O02 CRADLE OF PACAP: BELLE CHASSE, HEBERT CENTER

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As many of you know I had possibility to work in Dr. Arimura's laboratory for more than four years. When I arrived to New Orleans in 1987 with my 5 children, Dr. Arimura was surprised and frightened. Very soon he realized that the children did not hinder me in the laboratory work because my husband looked after them. During the first year of our stay in New Orleans PACAP, dreamed by Dr Arimura, was isolated and characterized by Dr. Miyata which means he helped PACAP to be born. I and other researchers like Paul Gottschall, Anikó Somogyvári-Vigh, Sándor Vigh and Ichiro Tatsuno rocked the cradle of PACAP until it was grown up.
When the amino acid sequence of PACAP was available and published in 1989, Dr. Arimura raised an antibody against this new peptide. I had a chance to investigate the distribution of PACAP in rat and sheep central and rat peripheral nervous system using immunohistochemistry. Later we found PACAP in the pituitary gland of proestrous rats using immunohistochemistry, *in situ* hybridization and cell immunoblot assay. PACAP immunoreactivity colocalized with that of LH and FSH. The occurrence in gonadotropes suggested its role in the regulation of the hypothalamo-pitutary-gonad axis. Indeed when PACAP was administered intracerebroventricularly on the day of proestrus before the so called „critical period" the LH surge and the expected ovulation on the next morning was blocked. PACAP administered intravenously had no similar effect. We also demonstrated that the inhibitory effect of PACAP was mediated by CRF and endogenous opioids.
We realized that a dense PACAP immunoreactive fiber network present in the suprachiasmatic nucleus suggests the presence of PACAP in retinal ganglion cells, that is, PACAP has to be present in the retinohypothalamic tract. We demonstrated these results in 1996 at a Regulatory Peptides Symposium. During the following years the role of PACAP in the biological clock was throughly investigated by Danish researchers (Hannibal and his co-workers). Utilizing the retrograde spreading of biotinylated dextran amine (BDA) tracer administered in the retina we found labeled cell bodies in several limbic structures and in the supraoptic and paraventricular nuclei. The BDA labeled cells in the dentate gyrus and supraoptic nucleus also showed PACAP immunoreactivity. It means that these PACAP immunoreactive cells send fibers directly to the retina forming a part of the centrifugal visual system. These fibers terminate around the retinal amacrine cells.

O03 CROSS-LINKING OF SP1 BY TRANSGLUTAMINASE2 SUPPRESSES PAC1 GENE EXPRESSION IN NEURONAL CELLS

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Pituitary adenylate cyclase–activating polypeptide (PACAP) function as a neuroprotective factor through the PACAP type 1 receptor, PAC1. Recently, we reported that nerve growth factor (NGF) augmented PAC1 gene expression through the activation of Sp1 via the Ras/MAPK pathway (Miura et al., 2012).

It was reported that the gene expressions of PACAP and PAC1 seems to be contradictory in ischemia, that is, the expression of PACAP gene of rat hippocampus is increased by *in vivo* ischemia (Stumm R et al., 2007). By contrast, that of PAC1 gene is decreased (Riek-Burchardt M et al., 2009), and the suppressive mechanism of PAC1 gene has been unknown so far.

As an initial attempt, we observed that PAC1 expression in Neuro2a cells or primary mouse cortical neurons was significantly suppressed during *in vitro* ischemic conditions – oxygen-glucose deprivation (OGD). Since endoplasmic reticulum (ER) stress is induced by ischemia, we tried to clarify how ER stress affects the expression of PAC1. Tunicamycin (TM), which induces ER stress, significantly suppressed PAC1 gene expression, and salubrinal, a selective inhibitor of PERK signaling pathway of ER stress cancelled its suppression.

Recently, it was reported that transglutaminase2 (TG2) is implicated in apoptosis of hepatocytes in alcoholic hepatitis by inactivation of Sp1 (Tatsukawa et al., 2011). This prompted us to clarify whether or not TG2 is involved in PAC1 gene expression. The pretreatment with cystamine, an inhibitor of TG activity significantly ameriolated the suppression of PAC1 gene expression due to OGD. Further, TG2-specific siRNA significantly also recovered attenuation of PAC1 protein expression by OGD. Thus we have demonstrated that ischemia or ER stress induces activation of the PERK pathway, which subsequently activates TG2 to crosslink Sp1, resulting in the suppression of PAC1 gene expression.

These findings regarding the suppression of PAC1 expression promises us that the maintenance of PAC1 gene expression would enable PACAP to elicit neuroprotection effectively during brain ischemia.

O04 PACAP EFFECTS ON CRMPS REGULATION IN PC12 CELLS

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The development of a functional neural circuitry involves several discrete steps. Newborn neurons must migrate to their proper locations, then extend axons and dendrites towards target regions, and form synapses with appropriate partners. Neuronal migration, neurite extension, and synapse formation are thus essential processes by which neurons acquire their polarity and characteristic functional morphology. These processes particularly rely on specific and coordinated dynamics and organization of the actin and microtubule cytoskeletons. Microtubules are now considered to be essential regulators of neuronal morphogenesis. They not only provide the support for active transport of the membranes, organelles, and macromolecules required for development, but also actively participate in controlling shape changes through its dynamism and restructuring capacity.
The family of the collapsin response mediator proteins (CRMPs), five cytosolic phosphoproteins, plays a significant physiological role in neuronal cell bodies and axons within the integrated mammalian central nervous system. Concretly they can bind tubulin heterodimers modifying the microtubule assembly.
Pituitary adenylate cyclase-activating polypeptide (PACAP), is a peptide which is involved in the regulation of neurogenesis, migration, apoptosis and differentiation in neurons. In all of these process the control of microtubule assembly is important. It is already known that in dorsal root ganglion neurons, CRMPs are involved in neurite extension induced by neurotrophins(Quach et al., 2004). So the aim of this work was to study the regulation by PACAP of the CRMP proteins and the functional implication of CRMP proteins in the neurotrophic effects of PACAP.

Quach, T.T., Duchemin, A.-M., Rogemond, V., Aguera, M., Honnorat, J., Belin, M.-F., and Kolattukudy, P.E. (2004). Involvement of collapsin response mediator proteins in the neurite extension induced by neurotrophins in dorsal root ganglion neurons. Mol. Cell. Neurosci. 25, 433–443.

O05 ACUTE ACTIVATION OF ASTROCYTES IN SPINAL DORSAL HORN VIA PAC1 RECEPTOR IS INVOLVED IN PACAP-INDUCED PERSISTENT AVERSIVE BEHAVIOR

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic neuropeptide regulating nociceptive transmission and pain-associated stress response. Previously we demonstrated that intrathecal (i.t.) administration of PACAP induces aversive behaviors such as biting, licking and scratching, which last more than several hours. Since vasoactive intestinal polypeptide (VIP) induced short-time aversive behavior, it was suggested that PACAP type 1 (PAC1) signaling might be involved in the persistent response (Shimizu et al, 2004). In this study, we aimed to clarify how PAC1 signaling induces prolonged aversive response. In mice (ddY male, 10 weeks old), the aversive behavior was dose-dependently mimicked by i.t. administration of PAC1 selective agonist maxadilan, and was attenuated by pretreatment of a PAC1 specific antagonist max.d.4. The maxadilan-induced aversive response was accompanied by the phosphorylation of mitogen-activated protein kinase, ERK in neurons of the spinal dorsal horn within 5-30 min, suggesting the induction of central sensitization in spinal dorsal horn, which is important for sustained aversive response. Within 30 minutes, maxadilan also augmented both the protein level of GFAP (grial fibrillary acidic protein) and the phosphorylation of JNK (c-Jun N-terminal kinase). When astroglial toxin L-a-aminoadipic acid was coadminsitrated with maxadilan, the aversive responses were potently decreased and the phosphorylation of ERK was suppressed. The cotreatment of a specific competitive inhibitor of cAMP-dependent protein kinase (PKA) Rp-8-Br-cAMP and maxadilan delayed the onset of aversive behavior together with the attenuation of ERK phosphorylation. Finally, both MEK inhibitor PD98059 and JNK inhibitor SP600125 significantly suppressed aversive response due to maxadilan. These results suggest that PACAP/PAC1 signaling induces pain-associated stress response by activation of PKA-ERK signaling in spinal dorsal horn, in which PAC1-dependent phosphorylation of ERK might be mediated by acute activation of spinal astrocytes.

O07 THE PREPARATION OF THE RECOMBINANT VIP-TAT AND ITS EFFECTS ON THE SCOPOLAMINE-INDUCED AMNESIA IN MICE

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Objective: Vasoactive intestinal Peptide (VIP) is a very important signal molecule of neurotransmitter, which participates in information transfer and physiological regulation. So it is a potential therapeutic neuropeptide, but unstable in structure .The 11-amino acid peptide TAT is a cell penetrating peptide, able to deliver protein cargoes across the cell membrane. In this study, the preparation of a recombinant VIP-TAT composed of VIP and TAT was achieved using genetic engineering principle and technology. After its bioactivity of cell penetrating and traversing biological barriers was detected, a mice model with amnesia induced by scopolamine was used to test the function of VIP- TAT and VIP.
Methods: Based on the natural sequence of VIP, VIP-TAT gene was designed and synthesized according to the expression bias of E.coli. VIP-TAT gene was obtained by two steps PCR using four primers and cloned into the expression vector pKYB. Engineering bacteria pKYB-VIP-TAT-ER2566 was constructed, and high-efficient preparation of VIP-TAT was achieved with the IMPACT (Intein Mediated Purification with an Affinity Chitin-binding Tag) protein expressing and purification system. Western blot was used to identify the immunological activity of recombinant VIP-TAT, and the fluorescence labeling technology was used to test its ability to penetrate into the cells and traverse the biological barriers into blood and brain. The inhibitory effects of VIP-TAT medicated by atomization on the food intake were also assayed. At last a mice model with scopolamine-induced amnesia was used to assay the effects of VIP-TAT. After one-time and long-term intervention trial with VIP-TAT and VIP, passive avoidance test was used to test the learning and memory of mice, and the antioxidant activities and oxidative stress indicators including MDA (malondialdehyde) and SOD (superoxide dismutase) in the brain and blood were dimtermined. The mechanism about the VIP-TAT against scopolamine-induced amnesia in mice was also explored from the content of ACHE (acetylcholin esterase) in the brain.
Results: The engineering strain pKYB-VIP-TAT-ER2566 was constructed, and the fermentation conditions acquired for the preparation of VIP-TAT were optimized. The recombinant VIP -TAT was obtained with IMPACT system. SDS-PAGE and western blot were used to characterize the recombinant VIP-TAT. VIP-TAT labeled with fluorescence FITC (fluorescein isothiocyanate) was found to penetrate into the CHO cells more effectively than the VIP labeled with FITC under the fluorescence microscope. VIP-TAT administrated by atomization was shown to get into blood through the respiratory tract and then traversed the blood brain barrier into brain more efficiently than VIP. After the administration by atomization, compared with VIP, VIP-TAT inhibited the food intake more effectively than VIP. These results indicated that VIP-

TAT had enhanced ability to traverse the biological barrier. During the treatment of the scopolamine-induced amnesia in mice, no matter whether administrated in one-time or long-term, in intraperitoneal injection or atomization, unlike VIP, VIP-TAT stably prolonged the incubation period of passive avoidance and ameliorated the amnesia in mice. VIP-TAT also decreased the levels of MDA and AChE in brain more effectively. It was also found that VIP-TAT not only promoted the SOD levels in blood and in brain, but also increased the red cells counts and hemoglobin (HGB) level in blood. These results indicated that VIP-TAT delivered by atomization had significantly efficient function on improving the learning and memory dysfunctions mice.
Conclusion: High-efficient preparation of recombination VIP-TAT with enhanced ability to traverse biological barriers was accomplished with genetic engineering principle and technology. In vivo experiment, VIP-TAT was proven more effective against mice with amnesia induced by scopolamine than VIP. The finding that VIP-TAT has the ability to improve the mice with learning and memory dysfunctions while medicated by atomization will lay the foundation for its further application.

