cardiomyopathic disease.
To examine the function of HGF/c-Met axis specifically in the heart, we generated two transgenic mouse models, with tetracycline-inducible expression of HGF or Tpr-Met, the constitutively activated form of c-Met receptor. This was achieved by crossing a responder mouse, carrying the HGF or Tpr-Met genes under the control of a Tet-responsive element, with a second transgenic mouse expressing the cardiac-specific transactivator α-Myosin Heavy Chain (α-MHC-tTA).
When HGF expression was switched on starting from the late prenatal stage, it caused increased Ki67 positive nuclei, reduced Connexin43 (Cx43) and low levels of sarcomeric proteins in newborn mice, as respect to control littermates. Interestingly, levels of Cx43 were maintained low throughout the whole lifespan. Moreover, adult bitransgenic mice developed systolic contractile dysfunction, with impaired fractional shortening, mild dilation, re-expression of beta-myosin heavy chain and no signs of fibrosis. When HGF gene was switched on in adult mice, no signs of disease were observed.
These data suggest that HGF/c-Met axis exerts not only beneficial effects, but acts as a two-edged sword, that might play important roles in the pathogenesis of progressive cardiac dysfunction.

Longo A[a]**.,** Oberto A[a]., Bertocchi I [a]., Mele P[a]., Palanza P[b]., Sprengel R[c]., Pisu M.G[d]., Serra M[d]., Biggio G[d]., Eva C[a].

[a] Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
[b] Università di Parma, Dip. di Biologia Evolutiva e Funzionale
[c] Max Planck Institute for Medical Research, Heidelberg, Germany
[d] Università di Cagliari, Dip.Biologia Sperimentale

Role of NPY-Y_1R transmission on stress response induced by group-housing in a model of conditional knock-out mice

NPY administration elicits an anxiolytic effect in several animal models and NPY knock-out mice develop an anxiogenic behaviour. NPY also inhibits the anxiogenic effect of CRH, suggesting that they may act as functional antagonists in limbic regions. NPY is co-express with GABA in the amygdala and they may interact in the regulation of emotional behaviour.
Y_1R and Y_5R for NPY coexist in the same neurons of several limbic and hypothalamic nuclei and might both be involved in the anxiolytic and antistress effects of NPY.
We have investigated the role of NPY-Y_1R neurotransmission and its interaction with CRH and GABAergic system in the emotional response to group housing, as a model of chronic stress exposure. For this purpose, we used conditional knockout male mice ($Y_1R^{Y5R-/-}$) in which Y_1R function is postnatally inactivated in Y_5R expressing neurons.
Group-housing induced an anxious behaviour in control ($Y_1R^{loxP/LoxP}$) and $Y_1R^{Y5R-/-}$ mice, as compared to social isolated mice. In the elevated plus-maze test, there was a significant effect of housing conditions for time spent and entries into the open arms. The anxious behaviour of group-housed mice was also associated with an increase of cerebrocortical neuroactive steroids concentrations and a decrease of CRH immunoreactive fibers in the central amydala.
Socially isolated $Y_1R^{Y5R-/-}$ mice also showed an anxious behaviour, as compared to socially isolated $Y_1R^{loxP/LoxP}$ mice. Conversely, conditional deletion of Y_1R failed to affect neuroactive steroids concentrations and CRH immunoreactivity.
These data suggest that activation of Y_1R, but not Y_5R, may be involved in development of anxiety in response to group-housing, whereby a mechanism that appears to be independent on GABAergic or CHR pathways.

Maestro N.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

An inducible muscle-specific mouse model of HGF expression to study the role of HGF/Met and satellite cells in rhabdomyosarcomagenesis

Rhabdomyosarcomas (RMS) are thought to arise predominantly from skeletal muscle precursor cells, even though this hypothesis has not yet been supported by robust data. To elucidate the mechanism of RMSgenesis, we generated an inducible, muscle-specific HGF transgenic mouse. We built a HGF "responder" mouse and crossed it with a "transactivator" expressing tTA under the muscle creatine kinase promoter (MCK, N.Raben). MCK-HGF mice (MH) showed a 50% increment in Ki67/Pax7-positive cells and enhanced muscle regeneration. In Ink4a-/- background these mice (MHI-/-) developed RMS with high penetrance and short latency. IHC analysis suggests that the RMS arose from activated satellite cells that were unable to differentiate. Rhabdomyoblasts derived from MHI-/- mice resembled normal satellite cells but formed colonies in soft-agar, thus demonstrating the acquisition of a high degree of malignancy. Remarkably, when this system was brought in a Pax7-/- background, the incidence of RMS was strongly reduced, highlighting the key role of satellite cells in the origin of this tumor. We will further investigate the nature and function of the rhabdomyoblasts obtained from the Pax7-/- tumors to identify the molecular pathways involved in their activation and commitment to the myogenic lineage. Altogether our results could improve the knowledge of the complex role of the HGF/Met axis in the physiopathology of muscle.