Keywords: VIP; VIP-TAT; scopolamine; amnesia; cell-penetrating pep

O08 HUMAN STEM/PROGENITOR CELLS FROM BONE MARROW IMPROVE SPINAL CORD INJURY VIA COMMUNICATING WITH PACAP

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Human stem/progenitor cells from bone marrow (mesenchymal stem cells, marrow stroma cells, MSCs) attract to rescue many diseases including CNS injuries by the transplantation. We have reported that implantation of hMSCs in ischemic mice decreased neural cell death, and induced microglia/macrophages to M2 alternative activating type. We also found an increase of PACAP gene by the microarray analysis. However, it has not been understood in detail for the role of PACAP expression. In this study, we determined that neuroprotective effect of hMSCs depends partially on mouse PACAP expression.
C57/BL6 mice (wild-type and PACAP +/- KO) were subjected to spinal cord injury by transection intervertebral between T9 and 10. The next day, the mice implanted hMSCs (5 x 10^5) or vehicle into intervertebral cord between T10 and 11. Then the mice were observed hind limb motor deficit for 7 days and obtained the spinal cord at 7days. The tissues determined injury size, hMSCs retention, mouse specific gene expression of PACAP, PAC1R, and pro-inflammatory and anti-inflammatory cytokines. The cord also determined human gene expression of some growth factors and anti-inflammatory cytokines.
Wild-type mice which implanted hMSCs improved significantly both motor deficit and injury volume to compare with that vehicle-treated mice. However, the wild-type mice implanted dead-hMSCs or PACAP +/- KO mice implanted hMSCs were abolished the effect. Retention of hMSCs did not different in both wild-type and PACAP +/- KO. The implanted hMSCs migrated toward to injury region for 7 days. Implantation of hMSCs increased significantly mouse PACAP gene expression, but not mouse PAC1R. Implantation of hMSCs into wild-type mice decreased mouse gene level of IL-1 beta, TNF alpha, IL-10 and TGF beta and increased mouse IL-4 level. However, implantation of hMSCs into PACAP +/- mice could not reproduce mouse IL-1 beta, TGF beta and IL-4 levels. PACAP +/- KO mice also influenced hMSCs gene expression level detected by human specific primer sets.
These results suggest that implanted hMSCs 1) migrated into injury region, 2) made recipient increase PACAP gene, 3) modified inflammatory balance to anti-inflammation with PACAP and 4) resulted in improvement of spinal cord injury.

O09 EFFECTS OF PACAP ON DIFFERENTIATION PROCESSES OF UMR106 OSTEOBLAST CELL LINE

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PACAP has important role in the regulation of differentiation of central nervous system and also of several peripheral tissues. However, little is known about the connection of PACAP signalling pathways and osteogenesis. Our goal was to clarify whether PACAP has any effect on the regulation of Hedgehog (Hh) signalling pathway during osteogenic differentiation.

In our experiments, we investigated the effects of PACAP neuropeptide on osteogenic differentiation of the UMR106 osteoblastic cell line. After application of PACAP 1-38 at 100 nM concentration as an agonist and PACAP 6-38 at 10 μM as an antagonist of PACAP receptors, we have monitored the morphological and molecular biological changes.

Administration of the neuropeptides did not alter the morphology and viability of UMR106 cells but resulted in an increased proliferation capability. The mRNA and protein expression of PKA, one of the most important classical downstream targets of PACAP signalling pathways, significantly increased after PACAP addition. It is known that PKA activation has regulatory function on osteogenic differentiation pathways, for which we have monitored the expression of Hedgehog signalling molecules and basic osteogenic transricption factors after administration of PACAPs. The expressions of Runx2 and CREB transcription factors, well known downstream targets of PKA signalling, were not altered. The protein expression and nuclear translocation of the active phosphorylated form of CREB decreased in the presence of PACAP 6-38, while it elevated the nuclear presence and expression of Runx2. The application of PACAP neuropeptides increased the mRNA and protein expression of PTHrP and Sonic Hedgehog but did not alter the expression of Indian Hedgehog. mRNA expression of osterix was elevated and increased amount of extracellular Ca^{2+} deposits were detected after administration PACAP 6-38 with Alizarin red staining. BMP expressions were also detected.

In our experiments we have shown that PACAP has effect on Hedgehog signalling pathways and increases cell proliferation in the UMR106 osteoblastic cell line. Augmentation of protein expression of SHH and PTHrP indicates that PACAP has positive effect on osteogenic differentiation.

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O10 CROSS-TALKS BETWEEN THE VIP-RECEPTORS SYSTEM, AKT/PTEN AND HEDGEHOG PATHWAYS IN THE REGULATION OF GLIOBLASTOMA MIGRATION AND INVASION

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Glioblastoma multiforme (GBM) is the most common and aggressive form of primary brain tumor in adults with a very bad prognosis. This may be due to the exacerbated migration and invasion properties of GBM cells leading to local or distant recurrences. Vasoactive intestinal peptide (VIP) and Pituitary adenylate cyclase-activating polypeptide (PACAP) are widely distributed in both central and peripheral nervous systems and are implicated in neural development and neuroprotection. VIP and PACAP also regulate proliferation and differentiation of numerous types of tumor cells as well as migration. We demonstrated that these peptides regulate migration of two GBM cell lines, the M059K and M059J cells, derived from a same tumor (Cochaud et al., Neuropeptides, 2010). The M059J cells poorly express the VIP-receptor system compared to M059K cells. Addition of the antagonist VIP10-28 or a polyclonal anti-PACAP antibody to the culture medium of M059K cells showed that endogenous neuropeptides of the VIP-receptor system reduced the invasive capacity of these cells. Data from our studies indicate that the more the VPAC1 receptor is expressed and activated by endogenous or exogenous agonists in these human GBM cells, the less these cells are able to migrate and invade in vitro. Recent studies demonstrate that PACAP inhibited proliferation of medulloblastoma cell lines and decreased expression of the Hedgehog (Hh) target gene Gli1 (Cohen J.R. et al., BMC Cancer, 2010). Suppression of the Hh pathway markedly inhibits glioma cell migration and invasion (Wang K. et al., Neurological research, 2010). However, little is known about the mechanisms linking the VIP-receptor system and the Hh pathway in GBM migration. In our studies to elucidate the mechanisms of the contribution of VIP and PACAP to the malignant behavior of GBM cells, we found that VIP and PACAP strongly inhibited expression of Gli1, in the human U87 and rat C6 GBM cells. Conversely, VIP10-28 increased Gli1 protein expression. VIP and PACAP also inhibited invasion of C6 GBM cells in rat brain slices cultured ex vivo. On the contrary, the VIP receptors antagonist VIP10-28 significantly stimulated C6 GBM migration and invasion, a process which was PKA, Akt and Hh-dependent. Taken together, these observations indicate that crossed interactions between the VIP-receptor system and the Hh and the Akt/PTEN pathways play key functions in GBM migration and invasion. In future prospects, prodrugs derived from cyclopamine that target and inhibit the Hh pathway, recently developed in our group will represent key molecules to further investigate these potential cross-talks and synergies.

O11 COMPARISON OF TOOTH DEVELOPMENT IN WILD TYPE AND PACAP-DEFICIENT MICE

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Teeth are derived from the ectoderm of the first branchial arch and the ectomesenchyme of the neural crest, therefore, their development shows similarities with the development of the nervous system. Pituitary adenylate cyclase activating polypeptide (PACAP) has protective effects in the nervous system and it plays a role in its development. PACAP-immunoreactive fibers have been found in the tooth pulp, but there is no data about the effect of endogenous PACAP on tooth development. Morphometric and structural comparison of the developing teeth was performed on native histological sagittal sections from the skull of 7-day-old wild type and PACAP-deficient mice. The structural analysis was carried out with Thermo Scientific DXR Raman microscope. On the same slides we examined the activation of the BMP, Sonic Hedgehog and Notch signaling pathways which play important role in the tooth development. In adult mice (1-year-old) morphometric, and hard tissue density measurements were done on prepared mandibles with Sky Scan Micro CT.
During the morphometric comparison of the 7-day-old samples we found that the dentin was significantly thinner in the molars of PACAP-deficient mice. The Raman spectra of the enamel in the wild-type mice demonstrated a broader range of 1240/1270 ratio, indicating a higher diversity in secondary structure of enamel proteins. In the dentin of PACAP-deficient mice higher intracrystalline disordering in the hydroxyapatite molecular structure was found. We found significant elevation in the expression of BMP (BMPR1, BMP2 and BMP7), Sonic Hedgehog (SHH, Gli1) and Notch (Notch1 and DLL) signaling pathways in PACAP-deficient mice compared to wild-type animals. With micro CT the tooth volume, the pulp chamber and the density of the dentin was significantly smaller in the incisors of the adult PACAP-deficient mice. These observations suggest that PACAP plays a role in tooth development. PACAP-deficient mice show alterations in the tooth development compared to wild-type animals.
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O12 VPAC2 RECEPTOR, A NOVEL TARGET IN THE TREATMENT OF MULTIPLE SCLEROSIS

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Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are potent anti-inflammatory factors that regulate T cell development during inflammation. They act through two common receptor subtypes, VPAC1 and VPAC2. Here, using (MOG_{35-55})-induced experimental autoimmune encephalomyelitis (EAE) model and VPAC2-deficient (KO) mice, we investigated the roles of VPAC2 in modulating T lymphocytes. We found that mutant mice showed higher EAE clinical scores than WT controls with enhanced immune cell infiltrations, as well as demyelination in the CNS. Moreover, consistent with the severe EAE pathology, gene expression of the proinflammatory cytokines TNF-α, IL-6, IFN-γ (Th1), and IL-17 (Th17) was significantly increased, whereas that of anti-inflammatory cytokines IL-10 and IL-4 (Th2) was dramatically reduced in mutant vs. WT spinal cord extracts after EAE. In ex vivo lymph node cultures on day 14 post-EAE, MOG-specific T effector cells responded much more (e.g. higher proliferation and increased IFN-γ/IL-17, but decreased IL-10 and TGFβ cytokine secretion) in receptor KO vs. WT mice. In addition, interestingly, we found that not only the proportion of CD4+CD25+FoxP3+ regulatory T cells (Tregs) but also their proliferative rates were drastically lower in VPAC2 KO vs. WT draining LN, thymus, and CNS. Furthermore, in vitro cell culture assays suggested that VPAC2 KO Tregs exhibited a defect in suppressing T cell proliferation and in expanding. Moreover, Th1/Th17 proportion was significantly increased with almost a complete blockade of Th2 cells in draining lymph nodes and CNS of VPAC2 KO vs. WT mice after EAE. In order to further dissect the actions of VPAC2, EAE-induced WT mice were treated with RO 25-1553, a specific VPAC2 agonist for 5 days at the onset (= day9-10). We demonstrated that: (1) the agonist was able to diminish the EAE symptoms by favoring the development of Th2 and Tregs and (2) its efficacy was optimal at the onset. Thus, the VPAC2 receptor : (1) appears to be critically required to control EAE severity, (2) is necessary for proper Treg expansion in the thymi and secondary sites during inflammation, and (3) may be identified as a valuable target for the development of new therapeutic strategies against multiple sclerosis and other inflammatory diseases.

O13 PACAP STIMULATES THE CORNEAL HEALING VIA LACRIMAL-MEDIATING AND DIRECT PATHWAYS IN MOUSE

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The dry eye syndrome is one of the most common eye ailments caused by volume reduction or the altered quality of tears with corneal damage, however, an effective treatment has yet to be established. Our study was started based on the finding of a new phenotype in PACAP null mice, which show dry eye-like symptoms, corneal keratinization and tear reduction. PACAP and its receptor were expressed in mouse lacrimal glands. PACAP and PAC1R were existed in mouse lacrimal gland, and PACAP eye drops stimulated tear secretion via an adenylate cyclase/cAMP/PKA cascade. PACAP stimulated phosphorylation of aquaporin 5, and its translocation from the cytosol to the membrane in lacrimal acinar cells. Moreover, AQP5 siRNA treatment to lacrimal gland attenuates PACAP-induced tear secretion. These results suggest a possible role of PACAP as an endogenous regulator of tear secretion through AQP5 translocation.
On the other hand, PACAP have direct pathway to affect the mouse cornea. PAC1R mRNA and its immunoreactivity was detected in mouse corneal epithelium, and PACAP was detected in mouse tear. In corneal injury model mice, PACAP eye drop significantly reduced the injured area at 12 hours, and the effect was disappeared by co-treatment with PACAP receptor antagonist. PACAP heterozygous knockout mouse delay the corneal healing. Although surgical removal of the lacrimal gland attenuates corneal healing, PACAP eye drop on the eyes significantly improved corneal damage. In vitro study, PACAP treatment to human corneal epithelial cells significantly decreased the injury area made by scratching.
These data suggest that PACAP suppressed corneal damage directly to corneal epithelial cells and indirectly to stimulating lacrimation. PACAP could be a good candidate for an eye-drop medicine for the dry eye syndrome.

O14 UROKINASE PLASMINOGEN ACTIVATOR SYSTEM IN SYNOVIAL FIBROBLASTS FROM OSTEOARTHRITIS PATIENTS: MODULATION BY INFLAMMATORY MEDIATORS AND NEUROPEPTIDES

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During osteoarthritis (OA), genetic, metabolic, biochemical and biomechanical factors activate an inflammatory response with interactions between cartilage, subchondral bone and synovium. In OA joint, synovial lining hyperplasia includes an increase of activated fibroblast-like synoviocytes (FLS), producing cytokines that perpetuate inflammation and proteases that contribute to cartilage destruction. Among the proinflammatory cytokines, IL-1β is a main player associated with cartilage destruction.
In synovitis, endocrine, immune and nervous system interact, releasing hormones, cytokines and neuropeptides. We have previously described the presence of vasoactive intestinal peptide (VIP) and corticotropin releasing factor (CRF) families of neuropeptides and their receptors in FLS from rheumatoid arthritis (RA) and OA patients. In animal models and in vitro studies, beneficial effects have been observed with VIP, as a potential therapeutic agent with protective effect upon cartilage and bone destruction in RA and OA.
Plasminogen activators (PAs) are specific proteolytic enzymes implicated in a variety of biological processes. The urokinase PA system is composed of urokinase-type plasminogen activator (uPA), uPA receptor (uPAR), and PA inhibitor-1 (PAI-1). These components are increased in some human diseases, including OA. uPA is a serine proteinase that catalyses the conversion from plasminogen to plasmin, degrading the extracellular matrix (ECM) directly or indirectly through activation of other proteolytic enzymes, as matrix metalloproteinases (MMPs). uPA is activated through binding to its receptor. uPA-uPAR signalling is inhibited by PA inhibitors, where PAI-1 forms a covalent uPA-PAI-1 complex. Fibronectin (Fn) is a glycoprotein in the ECM of many tissues, including cartilage and synovium. Proteolytic cleavage of Fn during cartilage degeneration liberates Fn fragments (Fn-fs) with proteolytic activities, enhancing MMPs.
Thus, we first examined the VIP and CRF effect on the constitutive expression of uPAR, uPA and PAI-1, in OA-FLS. VIP decreased constitutive uPA system by the reduction of uPA expression and the increase of PAI-1. Then, we analyzed the effect of Il-1β and Fn-fs on the uPA system, and how neuropeptides modulated it. VIP was able to counteract IL-1β and Fn-fs stimulated uPA system, decreasing uPAR and uPA expression and activity. However, CRF only had effect on the IL-1β stimulated uPAR, reducing its levels. Subsequently, we measured MMP-9 and MMP-13 levels as physiological consequence of the uPA system activation. Both, IL-1β and Fn-fs increased MMP-9 and MMP-13. In both cases, co-treatment with VIP resulted in a decrease of MMPs production.
All in all, uPA system points to be a promised target in the treatment of OA to block articular cartilage degradation. Furthermore, this study supports the therapeutic potential of VIP in the treatment of OA by the modulation of the uPA system.

O15 THE PATHOGENIC PHENOTYPE AND THE PLASTICITY OF TH17 PROFILE FROM RHEUMATOID ARTHRITIS PATIENTS ARE MODULATED IN VITRO BY VASOACTIVE INTESTINAL PEPTIDE

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Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation and tissue damage in joints. Several Th cells are involved in the inflammatory response. It has been shown a key role of Th17 cells in the pathogenesis of RA. Last reports indicate that depending on inflammatory microenvironment, Th17 cells could acquire pathogenic or non-pathogenic phenotype. It is important to know the specific phenotype of Th17 cells in this disease since, depending on pathogenic or non-pathogenic Th17 cells involved, RA could be exacerbated or ameliorated. In addition to heterogeneity of Th17 cells, this subset is also characterized by its inherent instability. Th17 cells can acquire Th1 phenotype and also it has been described that Treg cells can acquire Th17 phenotype. Therefore, it is very interesting to know the regulatory mechanisms involved in the heterogeneity and the plasticity of Th17 cells. Vasoactive Intestinal Peptide (VIP) plays important immunomodulatory functions and it is able to modulate Th17 cells. In several mouse models of inflammatory/autoimmune diseases, VIP has exhibited promising therapeutic actions. Moreover VIP has displayed anti-inflammatory/immunomodulatory effects in samples from human RA. Given the implication of Th17 cells in RA and the heterogeneity and the plasticity of Th17 cells, we tried to study the phenotype and the plasticity of Th17 from RA patients and if VIP, a neuropeptide present in the inflammatory microenvironment, is able to modulate them. Analysis of Th17 profile showed a largest presence of Th17 cells, a major pathogenic phenotype and further Th17/Th1 plasticity of Th17 cells from RA patients compared to healthy donors. VIP was also able to modulate these cells increasing their profile in non-pathogenic phenotype, decreasing their Th17/Th1 plasticity and rising the Treg/Th17 plasticity. In conclusion, we showed that VIP regulates the phenotype and the plasticity of Th17 cells in healthy donors and RA patients. Thus, we provide new insights to consider VIP as a good therapeutic candidate in the RA pathology.

O16 PACAP/PKA REDUCES POLYGLUTAMINE ANDROGEN RECEPTOR TOXICITY IN CELL MODELS OF SPINAL AND BULBAR MUSCULAR ATROPHY

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Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a neuromuscular disorder characterized by the dysfunction and loss of motor neurons from spinal cord and brainstem, together with weakness, fasciculation, and atrophy of skeletal muscle. SBMA is caused by expansion of the CAG trinucleotide repeat, encoding a polyglutamine tract, in the androgen receptor (AR) gene. In normal individuals, the repeat length ranges between 9 and 36 residues, and expansion over 38 residues causes disease. Here, we present evidence that pituitary adenylate cyclase activating peptide (PACAP)/protein kinase A (PKA) signaling is a novel modifier of SBMA pathogenesis. PKA activation protects cells from the toxicity of mutant androgen receptor in cultured cells. Treatment of SBMA cells with PACAP increases the production of cyclic AMP in motor neuron-derived cells expressing polyglutamine expanded AR. Treatment of these cells with PACAP reduces the toxicity of mutant protein via activation of PKA. Moreover, PACAP/PKA reduces mutant AR aggregation in cultured cells. Interestingly, PACAP/PKA activation results in a reduction of mutant AR phosphorylation, suggesting that the effect of PACAP/PKA is specific for SBMA. Our results show a protective role for PACAP/PKA in SBMA, suggesting this as a novel therapeutic approach for patient treatment.

O17 VASOACTIVE NEUROPEPTIDES IN CHRONIC FATIGUE SYNDROME/ MYALGIC ENCEPHALOMYELITIS (CFS/ME): POSSIBLE PATHOMECHANISMS IN A HUMAN DISEASE

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Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis (CFS/ME) may demonstrate characteristics of autoimmunity. CFS/ME is, in some patients, a severely disabling condition with multiple symptoms affecting neurological, cardiovascular, immunological, hormonal and gastrointestinal systems. While there is no single proven causative factor many cases have an association with recent infection. Symptoms may include, but are not limited to, incapacitating fatigue and severe post-exertional malaise, impaired memory and concentration, persistent sore throat, tender cervical or axillary lymph nodes, muscle pain, severe headaches and impaired and unrefreshing sleep. Suicide is a real risk in the most severely disabled group.

Importantly, immune changes in CFS/ME may be related to heightened or suppressed cell function, differential gene expression, changes of immune cell numbers and protein secretion promoting adverse inflammatory activation. Both innate and adaptive immune system perturbations persist in CFS/ME. These characteristics are similar to mechanisms of disease in autoimmune disorders suggesting that the changes in immune response may develop from cellular and molecular changes in immune cells and proteins. We present evidence of regulatory T cell (Treg) including Foxp3 anomalies, VPAC2R dysregulation and microRNA perturbations. We propose that the mechanism of CFS/ME may have an autoimmune component or perhaps the symptoms of CFS/ME are hallmarks of a novel autoimmune disorder yet to be identified possibly related to the vasoactive neuropeptide family.

O18 LOSS OF PACAP IN MICE SENSITIZES NIGROSTRIATAL DOPAMINERGIC NEURONS TO PARAQUAT-INDUCED INJURY AND MODULATES MICROGLIA AND PERIPHERAL T CELL ACTIVATION

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The risk of Parkinson's disease (PD) is increased by exposure to the pesticide paraquat (PQ), and the effect may be modulated by genetic or other environmental factors. It has been demonstrated that the PACAP can enhance tyrosine hydroxylase (TH) and VMAT2 expression, protect dopaminergic (DA) neurons against the neurotoxin 6-hydroxydopamine, regulate neuronal mitochondria, and inhibit inflammation. Diminished PACAP expression may thus interact with environmental factors such as PQ to increase the risk of PD. To mimic a low level environmental exposure to PQ, wild type (WT) and PACAP deficient mice were given a single [10mg/kg] dose of PQ, a regimen that did not induce loss of TH expression or DA neurons in WT mice. This treatment reduced the number of TH-positive cell bodies in the substantia nigra pars compacta (SNpc) of PACAP KO. Because inflammation is also a risk factor for PD, we performed a quantitative analysis of SNpc Iba+ microglia. As expected, PQ increased the number of larger microglial profiles, indicative of activation, in WT mice. Strikingly, microglial activation was already evident in PACAP-deficient mice in the basal state. PQ caused no further activation in these mice, although TNF-α mRNA levels were enhanced. PQ had no effects on the abundance of proinflammatory Th1 or Th17 cells in the lymph nodes of WT mice, but increased the numbers of anti-inflammatory regulator T cells (Tregs). PACAP-deficient mice, in contrast, had elevated numbers of Th17 cells after PQ, and the induction of Tregs was impaired. Endogenous PACAP thus acts to maintain the integrity of dopaminergic neurons during exposure to PQ, an action that may be linked to its ability to regulate microglia and/or other immune cells.

O19 VIP/PACAP-REGULATED ACTIVITY-DEPENDENT NEUROPROTECTIVE PROTEIN (ADNP) AND NAP: MICROTUBULE PROTECTION

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VIP and PACAP provide potent neuroprotection, which may reside in part by activation of ADNP. Neurodegenerations, including Alzheimer's disease (AD) and frontotemporal dementia (FTD) are characterized by tauopathy, with tau playing a central role in the promotion of microtubule assembly. Tau is characterized by the presence of a microtubule binding domain, which is composed of 3 or 4 repeats (3R and 4R tau) of a highly conserved tubulin binding motif. The 3R and 4R tau isoforms are expressed in a 1:1 ratio in most regions of the adult human brain, and deviations from this ratio are characteristic of frontotemporal degeneration associated tauopathies (1). While complete ADNP deficiency is lethal, ADNP heterozygous mice (ADNP+/) exhibit cognitive deficits, significant increase in phosphorylated tau, tangle-like structures (tauopathy), reduced neuronal survival and neurodegeneration (2). ADNP's binding partners include the SWI/SNF chromatin remodeling complex, associated with transcription and splicing. Brm is a component of the SWI/SNF complex and a known factor associated with alternative splicing and exon inclusion (3). Here, immunoprecipitations identified for the first time Brm-ADNP interaction. Furthermore, ADNP-PSF interactions were found as well, with PSF being a direct regulator of tau transcript splicing. Two-hybrid system analyses showed a potential direct interaction between of the microtubule-associated protein 1 light chain 3 (LC3B) and activity-dependent neuroprotective protein (ADNP)(4) with LC3 being one of the main autophagy markers and the question is if the ADNP snippet, drug candidate, NAP, modulates this interaction. Our previous studies have shown that NAP enhances tau – microtubule association (5) and protects axonal transport in vivo in the face of colchicine disruption of microtubules (6). Our further studies have shown NAP protection of function in microtubule-deficient models of disease (7,8). Together, these studies suggest VIP/PACAP/ADNP/NAP/microtubule relations, paving the path to better understanding and better treatments of prevalent neurodegenerative and neuropsychiatric diseases.