Manassero G.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

JNK-AP-1 pathway is involved in Sciatic Nerve Transection neuroregulation of CGRP dynamic in mice DRG

CGRP, a peptide that is widely distributed in the mammalian nervous system, is synthesized in DRG neurons and released from primary afferent neurons to mediate physiological and pathological states such as neuropathic pain. Previous studies demonstrated the effect of Sciatic Nerve Transection (SNT), a animal model to investigate neuropathic pain, on CGRP expression dynamic in primary sensory neurons of L4-L5 DRGs. JNK has been implicated in neuropathic pain development and maintenance: intrathecal infusion of a JNK peptide inhibitor, D-JNKI-I, prevented but did not reverse Sciatic Nerve Ligation–induced mechanical allodynia. The aim of this study was to investigate the involvement of JNK pathway in SNT and also which JNK isoform is responsible for CGRP regulation. Adult male 2-4 months-old ko mice for the different JNK isoforms and wild types (wt) underwent SNT. We excluded differences between wt and ko in i) footprint patterns ii) CGRP basal expression and showed by RT-PCR that all JNKs are expressed in wt DRGs before lesion. 72h post lesion, ipsi- and contralateral L4 DRGs were studied by IHC against CGRP. In a separate group of wt animals, to investigate whether JNK was involved in SNT CGRP expression, D-JNKI-I (0.3 mg/kg) was intraperitoneally injected 30 min before surgery. Treatment reversed CGRP downregulation ($P<0.05$), which was elevated after transection in wt ($P<0.001$). We found a marked decrease in the percentage of CGRP-IR neurons both in wt and JNK3 ko compared to the controls ($P<0.001$) whereas there was no downregulation both in JNK1 and JNK2 ko compared to the controls.
These results suggest that JNK-AP-1 pathway is involved in SNT downregulation of CGRP in DRG neurons and that both JNK1 and JNK2, and not JNK3, isoforms are responsible for this interaction.

Miceli, D.[1], Martini, M.[1], Franceschini, I.[3], Palanza, P.[3], Panzica, G.C.[1]

[1]Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
[2]UMR 6175 INRA/CNRS/ Tours University, France;
[3]Dept of Evolutionary and Functional Biology, Parma University, Italy

Organizational effects of bisphenol-A on kisspeptin expression in the hypothalamus of CD1 mouse

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 steroidsensitive 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 considered nuclei.
These results suggest that alterations of sexually dimorphic behaviors and reproduction, as well as early onset of puberty due to perinatal exposure to Bisphenol-A may be linked to modifications of Kisspeptin-GnRH circuits.
Supported by Regione Piemonte.

Piras A., Tonoli E. Vercelli A.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Scientific Institute of the Cavalieri-Ottolenghi Foundation, Neuroscience Institute of Turin Regione Gonzole 101043 Orbassano (TO) Italy

Activation of autophagy in a model of retinal ischemia following high intraocular pressure

Acute primary open angle glaucoma (POAG) is an optic neuropathy characterized by the elevation of intraocular pressure (IOP) that causes ischemia. Retinal ischemia results in a degeneration of the retinal ganglion cells (RGCs). Neuronal cell death can occur according different modes, of which necrosis and apoptosis represent the two extremes. A third mode of neuronal death has been put in evidence, autophagy. Autophagy is the process of bulk degradation and recycling of long-lived proteins, macromolecular aggregates, and damaged intracellular organelles. We investigated the activation of autophagy in RGCs following ischemia/reperfusion (I/R) produced by an acute IOP increase and evaluated its effect on RGC survival. We demonstrated that I/R induces the formation of autophagic vacuoles in the RGCs, which can be detected by their content of acid phosphatase, and that it promotes enhanced endocytosis characteristic of neurons dying by autophagy. We used acid phosphatase (AP) histochemistry and immunofluorescence staining of LC3 and LAMP1. Retinal I/R leads to the appearance of AP-positive granules and LAMP1-positive vesicle at 12 and 24 h, while LC3 labeling is present only 24 h after the insult. At 48 h there is no positivity for autophagic markers. We demonstrated that inhibition of autophagy by 3-methyladenine (3-MA) enhances the survival of RGCs, 24 h after the damage. Moreover our findings show that the I/R enhances autophagic lysosomal activity and endocytosis, as reflected by the increased endocytosis of both horseradish peroxidise (HRP) or fluorescent dextran into RGCs, one day after the damage. The present results may have clinical implications, because autophagy could be a novel and promising target in the treatment of diseases of the nervous system.

Rodriguez Gomez A., Panzica G.C.

Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy
Section: Anatomy

Effects of perinatal exposure to genistein on anxiety behavior of CD1 male mice

Genistein is a compound belonging to the family of phytoestrogens, produced by Leguminosae and particularly abundant in soybeans. This molecule is a tyrosine kinase inhibitor and shares structural features with the 17β-estradiol so that it can bind estrogen receptors and sex hormone binding proteins, exerting both estrogenic and antiestrogenic activity. In plants it shows an antimicrobial activity, specifically to protect them against insects. Genistein, as other phytoestrogens, is largely present in human and laboratory animal diets. Phytoestrogens have recently gained recognition for their beneficial effects on human health, but little is known about their effects on brain circuitries: experiments carried out on rats feeded with phytoestrogen rich diet have shown contrasting results on behavior and related brain areas. To understand if exposure to genistein during the critical period of differentiation of brain circuits and behaviors may alter this process, we orally administered to pregnant dames different doses of genistein (5 or 100 μg/g/day in sesame oil, or only the vehicle) from prenatal day 11 to postnatal day 8. Male puppies were tested from postnatal day 70 until the day 90 when they were sacrified for neurohistological investigations. Two tests were applied for anxiety behavior, the elevated plus maze and the open field. Males were also tested for aggressivity and sexual behavior. Statistical analysis reported no significant effects for the open field test, whereas we evidenced significant effects of higher doses of genistein (HG), in comparison to both controls (C) and low doses of genistein (LG) for the elevated plus maze test. In particular, HG males show lower level of anxiety spending more time in the open arms of the maze in comparison to LG and C. Other behaviors have been video recorded and are now under quantification. Thus, our preliminary results indicate that perinatal exposure to genistein has an organizational effect on anxiety and sexual behavior in male mice. Further analyses are required to understand what neural circuits have been permanently affected. Other experimental models of perinatal exposure to genistein evidenced organizational effects. For example, in rats, neonatal exposure can affect sexually dimorphic brain morphology and neuronal phenotypes in adulthood with regional and cellular specificity, as well as can decrease sexual behavior. Prenatal exposure to genistein of quail embryo is also inducing behavioral alterations (male copulatory behavior) as well as alterations of the vasotocinergic system.
In conclusion, present results confirm, in this murine model, that perinatal exposure to phytoestrogens may have life-long effects on differentiation of brain structures and behaviors.

Supported by Regione Piemonte

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

[1]*Neuroscience Institute of Turin, University of Torino, Corso M. D'Azeglio 52, 10126 Torino, Italy*
[2]*Primm s.r.l., San Raffaele Biomedical Science Park, Via Olgettina 58, 20132 Milan, Italy*
[3]*Vascular Research Laboratory, West Campus, BI-Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, USA*

Prourokinase mutant (M5) is a valid and efficient alternative to recombinant tissue plasminogen activator (tPA) in the acute treatment of stroke in the rat

Cerebral ischemia is one of the leading causes of death and disability worldwide; reperfusion by thrombolysis with (tPA) is the only approved therapy. Besides its narrow therapeutic time window, tPA displayed harmful side effects including haemorrhagic transformation and swelling due to increased permeability of neurovascular unit, which restricted its clinical use. Prourokinase (proUK) is a zymogenic plasminogen activator that induces fibrin-specific clot lysis without binding to fibrin; its unusual high intrinsic activity, however, caused important side effects like major bleeding. A proUK mutant (M5) was developed, in order to reduce spontaneous activation.
C1-inhibitor (C1I) was shown to limit both tPA and M5 non-specific activation. M5 vs Tpa mediated toxicity was initially tested in a rat model of permanent ischemia; mortality rate and histological appearance of ischemic sections were compared. Rats infused with tPA showed bleeding after infusion with highest mortality rate (57%) and frequent hemorrhagic infiltration. When treated with M5/C1I protocol mortality decreased to 16% and bleeding was particularly weak and short lasting. Recanalizations by tPA and M5 were compared in a second set of experiments using a thromboembolic model. As predicted by *in vitro* studies, reperfusion by M5/C1I was as much effective as tPA in dissolving blood clots and decreasing the volume of infarction, and displayed an improved clinical outcome. Neurological score in tPA-infused rats was lower than predicted by infarction size and significantly associated with brain oedema, which was larger than in M5 groups. Taken together, these data support M5/C1I as an alternative to tPA in the acute treatment of stroke, for its good thrombolytic properties and apparently reduced cytotoxicity.

ISTITUZIONI CHE HANNO COLLABORATO CON IL DIPARTIMENTO

- ***Banca del Sangue dell'Ospedale San Giovanni Battista, Torino***
- ***Harward University, Boston (USA)***
- ***IBD Onlus, Torino***
- ***Max Planck Institute for Medical Research, Heidelberg, Germany***
- ***Regione Piemonte***
- ***San Raffaele Biomedical Park, Milano***
- ***Scientific Institute of the Cavalieri-Ottolenghi Foundation, Torino***
- ***University of Parma, Italy***
- ***University of Tours, France***
- ***University of Cagliari, Italy***