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O20 IMPROVEMENT OF BEHAVIORAL DEFICITS AND TAU PHOSPHORYLATION BY NAP (DAVUNETIDE) IN THE THY1-ASYN MODEL OF PARKINSON'S DISEASE

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Alpha synuclein is a major risk gene for Parkinson's disease (PD) and the microtubule-associated protein tau is another one. A pilot study has previously shown that intranasal application of the microtubule protecting 8-amino acid peptide NAP (2µg/mouse/day/2 months) improved challenging beam performance at 2-3 months of age, and decreased alpha-synuclein aggregation in the substantia nigra of mice over-expressing human alpha-synuclein (Thy1-aSyn mice), a genetic model of pre-manifest PD (Fleming et al. 2011). Here, analyzing two doses in a 6 months application period starting at one month of age, we show that 5.5-6 months of treatment with 2µg/mouse/day (5 days a week) NAP significantly reduced hyperactivity and olfactory deficits in the Thy1-aSyn mice, and decreased Phospho-tau levels in the midbrain. While 2µg NAP treatment did not affect alpha-synuclein positive aggregates, 15µg NAP increased the number and surface area of proteinase-K resistant alpha-synuclein positive aggregates in the substantia nigra and shifted the distribution of aggregates to larger-sized aggregates in the ventrolateral and ventromedial substantia nigra. These data show that chronic administration of NAP at early disease stage can improve biochemical and behavioral outcomes in Thy1-aSyn mice, a model which recapitulates multiple aspects of PD. The current results provide further support for clinical development of neuroprotective/microtubule targeting drugs in PD, with NAP as a prototype.

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O21 PROTECTIVE EFFECTS OF PACAP AGAINST SALSOLINOL- AND INFLAMMATORY-MEDIATED TOXICITY IN SH-SY5Y CELLS: IMPLICATIONS FOR PARKINSON'S DISEASE

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Parkinson's disease (PD), a progressive degenerative disorder of the central nervous system is caused by a decline in dopaminergic cells in the substantia nigra (SN). Although the cause(s) of PD remain unclear, it has been suggested that endogenous neurotoxins as well as inflammation may play an important role. Pituitary adenylate cyclase activating polypeptide (PACAP) is an endogenous 38 amino acid containing neuropeptide with neuroprotective and anti-inflammatory properties. In this presentation, protective effects of PACAP against salsolinol- and lipopolysaccharide (LPS)-induced toxicity in SH-SY5Y cells will be provided. SH-SY5Y cells, derived from human neuroblastoma cells express high level of dopaminergic activity and are used extensively as a model to study SN neurons. Salsolinol is an endogenous dopamine metabolite with selective toxicity to nigral dopaminergic neurons, and LPS, derived from the outer membrane of gram-negative bacteria, has potent inflammatory effects. Both of these agents activate apoptotic pathways. Thus, antiapototic mechanism, as well as involvement of neurotrophic factors and PAC1 receptor in PACAP protective effects will also be presented. It is concluded that PACAP or PAC1 agonists may have therapeutic potential in PD caused by toxins or inflammation.

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O22 EFFECT OF PACAP ON CEREBRAL ENDOTHELIAL CELLS

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Cerebral endothelial cells (CECs) – coming in contact with pericytes and astrocytes – constitute the structural basis of the blood-brain barrier (BBB). The continuous belt of tight junctions (TJs) interconnecting CECs and the presence of specific transport systems, enzymes and receptors in the brain endothelium regulate the molecular and cellular traffic into the central nervous system.
Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide having several cellular protective effects. However, little is known about the effects of PACAP on the cerebral endothelium and BBB functions. Here we investigated the effects of PACAP on the barrier function and survival of CECs.
We have shown that PACAP has a protective effect on the tight and adherens junctions of brain endothelial cells. PACAP induces an increase in the transendothelial electrical resistance (TEER) in control conditions, and ameliorates Ca^{2+}-depletion induced decrease in TEER. Our immunofluorescence studies have shown that PACAP increases the amount of VE-cadherin and ZO-1 at the level of intercellular junctions. We have also observed that PACAP has a protective role against glucose-deprivation induced junctional damage and apoptosis.
In conclusion, our results show that PACAP protects cerebral endothelial cells against junctional disruption and apoptosis.
This work was supported by PTE-MTA „Lendulet" Program, Arimura Foundation, OTKA K104984, TAMOP 4.2.2.A-11/1/KONV-2012-0024, TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program".

O23 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL THIOREDOXIN REDUCTASE INVOLVED IN THE NEUROPROTECTIVE EFFECT OF PACAP

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The neuropeptide PACAP exerts neurotrophic activities by regulating the expression of various genes and pathways in a coordinated manner. Analysis of the transcriptome of PC12 cells after treatment with PACAP allowed the identification of several genes with unknown function but which may play a crucial role in survival and neuritogenesis in neuronal cells. Among the genes up-regulated by PACAP, we identified a new member of the selenoprotein family named selenoprotein T (SelT). Selenoproteins are selenium-containing proteins involved in the control of redox homeostasis thanks to the nucleophilic activity of the oligoelement. Using a recombinant protein, we could demonstrate that SelT is a new thioredoxin reductase residing mainly in the endoplasmic reticulum. Our initial studies showed that SelT is strongly expressed in the nervous system during development and following neuronal injury. Transient transfection experiments performed in PC12 cells showed that a 250-bp SelT promoter sequence was able to confer regulation by PACAP, forskolin and H2O2 to a reporter gene. This sequence encompassed a recognition site for nuclear respiratory factor 1 (NRF1), an important factor controlling the expression of numerous antioxidant genes. Targeted mutagenesis of the NRF1 site reduced the SelT promoter activity in basal and PACAP-stimulated conditions, suggesting that a single cis-regulatory element binding NRF1 may act as a major switch to control SelT gene expression during neuronal differentiation, most likely to participate to the neuroprotective action of PACAP. In order to determine precisely the physiologic function of SelT, we developed a conditional knockout using the Cre-Lox system. Global ablation of the SelT gene resulted in early lethality of mice, indicating that SelT plays a crucial role during embryogenesis. We then generated mice with a targeted knockout of SelT in the brain which were viable but exhibited a reduction in the volume of most brain structures. In fact, we found higher ROS levels and caspase-3 activity in the brain of KO neonates, indicating that SelT is involved in neuroblast survival by protecting the cells against oxidative stress. Behavioral studies showed that adult KO mice display a hyperactive behavior. Remarkably, treatment of these mice with the neurotoxin MPTP led to a Parkinsonian-like phenotype, culminating at animal death within few hours. Analysis of the substantia nigra compacta (SNc) revealed an accumulation of ROS in KO mice, suggesting that SelT plays a crucial role in the protection of catecholaminergic neurons against oxidative stress. Together, these data highlight a new PACAP-regulated pathway involving an unprecendently characterized enzyme whose deficiency is associated with high oxidative stress, induced Parkinsonism-like phenotype and abnormalities in the establishment of cognitive networks.

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O24 MOLECULAR MECHANISMS UNDERLYING THE NEPHRO-PROTECTIVE EFFECTS OF PACAP IN DIABETES

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Introduction: We have recently revealed that PACAP has nephroprotective effect in diabetic kidney disease, however, the molecular mechanism leading to less severe damage in the PACAP-treated kidneys remains unknown. Cytoprotective effects of PACAP are known to be mediated mainly through its specific Gs-protein-coupled receptor, the PAC1 receptor. Excessive fibrosis is one of the key events in the pathogenesis of diabetic nephropathy, however, the effect of PACAP on the profibrotic factors was not fully elucidated. Therefore, our aim was to get further insight into the protective mechanism of PACAP in experimental diabetic nephropathy.
Methods: Diabetes was induced by a single iv. injection of streptozotocin (65mg/kg) in male Wistar rats. PACAP-treated animals were administered ip. 20ug PACAP every second day. Expression of the proapoptotic pp38MAPK and the antiapoptotic pAkt, pERK1/2 and XIAP family (livin, survivin and XIAP), and also that of caspase 3,6 and NFκB was determined by Western blot. PCR and Western blot were used to measure the levels of fibrotic markers, like collagen IV and TGFβ1 in the kidney samples. Changes in the GLUT1, PAC1 receptor and connexin 43 protein were evaluated by immunohistochemistry.
Results: Diabetes resulted in a remarkable increase in the expression of the proapoptotic pp38MAPK and PACAP treatment successfully counteracted this increase. The examined antiapoptotic factors, including pAkt and pERK1/2 showed a slight increase in the diabetic kidneys, while PACAP treatment resulted in a notable elevation of these proteins. Levels of caspase 3 and 6, the corresponding cleaved caspases and also NFκB decreased due to PACAP treatment. PACAP attenuated the production of fibrotic markers – collagen IV and TGFβ1 – which play important roles in the pathogenesis of diabetic nephropathy. Immunohistochemistry revealed a significantly higher expression of PAC1 receptors in diabetic kidneys, and further elevation was observed in PACAP-treated diabetic samples. PACAP did not change the altered expression of GLUT1 in diabetic nephropathy.
Conclusion: The protective effect of PACAP is, at least partly, due to its antiapoptotic and antifibrotic effect in addition to the previously described antiinflammatory effect.
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O25 EXAMINATION OF THE PROTECTIVE EFFECTS OF PACAP IN RAT DIABETIC NEPHROPATHY

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Introduction: Diabetic nephropathy is the leading cause of end-stage renal failure and accounts for 30-40% of patients entering renal transplant programmes. Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide consisting of 38 amino acids. Its nephroprotective effect was proved in numerous in vivo and in vitro studies. The aim of our study was to investigate the effect of PACAP in diabetic nephropathy.
Methods: Diabetes was induced by a single intravenous injection of streptozotocin (65mg/kg) in male Wistar rats. PACAP-treated animals were administered 20ug PACAP intraperitoneally every second day. Body weight and blood sugar levels were monitored weekly. Kidneys were removed after 8-weeks survival, kidney/body weight ratio was determined and a complex histological analysis was performed. Expression of inflammatory cytokines was evaluated by semiquantitative cytokine array and Luminex assay. Oxidative stress was determined using the colorimetric analysis of stress markers (MDA, GSH and SOD).
Results: There was no difference in the weekly blood sugar level in the intact control and PACAP-treated groups or between the diabetic control and PACAP-treated groups. Diabetic animals showed a significant decrease in their body weight, and PACAP treatment was unable to significantly counteract the weight loss. Histological analysis revealed severe diabetic nephropathy in kidneys of control diabetic animals (glomerular PAS-positive area expansion, tubular damage, Armanni-Ebstein phenomenon, vascular hyalinosis). PACAP-treatment significantly diminished the damage. Diabetic kidneys showed strong cytokine activation compared to their healthy controls. PACAP was effective in counteracting the changed cytokine expression pattern (e.g. L-selectin, TIMP-1, CINC-1), moreover, it elevated the decreased level of GSH in diabetes.
Conclusion: To conclude, PACAP is effective in ameliorating diabetic nephropathy. These results raise the opportunity for the use of PACAP as a possible therapeutic or preventive method in treating renal complications of diabetes.
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O26 VASOACTIVE INTESTINAL PEPTIDE DEFICIENT MICE EXHIBIT AMELIORATED IMMUNE RESPONSES IN EXPERIMENTAL MODELS OF INFLAMMATION

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Vasoactive intestnal peptide (VIP) is a neuropeptide with well-described anti-inflammatory properties demonstrated with *in vitro* and in vivo experimental models. The recent development of VIP deficient mice (KO) allowed us to study the role of the endogenous source of this peptide in inflammation. Contrary to our expectations, we found that female VIP deficient mice exhibited a remarkable reduced clinical course of experimental autoimmune encephalomyelitis (EAE) induced by myelin olygodendrocyte protein (MOG) administration. Nevertheless, lymphocytes from VIP KO mice were capable to transfer EAE to WT mice, and presented a robust response to in vitro, suggesting that they present encephalitogenic potential, and that adaptive immunity may not be impaired in these mice. We postulated that VIP KO mice may present defects in the innate arm of immunity, and tested this hypothesis by studying their response to lipopolysaccharide (LPS). Similar to what we found in the model of EAE, VIP KO female mice exhibited a significantly higher survival than WT mice in response to LPS, and lower levels of TNFa, IL-6 and IL-12 in the sera and peritoneal suspensions. In addition, peritoneal cells from these mice produced less IL-6 and TNFa than WT cells when stimulated in vitro with LPS. The reduction in cytokines was accompanied by decreased levels of P-ikB in the KO mice. Although the mechanisms for this immunological phenotype of , VIP KO mice remain to be elucidated, our results suggest that long-term absence of VIP may be protective from inflammation.

O27 ROLES OF PITUITARY ADENYLATE-CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IN NOCICEPTION AND INFLAMMATION IN AN IMMUNE MEDIATED ARTHRITIS MODEL

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Pituitary adenylate-cyclase activating polypeptide-38 (PACAP-38) is expressed in the spinal dorsal horn, capsaicin-sensitive sensory neurons and immune cells. We have previously demonstrated its important roles in several pain and inflammation models, as well as its divergent peripheral functions depending on different neuronal and immune mechanisms. In the present study we investigated the involvement of this peptide in the serum transfer model of rheumatoid arthritis using gene-deficient mice.

PACAP gene deleted (PACAP-/-) and wildtype (PACAP+/+) mice were treated i.p. with arthritogenic K/BxN or control negative serum. The severity of the joint inflammation was assessed with semiquantitative scoring, edema was measured by plethysmometry, and the mechanonociceptive threshold of the hindpaws by dynamic plantar esthesiometry during the 11 days of the experiment. The thermonociceptive threshold was determined on an increasing temperature hot-plate. Body weight was also measured and motor functions were studied with the Rota-Rod and horizontal wire grid grip test. In vivo positrone emission tomography (PET) was performed on days 5 or 10 using [18F]Fluoro-desoxyglucose (FDG) (average 4MBq/animal) by a Mediso nanoScan(r) PET/MRI device.

PACAP+/+ mice developed remarkable joint inflammation and hindpaw edema, which reached its 40% maximum on day 3, body weight decreased by 10%. The mechanonociceptive threshold decreased by 10-15% till day 5., which normalized by day 9. In the wire-grid grip test the performance decreased by 25%. In PACAP-/- mice the inflammatory score and edema were significantly less severe than observed in wildtypes, mechanical hyperalgesia, weight loss and motor impairment were not observed. During the PET scans we found increased FDG accumulation in the inflamed frontpaw and hindpaw joints of wildtype mice, which was smaller in the PACAP-deficient group. The motor coordination and the thermonociceptive threshold did not change in this model in either group.

We provided evidence for important pro-inflammatory and nociceptive roles of PACAP this arthritis model. Identifying its target and unraveling the precise mechanisms could provide promising new therapeutical perspectives for chronic joint inflammation and related pain.

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O28 CAPSAICIN INDUCES SKIN INFLAMMATION VIA TRPV1-MEDIATED UP-REGULATION OF PACAP

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Pituitary adenylate-cyclase activating polypeptide (PACAP) has been described in the human skin. However, there are few data concerning its expression in the mouse skin and its role in neurogenic/non-neurogenic acute cutaneous inflammation models. In the present study we demonstrate that PACAP-immunoreactivity (PACAP-IR) can be measured in the homogenates of different mouse skin areas with radioimmunoassay. Its concentration was relatively similar in the plantar and dorsal paw skin as well as the ear, but significantly smaller in the back skin. PACAP and its specific receptor, PAC1, have also been detected at the mRNA level with RT-PCR; their relative expression was almost the same in these skin regions. Injection of capsaicin, agonist of the transient receptor potential vanilloid 1 (TRPV1) ion channel, into the plantar surface of the paw (50 µl, 100 µg/ml s.c.) increased PACAP-IR, as well as PACAP and PAC1 mRNA levels in the plantar skin. In contrast, intraplantar complete Freund's adjuvant (CFA; 50 µl, 1 mg/ml) did not alter either PACAP-IR or PACAP/PAC1 mRNA expression. Neurogenic edema induced by intraplantar capsaicin was significantly smaller in PACAP deficient mice than in their wildtype counterparts throughout a 24-hour experimental period, but CFA-evoked paw swelling was not influence by the genetic deletion of the PACAP gene. These results provide evidence for the presence of PACAP mRNA and immunoreactivity in different mouse skin samples. Their capsaicin-induced up-regulation can be either due to direct effect of capsaicin at extraneural TRPV1 receptors or an indirect action in response to sensory-nerve derived inflammatory mediators. Therefore, PACAP may function as a pro-inflammatory mediator and increase edema formation in the mouse skin.

O29 PACAP TREATMENT AMELIORATES *TOXOPLASMA GONDII*-INDUCED ENCEPHALITIS IN MICE

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Background: Pituitary adenylate cyclase-activating polypeptide (PACAP) is well known to play crucial roles in immunity and inflammation. For the first time, we investigated the potential anti-inflammatory and immuno-modulatory properties of PACAP in a murine parasite-induced encephalitis model.
Methodology/Principal Findings: Three weeks following intraperitoneal *Toxoplasma gondii* infection (3 cysts, ME49 strain), C57BL/6 mice start to develop encephalitis. Therefore, we administered PACAP intraperitoneally (1.5 mg / kg body weight) for 6 days starting at day 21 p.i. PACAP treated animals displayed reduced signs of intracerebral inflammation as compared to placebo treated controls. Examination of brain tissues on day 28 p.i. revealed reduced parasitic cyst and inflammatory cell numbers accompanied by fewer CD3+, F4/80+, Caspase3+ cells within the brain parenchyma. Furthermore, PACAP treated animals exhibited significantly lower intracerebral IL-6 and IFN-g mRNA expression levels (quantitative RT-PCR) as compared to Placebo control mice. In ongoing *in vitro* studies, we are currently investigating the underlying immunomodulatory mechanisms exerted by PACAP.
Conclusion/Significance: PACAP treatment ameliorates *T. gondii* induced encephalitis in a murine model. These findings might provide beneficial treatment options for encephalitis patients.
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O30 ANTIMICROBIAL ACTIVITY OF A STABLE ANALOG OF VASOACTIVE INTESTINAL PEPTIDE

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Currently we faced an alarming resurgence in infectious diseases caused by antimicrobial resistance. This justifies the urgent necessity for identifying therapeutic factors with new ways of actions acting on critical/vital structures for microbes. In this sense, in the last years it has been increased interest in the development of novel strategies based on our natural immune defenses. Current therapeutic approaches are focused on the characterization and research about antimicrobial peptides, now called host defense peptides (HDPs) due to their combination of antimicrobial activity against diverse microbes and the diverse range of functions in modulating immunity. Vasoactive intestinal peptide (VIP) is a major neuropeptide involved in a wide range of biological functions. Recently, based on its cationic and amphipathic structure resembling antimicrobial peptides, it has been demonstrated its antibacterial and antiparasitic activity. This suggests VIP as an attractive candidate to develop new and efficient antibacterial/antiparasitic therapies. However, some limitations such as half-life in serum and proteolytic degradation must be overcome. Here, we investigate by the first time the antimicrobial and antiparasitic activity of the synthetic derivative of VIP [A8,24,25, R15,20,21, L17, des-N28]-VIP-GRR, called as VIP51. Also, we design a fragment derived from VIP51 (called as VIP51F6-30) in order to analyze the requirements for the peptide/membrane interactions. VIP51/VIP51F kill Gram(+) and Gram(-) bacteria and show a significant leishmanicidal effect. Both analogs disrupt the surface-membrane of bacteria and parasites leading to pore formation and cell death. Using specific mutants for bacterial lipopolysaccharide and mutants for the lipophosphoglycan component of the parasite surface, we show that there is a specific effect of these peptides depending on the surface structure and pathogen. Interestingly, these peptides were no lytic when incubated with mammal cells. Treatment with VIP51/VIP51F prevented mortality, decrease bacterial load and reduce inflammation in mice suffering polimicrobial sepsis induced by cecal ligation and puncture. Although both peptides show similar antiparasitic effects in vitro, only VIP51F was effective as a treatment of cutaneous leishmaniasis, decreasing footpad swelling, lesion size, and parasite burden. Interestingly, these differences correlated with differences in the immune response of infected mice after treatment. Together, these results indicate that, VIP51 and VIP51F, novel derivatives from VIP with improved stability and longer half-life when compared with the endogenous peptide, also show higher antibacterial/antiparasitic effects suggesting that they could be an attractive alternative as treatment for these diseases.

O31 ALTERATIONS IN PACAP-38-LIKE IMMUNOREACTIVITY IN THE PLASMA DURING ICTAL AND INTERICTAL PERIODS OF MIGRAINE PATIENTS

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Background: Recent studies on migraineurs and our own animal experiments have revealed that pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) has an important role in activation of the trigeminovascular system. The aim of this study was to determine the PACAP-38-like immunoreactivity (LI) in the plasma in healthy subjects, and parallel with the calcitonin gene-related peptide (CGRP)-LI in migraine patients in the ictal and interictal periods.
Methods: A total of 87 migraineurs and 40 healthy control volunteers were enrolled in the examination. Blood samples were collected from the cubital veins in both periods in 21 patients, and in either the ictal or the interictal period in the remaining 66 patients, and were analysed by radioimmunoassay.
Results: A significantly lower PACAP-38-LI was measured in the interictal plasma of the migraineurs as compared with the healthy control group ($p<0.011$). In contrast, elevated peptide levels were detected in the ictal period relative to the attack-free period in the 21 migraineurs ($p_{PACAP-38}<0.001$; $p_{CGRP}<0.035$) and PACAP-38-LI in the overall population of migraineurs ($p<0.009$). A negative correlation was observed between the interictal PACAP-38-LI and the disease duration.
Conclusion: This is the first study that has provided evidence of a clear association between migraine phases (ictal and interictal) and plasma PACAP-38-LI alterations.

O32 VIP-DEFICIENT MICE EXHIBIT SEVERE BUT REVERSIBLE ALLODYNIA TO MECHANICAL AND COLD STIMULI

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The vasoactive intestinal peptide (VIP) has long been implicated in a plethora of neural functions ranging from cortical development at early embryological stage to regulation and modulation of neuroneurosecretion, biological rhythms and behavior in adulthood, as VIP-deficient mice revealed. Back in 2006 we reported that these mice exhibited locomotor deficit and poor performance in specific tasks of the SHIRPA test as a consequence of muscular weakness. Another plausible explanation is that lack of VIP is affecting sensory signal integration. Therefore we first assessed both mechanical and thermal (hot and cold) nociception using Von Frey and conventional or dynamic plate tests, respectively. Compared to wild-type and heterozygous adults, VIP null mice displayed a rather low sensory threshold on cold and mechanical paradigm whilst their sensitivity to heat remained unaffected. In vivo electrophysiology performed on intact spinal cord preparation revealed an exacerbated activation of C-type fibers in response to physiological mechanical and electrical stimuli, mimicking allodynia. We then ask if this phenotype was permanent (as a result developmental sensory defect) or if it could be reversed. Experiments revealed that short term intraperitoneal administration of VIP to deficient animals, induced a full and long-lasting return to nociceptive baseline. this strongly suggests that hyperalgia/allodynia is more likely to originate from epigenetic remodelling. To further validate this finding, we performed gene expression screening by quantitative RT-PCR on selected gene candidates implicated in pain. Thus we isolated a very small subset of genes whose expression is directly controlled by VIP and that may account for the very specific allodynia observed in VIP deficient mice. All together, these results strongly support a role for VIP in physiological control of nociception and reveal some unexpected analgesic property to be further evaluated.

O33 APPLICATION OF THE THREE HIT THEORY IN PACAP HETEROZYGOUS MICE: MATERNAL SEPARATION AND CHRONIC STRESS INFLUENCE BNST CRF AND CPEW UCN1 IN AN INVERSE MANNER

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According to the three hit theory of depression genetic predisposition, epigenetic factors and stress precipitate the symptoms of major depression. There are several animal models widely used to study the neurobiology of mood disorders, their validity is still not unequivocally accepted. In this work we aimed to set up and validate a mouse model for depression by the evaluatation of the activity of the hypothalamus pituitary adrenal (HPA) axis. We also planned to study the contribution of corticotropin releasing factor (CRF) producing neurons in the bed nucleus of the stria terminalis (BNST), and in the central amygdala. The possible role of the CRF-related urocortin1 (Ucn1) peptide containing neurons of the central projecting Edinger-Westphal nucleus (cpEW) was also studied. According to the three hit theory for the genetic predisposition we used mice heterozygous for the gene of pituitary adenylate cyclase-activating polypeptide (PACAP). Litters of PACAP heterozygous pairs were exposed to severe maternal separation to induce epigenetic changes vs. non-deprived or briefly separated controls. Half of adult mice later were subjected to the chronic variable mild stress paradigm. We hypothesized that mice carrying all three risk factors will fail to adapt or show some maladaptive alterations supporting the validity of the model, and both the CRF and Ucn1 systems will be affected in their peptide content and/or neuronal activity. According to our results our stress paradigm was effective as in stressed groups the adrenal gland weighs significantly increased, which rise was the greatest in with maternal separation history. Corticosterone measurements supported this, indicating the over activity of the HPA axis. Histological results revealed that maternally deprived mice exposed to chronic stress reacted with an increase in CRF immunoreactive cell counts and specific signal density in the oval nucleus of the BNST. In contrast, in the central nucleus of the amygdala, the chronic stress-induced increase in the CRF specific signal density was in maternally non-derived mice observed only. Similarly, in maternally deprived mice we did not find increased neuronal activity by FosB in Ucn1 neurons and the stress induced increase in Ucn1 was abolished. The three hit theory of depression seems to be applicable in PACAP heterozygote mice, and it could be a promising model to study the pathophysiology of stress-related mood disorders. The elevated CRF contents in neurons of the oval nucleus of the BNST and decreased Ucn1 neuronal activity suggests that both systems are affected in mood disorders, and their inversely altered expression and/or activity could contribute to the psychopathology.
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O34 LOCALIZATION AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF THE DORSAL VAGAL NUCLEUS (DMX) NEURONS PROJECTING TO THE PORCINE STOMACH PREPYLORIC REGION IN PHYSIOLOGICAL STATE, FOLLOWING STOMACH PARTIAL RESECTION AND AFTER PROLONGED ACETYLSALICYLIC ACID SUPPLEMENTATION

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To investigate localization and chemical coding of the parasympathetic DMX neurons supplying the porcine stomach prepyloric area Fast Blue was injected into the studied region of control, resection (RES) and acetylsalicylic acid (ASA) group. Following paraformaldehyde perfusion fixation the DMX sections were stained immunocytochemically to ChAT, PACAP,VIP, NOS, GAL, CART, SP and LENK.
Fluorescence microscopy revealed 485,2 ± 42,7, 575,2 ± 76,22 and 705,8 ± 61,04 of the FB+ perikarya in DMX of control, RES and ASA group, respectively. All FB+ cells were ChAT+. In the control DMX 30,08 ± 1,97% of the FB+ neurons expressed PACAP, while no other peptides were found in the FB-labeled perikarya. In the RES DMX PACAP was found in 45,58 ± 2,2%, VIP in 28,83 ± 3,63%, NOS in 21,22 ± 3,32% and GAL in 5,67 ± 1,49% of the FB+ perikarya. In the ASA DMX PACAP was revealed in 49,53 ± 5,73%, VIP in 40,32± 7,84%, NOS in 25,02 ± 6,08% and GAL in 3,37 ± 0,85% of the labeled neurons.
Our research for the first time revealed:
I. Expression of PACAP in the porcine vagal parasympathetic neurons projecting to the stomach prepyloric region.
II. Numerical increase of the FB+/PACAP+ somata in the DMX of the RES and ASA group.
III. De novo synthesis of the VIP, NOS and GAL in the retrogradely traced neurons following resection of the FB-injected area of the prepyloric stomach region as well as a result of the long term acetylsalicylic acid administration. Acquired data indicate possible participation of PACAP, VIP, NOS and GAL in neural response to studied pathological states.

O35 PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE (PACAP) IN THE TELEOST FISH IMMUNE SYSTEM: FROM ITS DISCOVERY TO THEIR FUNCTION

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Together with genetic and environment factors, health depends on regulatory interactions between the three systems involved in homeostasis: nervous, endocrine and immune systems. A significant point for the behavior of this framework is the sharing of common ligand-receptor-effectors molecular systems. For lower vertebrates, there is, as yet, scarce information available regarding this inter-system communication. Recently, we have made a start through studies on the role of the pituitary adenylate cyclase-activating peptide (PACAP) in innate and adaptive immunity in fish. We have shown that administration of this neuropeptide increases deferent humoral immune parameters in fish larvae and juveniles. This immunological status was correlated with higher growth hormone concentration in serum and with an improvement of the fish antioxidant defense mechanisms. Current work provides new insights about the effects of PACAP on the fish immune system. It demonstrated for the first time both the occurrence of the two PACAP transcriptional variants (PACAP and PRP/PACAP) together with their receptors (PAC-1, VPAC-1 and VPAC-2) in diverse lymphoid organs of the salmonid fish. Additionally, their expression levels were assessed in head kidney and spleen leukocytes, and in the monocyte/macrophage cell line RTS11 at different time points after infection with important pathogens for aquaculture: such as the viruses viral hemorrhagic septicemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV). The results disclosed a differential regulation of the PACAP transcripts and their receptors after infections. These findings added PACAP and its receptors to the growing list of mediators shared by the nervous, endocrine and immune system in fish, and suggest a possible role of these molecules in antiviral immunity. To support the previous hypothesis, a direct action of PACAP on the regulation of different immune genes and cytokines in fish lymphoid tissue was evaluated. We have observed that PACAP modulates the IL-1β, TNFα, IL-15, Mx, INF gamma and TLR9 mRNA levels in fish peripheral blood and head kidney leukocytes in vitro. This effect was associated with its ability to enhance the MHC-II, CD4 co-receptor and IgM transcripts. The overall results corroborated the existence of diverse mechanism of modulation of the immune functions in fish mediated by the VIP-PACAP system.

O36 REVERSAL OF AGE RELATED LEARNING DEFICIENCY BY THE VERTEBRATE PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IN *LYMNAEA*

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The common pond snail *(Lymnaea stagnalis)* has been extensively used as a model system for studying the cellular and molecular mechanisms of associative learning and memory. The main advantage of this system is that animals can learn after a single trial food reward conditioning and the memory can be recalled even after 3 weeks. However this robust, „flash-bulb" like memory can only be induced in young adults (3-4 months old); aged snails (over 6 months) cannot learn the association after only one training trial. Recently we have shown that the homolog of the vertebrate PACAP and its receptors (PAC1-R, VPAC1 and VPAC2) exist in *Lymnaea* and PACAP activates the adenylate cyclase enzyme, just like in the vertebrate nervous system. Previous work already has demonstrated the role of highly conserved molecular pathways, both upstream and downstream of adenylate cyclase, in long-term memory resulting from single-trial food-reward clasical conditioning in *Lymnaea*. These include NMDA receptor activation and CaMKII-mediated mechanisms, the cAMP-PKA-CREB pathway and the NOS-NO-guanylate cyclase-cGMP-PKG cascade.

Our recent work has tested the hypothesis that PACAP plays an important role in the formation of robust LTM after classical food-reward conditioning and provided the first evidence that PACAP is both necessary and instructive for fast and robust memory formation after reward classical conditioning in young animals. Here we tested the role of PACAP in learning in aged animals by looking at its effect on the formation of long-term memory after single trial appetitive conditioning. Our new results show that systemic injection of synthetic PACAP 1h before training boosts memory formation in old animals. Since PACAP is a highly conserved molecule, our results indicate that it has an important role in learning and memory in general and it can also be used as a memory „rejuvenating" agent during normal biological ageing.

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O37 TRANSMEMBRANE DOMAIN PEPTIDES AS A NEW CLASS OF DRUGS TO DEMONSTRATE THE IN VIVO FUNCTION OF GPCR HETERO-OLIGOMERIZATION IN WATER INTAKE

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Angiotensin (ANGII) and secretin (SCT) share overlapping osmoregulatory functions in the brain. The observation that remains highly elusive and hence controversial is that a functional SCT/SCTR axis in the brain was found a pre-requisite for carrying out central actions of ANGII. As both angiotensin receptor type 1A (AT1aR) and secretin receptor (SCTR) are co-expressed in brain osmo-regulatory centers, a possible mechanism to explain these data is the formation of functional SCTR and AT1aR oligomers, leading to subsequently modulation in physiological responses. In this report, we initially establish that SCTR and AT1aR can homo- and hetero-oligomerize, and also that several transmembrane (TM) peptides of SCTR and AT1aR are able to inhibit receptor oligomerization, and to modulate cAMP responses of SCTR. One of these peptide, ATM-1, corresponding to the first transmembrane domain region of AT1aR, inhibits only hetero-oligomer formation. When injected into the later ventricle of mouse, this peptide is capable of suppressing water-drinking behavior upon hyperosmotic shock, similar to what is observed in SCTR knockouts and in H-89 injected mice. Here we show the *in vivo* action of SCTR/AT1aR receptor oligomerization in central neurons, in this case, in inducing water intake. Using the constitutively active mutant of AT1aR, we show AT1aR/SCTR hetero-oligomer possesses a functional bias of SCTR, in which the active conformation of AT1aR is a key to regulate SCTR in mediating cAMP responses. This therefore provided a molecular model to the potentiation action of ANGII on SCT, and that SCTR/AT1aR oligomerization plays a crucial physiological function in our body to distinguish elevated levels of SCT as a consequence of hyperosmolality or food intake by the difference in ANGII levels. We also demonstrate that TM peptides are potentially a new class of drugs that can modulate GPCR functions via the disruption of receptor oligomerization. These peptides are highly specific in its action since they are structurally homologous to part of the target GPCR. These peptides can be used to disrupt all oligomerization events of the target receptor or can be used to specifically inhibit physiological functions due to hetero-oligomerization. The potential of this new class of drug in biological studies is therefore tremendous, as we are only in the beginning to understand the importance of receptor oligomerization in our body.

O38 THERAPEUTIC EFFECT OF VIP ON INFLAMMATORY CARDIOVASCULAR DISORDERS: ATHEROSCLEROSIS AND AUTOIMMUNE MYOCARDITIS

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Evidence indicates that many cardiovascular disorders of high incidence in occidental world, such as atherosclerosis and dilated miocarditis, are consequence of an exacerbated chronic inflammation that drives tissue-specific autoimmune responses. It is widely known that VIP is a potent anti-inflammatory factor which shows protective actions on Th1-driven self-reactive response in many experimental autoimmune disorders. Here, we investigated the potential therapeutic effect of VIP in two preclinical models of atherosclerosis and autoimmune miocarditis. Systemic infusion of VIP reduced significantly the appearance of atherosclerotic plaques in aortic arch and descending aorta artery of APO-E-KO mice fed with a high-cholesterol diet. This therapeutic effect was exerted at multiple levels. First, VIP inhibited the infiltration of macrophages and CD4 T cells into the atherosclerotic plaque and the local production of inflammatory and Th1/Th17 cytokines by the developing plaque. Second, VIP reduced the formation of foam cells (infiltrating macrophages that capture oxidized lipids such as ox-LDL) by increasing the expression of ABCA proteins and out-fluxes of cholesterol in these cells. Third, VIP decreased the activation/differentiation of Th1 and Th17 cells in draining lymph nodes through an antigen-specific mechanism that could involve regulatory T cells. Fourth, VIP inhibited the proliferation of smooth muscle cells and their migration to the developing plaque and reduced the formation of neointima lesions and vascular stenosis in the carotid artery of atherosclerotic animals. Alternatively to chronic VIP treatment, we also designed a cell-based therapy with VIP-expressing adipose-derived mesenchymal cells, in which a single injection of these „Trojan horses" was enough to reduce the number and size of atherosclerotic plaques in the arterial system. Similarly to atherosclerosis, systemic VIP treatment reduced clinical signs of dilated myocarditis induced by immunization of Balb/c mice with a fragment of cardiac myosin heavy chain. VIP treatment decreased the infiltration of inflammatory cells, the content of fibrotic deposits and the levels of inflammatory and Th1 cytokines in the myocardium. Moreover, VIP reduced the amounts of circulating anti-myosin self-antidodies. The effect of VIP in myocarditis was partially exerted peripherally, because draining lymph node cells from VIP-treated mice responded less to self-antigen specific recall activation. In summary, VIP emerges as an attractive candidate to treat the immunopathology of atherosclerosis and myocarditis, and consequently, to reduce the risk of brain stroke, ischemia and myocardial infarct in these disorders.

O39 LOW BASELINE SERUM LEVEL OF VIP IS A MARKER OF WORSE PROGNOSIS IN PATIENTS WITH EARLY ARTHRITIS

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At present, available biomarkers are insufficient to predict disease outcome in patients with rheumatoid arthritis (RA). New prognostic factors that correlate with progressive disease are needed to identify those patients with the worst potential outcomes, who will require more intensive treatment for their disease. Vasoactive intestinal peptide (VIP) is a peptide with anti-inflammatory and immunomodulatory properties in RA. Our aim was to study serum levels of VIP during the follow-up of an early arthritis (EA) cohort and to analyze its value as a biomarker in RA.

Data from 91 patients (76% fulfilling RA criteria and 24% undifferentiated arthritis) from an EA register were analyzed (73% women, median age 54 years, 5.4–month median disease duration at entry).Sociodemographic, clinical and therapeutic data were collected in a two years follow-up protocol. VIP levels were determined by ELISA in sera harvested from 353 visits (3.5 visit per patient) and from healthy controls. VIP values below 25th percentile of the healthy population were considered low. To determine the effect of independent variables on VIP levels, a longitudinal multivariable analysis nested by patient and visit was performed. A multivariate ordered logistic regression was modeled to determine the effect of low VIP serum levels on disease activity at the end of follow-up.

VIP concentration was considerably heterogeneous in EA patients and did not significantly vary along the follow-up. The patients fulfilling RA criteria showed the lowest VIP concentration values although, in average, no significant differences were observed compared to healthy donors. Along the follow-up VIP levels were lower in individuals with higher disease activity measured by DAS28 (coef. beta: -0.043 ± 0.019; p=0.026).In addition, at the end of the follow-up, those patients with low baseline levels of VIP and negative anti-citrullinated peptide antibodies (ACPA) displayed higher disease activity (OR: 6.11; p=0.023) despite receiving more intensive treatment than those with normal VIP levels and negative ACPA.

Patients who are unable to up-regulate VIP seem to have a worse clinical course despite receiving more intense treatment. These findings indicate that measurement of VIP levels may be suitable as a prognostic biomarker.

O40 VIP IS A NEGATIVE REGULATOR OF MEDIATORS INVOLVED IN THE CROSS-TALK OF SYNOVIAL FIBROBLASTS AND TH1/TH17 CELLS IN RHEUMATIC DISEASES

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Rheumatoid arthritis (RA) and osteoarthritis (OA) are two rheumatic diseases of unknown aetiology which development is associated with a chronic inflammatory response localized in the synovium of diathrodial joints, leading to a progressive destruction of articular cartilage and bone. Fibroblast-like synoviocytes (FLS), an abundant resident cell type in synovial tissue, play a crucial role in the pathogenesis of both rheumatic diseases because of their ability to produce a variety of mediators involved in joint inflammation and destruction. This pathogenic behavior is activated and enhanced in response to pro-inflammatory factors and Toll-like receptor (TLR) agonists. In recent years Vasoactive intestinal peptide (VIP) has emerged as a potential candidate for treatment of inflammatory and autoimmune diseases. According to previous studies by our group, VIP modulates different pro-inflammatory pathways ex vivoin human RA synovial cells. Specifically, we have described that VIP anti-inflammatory signalling is functional in human FLS wherein it is able to interfere with TNFα signalling and acts as a negative regulator of the signalling triggered by TLR2, TLR4 and RNA sensors of innate immunity.
Our aim was to examine whether inflammatory mediators present in rheumatic joints modify the capacity of FLS to respond to immune signalling and also whether they affect their contribution to synovial inflammation and joint destruction. We examined the potential ability of VIP to modulate the effect of these mediators in FLS immune activity and in their pathogenic production of factors that exacerbate joint destruction and inflammation. Our data shown that TNFα, IL-17 and TLR ligands modulate the expression of IL-17 receptors and the production of IL-12 and IL-23, two cytokines involved in the facilitation of Th1 and Th17 differentiation respectively. Besides, IL-22 stimulated the up-regulation of alarmins S100A8/A9 and MMP1 production as well as FLS proliferation, which are related to destructive processes in the joint. VIP treatment diminished the stimulatory action of IL-22 on FLS activation and was able to counteract the enhancing effect of pro-inflammatory molecules on IL-17 receptors and IL-12 family of cytokines expression. Our results corroborate the role of VIP as a negative regulator of pro-inflammatory pathways and demonstrate its capacity to modulate the expression of several molecules potentially involved in the cross-talk between FLS and Th1/Th17 cells. These data expand the beneficial effects of this endogenous neuroimmunopeptide in rheumatic diseases, reinforcing its potential as a therapeutic agent.

O41 VASOACTIVE INTESTINAL PEPTIDE MAINTAINS THE NON-PATOGENIC PHENOTYPE OF HUMAN TH17-POLARIZED CELLS FROM NAIVE T CELLS AND DECREASES THEIR TH1 POTENTIAL

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Classically, T helper (Th) cells have been subdivided into different subsets, including Th1, Th2 and Treg. Recently, a novel subset has been identified, Th17 subset. The inflammatory microenvironment determines the differentiation of naive T cells to a committed lineage. In contrast to Th1 or Th2 subsets, several studies have showed that Th17 subset is a lineage less committed. Therefore, the inflammatory microenvironment also might cause changes in the Th17 acquired lineage becoming, for example, Th1 cells. In addition to their inherent plasticity, Th17 cells are also characterized by their functional heterogeneity. Last reports indicate that Th17 cells could have pathogenic or non-pathogenic phenotype. Pathogenic Th17 cells are characterized by IL-17, IL-21, IL-22, IL-2, INFγ and GM-CSF secretion. Meanwhile, non-pathogenic Th17 cells secrete IL- 17, IL-21, IL-9 and IL-10. Given this heterogeneity, it is very interesting to know the regulatory mechanisms involved in differentiation, function and plasticity of Th17 cells. Vasoactive Intestinal Peptide (VIP) is one of the best-studied immunomodulatory peptides. This peptide plays important regulatory functions through union to its specific receptors, VPAC1 and VPAC2. It has been showed that VIP is able to modulate mouse Th17 cells. However, it is not clear the role of VIP on human Th17 cells. Therefore, we tested if VIP modulates the human Th17 differentiation. Analysis of VIP effect showed that it modulates the human Th17 differentiation, maintaining the non-pathogenic phenotype, increasing the proliferation, and decreasing the Th17/Th1 plasticity of Th17 cells. In addition, we studied the expression and function of VPAC receptors in these cells. Data showed that Th17 differentiation caused a switch in the VPAC1 and VPAC2 expression pattern. Analysis with specific agonists and antagonist of these receptors showed that both are differently involved in the VIP modulation of Th17 cells. In conclusion, we describe for the first time the differentiation of human naive T cells towards Th17-polarized cells under VIP and demonstrate how this differentiation affects the expression of the VIP receptors.

O42 PET IMAGING OF KRAS2 ACTIVATED LUNG CANCER IN TRANSGENIC MICE USING VPAC1 RECEPTOR SPECIFIC CU-64-TP3805

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Introduction and Hypothesis: Each year in the USA, 228,000 new cases of lung cancer (LC) are diagnosed and more than 160,000 people, both men and women die of the disease. New approaches to drug discovery are increasingly based upon the better understanding of biochemical pathways that govern the genesis of the disease at a molecular level. VPAC1 receptors are expressed in high density on LC [1-2]. A peptide molecule has been desized, synthesized, labeled with a radioactive metal ion copper-64 (Cu-64), and evaluated in vitro (kd = 3.1 x 10-9M) and in experimental animals as well as in humans diagnosed with breast cancer [3-9]. Our data suggest that Cu-64-TP3805 PET (positron emission tomography) or PEM (positron emission mammography) can image all malignant lesions that express VPAC1 receptors with high sensitivity (100%) in animals (n=10) and in humans (n=24) , but not the benign masses that do not express VPAC1 receptors [10]. We therefore hypothesized that Cu-64-TP3805 will PET image LC early, accurately and with a high sensitivity.
Method: KRAS2 is most frequently a mutated oncogene found in 25% - 50% of LC. KRAS2 mutant G12D transgenic mice are born with microscopic deposits of LC. More than 90% of these mice die at the age of 7-8 months by the burden of LC [11]. These mice were PET imaged longitudely from 1.5 to 6 months of age. Once every two weeks they were administered, via a lateral tail vein, 150±10 μCi of F-18-FDG, anesthetized with 1.5% halothane in 98.5% oxygen and imaged using PET/CT (Siemens, Nashville, TN) one hr later. All animal protocols were approved by the institutional animal care and use committee. After a complete decay of F-18 radioactivity, animals were injected 110±10% μCi of Cu-64-TP3805 and imaged similarly at 4 hr and 24 hrs after injection. Images were reconstructed and analyzed for quantification. Normal mice (n=3) were also imaged similarly as a control. After final imaging, animals were sacrificed, lungs extirpated, for histology. RTPCR studies are in progress.
Results: Greater than 98% of the Cu-64 activity was bound to 20 μg (~ 0.5 x 10-8M) of the peptide. All lungs of normal mice were free of any uptake of radioactivity either F-18-FDG or Cu-64-TP3805. As seen by CT and PET scans, the lung nodules continued to grow in size. In two of the four KRAS2 mutant G12D mice, no nodular uptake of F-18-FDG was seen. Contrary to this, lung nodules in all mice were unequivocally delineated by Cu-64-TP3805. Histological examinations confirmed the malignancy and RTPCR studies to validate the presence of VPAC1 are ongoing.
Conclusion: VPAC1 Specific Cu-64-TP3805 peptide analogue can PET image spontaneously grown LC lesions with high accuracy as compared to the current gold standard F-18-FDG. These results are consistent with pathologic findings. Targeting genomic biomarkers with specific biomolecules demonstrates novel approach to image LC.
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O43 PEPTIDOMIC/PROTEOMIC PROFILING OF HUMAN EMBRYO SECTRETOME

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The presence of PACAP has been shown in various endocrine and reproductive organs. In our previous study the presence of PACAP-38 was detected from follicular fluid to predict its possible function in oocyte development. The objective of our present study was to discover molecular alterations during in-vitro fertilization treatment to establish their predictive value for embryos viability. The peptides and proteins were measured by matrix-assisted laser desorption ionization tandem time-of-flight mass spectrometry after 3rd and 5th days from the micro-droplets of embryo culture as well as in control media. The mass spectrometric results were statistically evaluated by ClinProTools (Bruker Daltonics) clustering software. Our results demonstrated that analysis of peptidomic/proteomic profiles of the blastocyst secretome can be predictive to distinguish between higher and lower viability of early stage human embryos. Based on our statistical analysis the molecular differences of embryos with good and unsatisfactory implantation properties were significantly detectable after 3rd and 5th days as well.

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O44 EFFECTS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE ON SPERMATOGENESIS

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Pituitary adenylate cyclase activating polypeptide (PACAP), a neuropeptide with diverse effects, was originally isolated as a hypothalamo-hypophyseal peptide. Subsequent studies showed highest levels of PACAP in the testis after the brain, suggesting that it influences the development and functioning of spermatozoa. Indeed, it has been proven that PACAP has an effect on spermatogenesis, both locally and via influencing the hypothalamo-hypophyseal-gonadal axis. The aim of the present study was to investigate sperm motility, morphology and expression of key determinants of spermatogenesis in the testis of mice lacking endogenous PACAP. Motility of sperm cells was investigated using a computer aided sperm analysis system. Sperms isolated from the epididymis of PACAP KO mice showed a decrease in sperm motility. The morphological analysis of spermatozoa isolated from wild type and PACAP KO mice showed that the sperm head diameter was significantly smaller in PACAP KO mice. The shape of the heads investigated with transmission and scanning electronmicroscopy, did not show marked differences between the two groups, but the size of the heads was smaller in PACAP KO animals. However, we found more abnormal tail forms among PACAP KO cells. The family of Sox transcription factors play key roles in spermatogenesis. We investigated Sox 9 and Sox 10 in the testis of PACAP KO mice by immunohistochemistry and Western blotting. We found that while Sox 9 expression was markedly reduced, Sox 10 was significantly increased in PACAP KO mice. The phosphatase PP2A was also increased in mice lacking PACAP. Our results show that there are marked differences in sperm morphology, biochemistry and function between wild type and PACAP KO mice, suggesting that endogenous PACAP plays an important role in spermatogenesis.

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O45 RADIOIMMUNOASSAY EXAMINATION OF PACAP38-LIKE IMMUNOREACTIVITY IN DIFFERENT MILK AND INFANT FORMULA SAMPLES

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide with important role in reproductive and developmental processes. Recently, we have described that PACAP38 is present in high levels in the milk of humans and ruminant animals. The aim of the first part of the study was to investigate PACAP38-like immunoreactivity (PACAP38-LI) in human colostrum, transitional and mature milk, and we planned to detect changes of PACAP38-LI during different periods of lactation by radioimmunoassay (RIA). In the second part of the experiment we aimed to measure PACAP38-LI in fresh cow milk, pasteurized cow milk and commercial infant formula samples by RIA and to prove the presence of PACAP38 in infant formulas by mass spectrometry (MALDI TOF/TOF) analysis.

We found that PACAP38-LI was significantly higher in human colostrum samples than in mature milk. PACAP38-LI did not show significant changes within the first 10-month period of lactation, but a significant increase was observed thereafter, up to the examined 17 months. We found that PACAP38-LI did not show any alteration in the foremilk and hindmilk samples. There was no difference in the PACAP38 content of fresh and pasteurized cow milk and in infant formula samples either. However, the hypoantigenic infant formulas contained significantly higher levels of PACAP38-LI. The result of mass spectrometry indicates that the measured PACAP38-LI represents PACAP38 molecule in the infant formula.

Our present data show that PACAP38 is relatively stable in the milk and it can withstand the manufacturing processes. The importance of PACAP in human milk is not known exactly, probably it plays a role in the development of the newborn nervous system, immunsystem and in the regulation of the growth/secretory function of mammary gland. Further investigations are needed to evaluate the exact function of this neuropeptide in the milk.

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O46 PRESENCE OF PACAP IN HUMAN FEMALE GENITAL SYSTEM

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Pituitary adenylate cyclase activating polypeptide (PACAP) plays an important role in the regulation of several reproductive processes, including female endocrine functions and intrauterine growth. The presence of PACAP has been shown in the placenta, ovary, uterus and mammary gland. It has been shown that PACAP influences follicular and placental growth. A few studies have documented on the presence and effects of PACAP in human reproductive processes. In the present study we report on studies related to PACAP-like immunoreactivity (LI) in human follicular fluid (FF), placenta and amniotic fluid. We investigated whether there is correlation between PACAP-like immunoreactivity (LI) of the human follicular fluid obtained from women undergoing superovulation treatment and the number of retrieved oocytes. Furthermore, we investigated whether there is any tendency of PACAP-LI in the human amniotic fluid and malformations. Finally, we report on the PACAP-LI in the placenta obtained from first and third trimesters. We found that differences in PACAP-LI in the follicular fluid were significant, indicating correlation between concentration of PACAP in FF and the number of recruited oocytes. Higher concentrations of PACAP in FF might be associated with lower number of developing oocytes while low concentrations of PACAP might correlate with a markedly higher number of ova retrieved, thus predicting a higher chance for ovarian hyperstimulation. In the amniotic fluid we found that markedly lower PACAP-LI levels were detected in chromosomal abnormalities such as Down and Edward syndrome. Finally, we found that the level of PACAP-LI in the placenta increases as pregnancy is progressing. In summary, our results indicate that PACAP occurs in the human female reproductive system and probably plays a role in pathological processes, the details of which need to be further investigated.

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O47 PERSONALIZED EXAMINATION OF VPAC1 BIOMARKER FOR DETECTING GENITOURINARY CANCER

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Introduction: In 2010, more than 30,000 men succumbed to prostate cancer (PC) and more than 240,000 new PC cases were identified in the USA [1]. Digital rectal examination, MRI, and a blood test for prostate specific antigen (PSA) determination play a significant role in detecting advanced PC. However, they are not considered reliable tools for early warning of PC to detect recurrent cancer or to determine metastatic status of the disease [2-4]. Unreliable diagnosis results in undertreatment or overtreatment of patients with minimal benefit, enormous morbidity, incontinence, and/or impotence. Histology remains the mainstay of PC confirmation. However, out of >750,000 biopsies performed each year in the USA, >65% show benign pathology, and cost hundreds of millions of healthcare dollars. Similarly, in the USA, bladder cancer (BC) kills more than 15,200 and inflicts more than 72,500 new cases each year [5]. Although many types of urinary markers have been explored, none has yet become useful due to the lack of specificity and sensitivity [6]. VPAC1 cell surface receptors, express themselves at the onset of the malignancy, and may be prior to elevation of PSA, and well before cell morphology is altered [7, 8]. We have successfully initiated the use of Cu-64 labeled VPAC1 receptor-specific peptide constructs to image disease specific oncogene products in experimental animal models, and in humans [9-16]. We hypothesized that VPAC1 receptors expressed in high density on PC and BC can be targeted for detection of shed tumor cells (STC) in patient urine, using TP4303, a VPAC1 specific biomolecule labeled with a near infrared fluorophore.
Method: Urine samples (n=42) were collected from normal volunteers (n=23) and from patients with PC (n=7), BC (n=2), elevated PSA (n=1), renal stone (n=1), urethral trauma (n=1), testicular pain (n=1), overactive bladder (n=1), minimal urinary frequency (n=1) and benign hyperplasia (BPH) (n=1). Samples were cytospun onto microscope slides. The immobilized cells were then incubated with TP4303 (1 nM in PBS), washed, incubated with a nucleic acid stain, DAPI (200 nM), and a cover slip was placed. Slides were allowed to dry overnight in a dark room and then observed by confocal fluorescence microscopy (Ex: 633 nm. Em: 754 nm).
Results: Cells could be detected at a concentration of 5 cells per ml. All patients (100%) with PC and BC cells had STC. No STC were detected in the urine samples of normal volunteers or patients with BPH.
Conclusion: The method is simple, noninvasive, rapid and thus far detected STC in patients with a known disease. No STC were found either in normal volunteers or those with biopsy proven BPH.
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O48 SURVIVAL PROMOTION OF CELLS EXPRESSING AMYLOID-BETA AND PRESENILIN BY NICOTINE, AMPA AND KETAMINE: IMPLICATIONS FOR ALZHEIMER'S DISEASE

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The causes of Alzheimer's disease (AD), a progressive neurodegenerative disease, characterized by cognitive impairments and formation of plaques and tangles remain elusive. Cellular models whereby expression of beta amyloid (Aβ), the major component of plaques, is exaggerated are commonly used to test the efficacy of novel neuroprotective compounds. In addition to Aβ, mutation in the protein presenilin has also been shown to contribute to Alzheimer's pathology. Recently, a cellular neuroblastoma model where both beta amyloid and mutated presenilin are expressed has become available. In this presentation, survival promotion of nicotine, ketamine and AMPA in these single and double transfected cells will be provided. It is concluded that nicotinic or glutamatergic based drugs are of therapeutic potential in AD.
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O49 PACAP27 IS NEUROPROTECTIVE AGAINST HIV-TAT NEUROTOXICITY

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Human Immunodeficiency Virus (HIV) causes neuronal atrophy and synaptic simplification. HIV proteins, such as transactivator of transcription (Tat), have emerged as leading candidates to explain HIV-mediated neurotoxicity. Using rat cortical neurons we determined that Tat-mediated toxicity is caused by massive mitochondria perinuclear accumulation combined with mitochondrial destabilization, as measured by MTT and cytochrome c release and accumulation of free radicals. In addition, Tat causes a shortening of neuronal processes. Pituitary adenylate cyclase-activating polypeptide 27 (PACAP27) is expressed within the CNS, inhibits programmed cell death and stimulates neurite outgrowth. Therefore, we examined whether PACAP prevents Tat-induced neurotoxicity. We report that PACAP inhibits Tat-mediated mitochondrial destabilization and cytochrome c elevation. PACAP neuroprotective activity appears to be mediated by TrkB, the high affinity receptor for BDNF. Overall our data identify PACAP27 as a potential therapeutic agent against the neurotoxic effects of HIV.
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O50 EVOLUTION OF 'SELECTIVE' NEUROTOXINS

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Through most of history, neurotoxins were equivalent to neuropoisons – substances that nonselectively damaged or destroyed nerves. The rational deduction of nerve function was achieved by surgical axotomy, electrolytic lesioning or discrete application of a noxious chemical to neuronal nuclei. Starting in the 1950s with development of an antibody to 'nerve growth factor', it became possible to produce phenotypically selective destruction of nerves. Over the course of the next 60 years there has been a revolution in discovery and development of selective neurotoxins – capable of destroying specific neuronal phenotypes; or populations of neurons with unique types of receptors. Simultaneously, the definition of a 'selective' neurotoxin has blurred, as nonselective chemical agents are becoming termed as 'selective' neurotoxins. For example, the mitochondrial poison rotenone has become accepted as a viable means to model Parkinson's disease in rodents. N-methyl-D-aspartate receptor (NMDA-R) antagonists, regarded as neuroprotectants, become overly neurotoxic when administered in ontogeny. The dopamine D2-R agonist quinpirole, administered chronically, produces lifelong D2-R supersensitization – a neurotoxic phenomenon, without accompanying neuronal damage. This meaning of a 'selective' neurotoxin will continue to evolve according to whether it suitably models neurological disorders and/or achieves a desired end-point in studies delving into neuronal mechanisms that attend neurodegenerative or neuroprotective phenomena.

Chemical neurotoxins, historically, have been regarded as agents able to produce nerve damage or overt neurodegeneration. Starting with the era of „selective neurotoxins" in the 1950s, neurotoxins have taken multiple forms, acting by a variety of mechanisms: a) suppression of neurotrophins (anti-Nerve Growth Factor); b) production of intracellular reactive oxygen species (6-OHDA); c) formation of a toxic metabolite with specificity for mitochondrial complexes (MPTP → MPP+); d) impairment of neurotransmitter synthetic enzymes (DSP-4); e) inactivation of exocytosis (botulinum toxin); e) excitotoxin action at unique receptor types (kainate); e) evolution to a toxic species during continuous administration (cocaine); f) suicide inhibition of an intraneuronal enzyme (3-nitropropionate); inactivation of ribosomal protein (IgG-saporin); g) alkylation of the neuronal transporter site (ethylcholine aziridinium); h) desensitization of membranous receptors (capsaicin). The list is not exclusive, and it has the caveat that many neurotoxins act by multiple means. Surely, many other neurotoxins are yet to be discovered, and actions are destined to be at sites not yet known. Also, the term „selective neurotoxin" is entering a grey zone. Rotenone, a mitochondrial poison in any cell, is now given long-term to specifically model Parkinson's disease – an outcome marked by dopaminergic neuronal damage accompanied by alpha-synuclein deposits. N-

methyl-D-aspartate receptor (NMDA-R) antagonists, known neuroprotectants, become neurotoxic when administered during ontogeny. And the dopamine D2-R agonist quinpirole, when administered repeatedly, produces life-long D2-R supersensitivity – a neurotoxic outcome unaccompanied by any sign of overt neuronal damage. The character and definition of a selective neurotoxin is amorphous, and is likely to become more uncertain in the future.

O51 NEUROPROTECTIVE ABILITY OF PACAPS AGAINST OXIDATIVE STRESS AND EXCITOTOXICITY IN HUMAN PRIMARY CORTICAL NEURONS

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a member of the vasoactive intestinal polypeptide superfamily. PACAP is found in numerous brain areas and neuron types throughout the central and peripheral nervous system including hippocampus, substantia nigra, and amygdala. Both PACAP has been shown to be neuroprotective in several pathophysiological contexts. PACAP can act in both autocrine and paracrine manner through specific receptors such as PAC1 which is predominantly expressed in brain. Two biologically active PACAP isoforms of different length exist, PACAP1-38 and 6-38. Most of the *in vitro* studies have been performed using animal cells or human cell lines. In this study, we have tested the neuroprotective ability of both PACAP isoforms on primary cultures of human cortical neurons against oxidative stress (H2O2) and excitotoxicity (Quinolinic acid). We have also looked at the expression of PAC1 in human primary brain cells e.g. neurons, astrocytes, and microglial cells. Finally, we have examined the potential anti-inflammatory effects of PACAPs against activation of the kynurenine pathway in human primary monocytic cells. This first study with human primary brain cells will bring new insight in PACAPS neuroprotective and anti-inflammatory abilities.

O52 INFLAMMATION, GLYAPSE FORMATION AND INHIBITION OF MICROGLIAL MOTILITY IN PARKINSONISM

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In the context of neurodegeneration, where the phagocytic domain might be exacerbated, microglia could also eliminate neurons unnecessarily in a vicious circle, and create a chronic pathological environment.
Using MPTP intoxication as a model of Parkinsonism we analyzed dopaminergic cell death as well as the cellular polarization of microglia and the formation of gliapses (body-to-body neuron-glia contacts) where microglial motility was mediated by ROCK/Cdc42. Additional treatment with ROCK inhibitors (HA-1077, fasudil) sacrificing animals at three different time windows (24, 48 and 72 h after MPTP) allows us to observe a quantifiable and evident neuronal death after the administration of MPTP which was prevented by blocking ROCK. High-resolution confocal images demonstrate that microglia engulf entire neurons at one-to-one ratio, and the microglial cell body participates in the formation of the phagocytic cup, engulfing and eliminating neurons in areas of dopaminergic degeneration in adult mammals. The process of microglial polarization in dopaminergic neurodegeneration undergoes different steps in adult mice, beginning with the polarization of filopodial processes toward neurons (occurring at 24h after MPTP treatment), and followed by the polarization/migration of the microglial cellular body (occurring 48h after MPTP). The velocity of the approach of the microglial cell to the damaged dopaminergic neuron after MPTP administration suggests that microglial cells might have a specific time window, within 24h, to recognize the state of the neuron and ÒdecideÓ and resolve its final fate. Our data show that body-to-body contacts increased at 48h after MPTP and precede the neuron elimination observed at 72h, suggesting that the elimination of dopaminergic neurons requires prior gliapse formation followed via microglial phagocytosis. ROCK inhibition diminishes most microglial activation properties, such as the increased cell body size, number of filopodial processes and size of Golgi apparatus, after MPTP insult. Then, ROCK inhibitors might be a promising alternative for the treatment of some neurodegenerative diseases acting as disease-modifying drugs.