P01 PAC1 RECEPTOR INTERNALIZATION IS REQUIRED FOR ACTIVATION OF THE MEK/ERK INTRACELLULAR SIGNALING CASCADE IN PAC1 RECEPTOR EXPRESSING HEK 293 CELLS

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Prior studies indicate that internalization of the PACAP/PAC1 receptor complex and formation of a signaling endosome mediates multiple cellular functions following receptor activation (May et al, J Biol Chem 285: 9749, 2010; Merriam et al., J Neurosci: 33: 4614, 2013). Using HEK 293 cells stably expressing the GFP-tagged PAC1 receptor, we have currently examined whether PACAP activation of the MEK/ERK kinase signaling cascade was affected by environmental or pharmacological interventions that blunted PAC1 receptor internalization. Fluorescent imaging documented a PACAP-induced internalization of the PAC1 receptor at 37 C; however, PAC1 receptor internalization was suppressed at room temperature (~25 C), or by treatments with the small molecule clathrin inhibitor Pitstop 2 or the dynamin I/II inhibitor dynasore. Although none of these treatments inhibitedPACAP-induced increase in HEK PAC1 receptor cell cAMP production, PACAP-stimulated ERK phosphorylation was significantly decreased under pharmacological and temperature conditions that suppressed PAC1 receptor endocytosis. In the pharmacological/temperature paradigms, forskolin-stimulated activation of adenylyl cyclase in the HEK PAC1 receptor cells increased cellular cAMP levels comparable to those seen with PACAP, but failed to recapitulate the PACAP-induced ERK phosphorylation. Further, PACAP (25 nM) consistently initiated intracellular calcium transients from fura-2 measurements regardless of temperature conditions. These results suggest that the PAC1 receptor-stimulation of adenylyl cyclase and transient elevation of intracellular Ca^{2+} is mediated at the plasma membrane, whereas PACAP-induced activation of the MEK/ERK kinase pathway is PACAP/PAC1 receptor internalization dependent.

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P02 ACTIVATION OF ATP-SENSITIVE POTASSIUM (KATP) CHANNELS UNDERLIES VASODILATION TO PACAP, BUT NOT CGRP, IN PRESSURIZED RAT MIDDLE MENINGEAL ARTERY

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Migraine is a complex neurological disorder that often presents as an intense unilateral headache accompanied by nausea, photophobia and other neurological symptoms. Activation of the trigeminovascular system and/or the sphenopalatine ganglia involving the release of the neuropeptides pituitary adenylate cyclase activating polypeptide (PACAP) and calcitonin gene related peptide (CGRP) has been implicated in vasodilation of the middle meningeal artery (MMA) and the sensation of migraine headache. However, the mechanism by which these two peptides exert their vasodilatory effect on the MMA is unclear. Activation of distinct receptors for PACAP and CGRP have been linked to activation of adenylyl cyclase in vascular smooth muscle. Further, CGRP receptors have also been identified in vascular endothelial cells. Activation of cyclic AMP-dependent protein kinase has been shown to induce vasodilation via multiple mechanisms including phosphorylation and activation of smooth muscle KATP channels in a variety of vascular beds. In the present study our goal is to determine the role of K_{ATP} channels in vasodilation mediated via PACAP and CGRP in rat MMA. In isolated, pressurized MMAs both PACAP and CGRP induced significant vasodilation, although PACAP (EC_{50} ~ 1 pM) exhibited ~ 1,000-fold greater potency compared to CGRP (EC50 ~ 1 nM). PACAP-induced MMA dilation was abolished by the K_{ATP} channel inhibitor, glibenclamide (10 μM), but in marked contrast, glibenclamide had no apparent effects on CGRP-induced MMA dilation. The nitric oxide synthase inhibitor N-nitro-L-arginine (L-NNA) had no effects on either PACAP- or CGRP-mediated dilation. These observations demonstrate that PACAP dilates MMA via activation of vascular K_{ATP} channels, while CGRP acts through an alternative pathway. Thus, PACAP and CGRP may contribute to the etiology of migraine via two distinct mechanisms. Therapeutic approaches targeting a combination of both PACAP and CGRP may be more effective than targeting either of these peptides alone in alleviating migraine headache.

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P03 COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT PEPTIDE (CARTP): DISTRIBUTION AND FUNCTION IN RAT URINARY BLADDER

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CART is a biologically active peptide implicated in variety of physiological processes including sensory processing and autonomic regulation. We investigated the distribution of CARTp 55-102 in rat lower urinary tract (LUT) and evaluated CART-induced changes in nerve-evoked urinary bladder detrusor contractility *in vitro.* CARTp immunohistochemistry in LUT tissues and CART-induced changes in nerve-evoked urinary bladder detrusor contractility using vertical, isolated myography. CARTp 55-102 stained neuronal cell bodies, clusters of non-neuronal cells and nerve fibers in rat urinary bladder wall primarily in the region of the bladder neck and urethra. A dense plexus of CARTp-immunoreactive (IR) nerve fibers was detected also within ureters and small diameter blood vessels. The majority of CARTp-IR neuronal elements were also nNOS-IR (18.9%) while non-neuronal elements stained positively for TH (100%). CARTp significantly ($p \leq 0.05$) increased the amplitude of detrusor contractions elicited by low frequency field-stimulation (<15 Hz; $p \leq 0.001$) as well as the amplitude and frequency of spontaneous phasic urinary bladder smooth muscle contractions ($p \leq 0.05$). The responses to CARTp stimulation were dose-dependent and were increased by the presence of urothelium. Inhibitory effects of atropine on detrusor contractility were reduced in the presence of CARTp ($p \leq 0.001$). CARTp is highly expressed in rat LUT. The distribution of CARTp-immunoreactivity and colocalization with TH and nNOS suggests neurohumoral functions in rat LUT. CARTp increased the amplitude of detrusor contractions elicited by low frequency field-stimulation. CARTp exhibited an antagonizing action on atropine-induced reductions in detrusor contractility. These data suggest that CARTp may play role(s) in the control of parasympathetic outflow to LUT and detrusor contractility.
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P04 ANALYSIS OF PACAP SIGNALING-MEDIATED RECEPTOR INTERNALIZATION USING THE HALOTAG SYSTEM

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Pituitary adenylate cyclase-activating polypeptide (PACAP) plays a role as a neurotransmitter, neuromodulator, and neurotrophic factor. Previously, we demonstrated that PACAP-deficient (PACAP-/-) mice showed notable psychomotor abnormalities, most of which were reversed by the atypical antipsychotic risperidone and a selective serotonin (5-HT) 2A receptor antagonist, and that head-twitch and ear-scratch responses induced by the 5-HT2 agonist DOI were significantly increased in PACAP-/- mice. These findings suggest altered 5-HT2 receptor signaling in PACAP-/- mice and a functional interaction between these two receptor types. Since it has been shown that 5-HT2A receptor functions are affected by desensitization that involves receptor internalization, in this study, we have examined the effect of PACAP on 5-HT2A receptor internalization in HEK293T cells using the HaloTag system. As expected, PACAP induced internalization of PAC1 receptor. Interestingly, PACAP induced 5-HT2A receptor internalization in a dose-and time-dependent manner. In contrast, PACAP showed no effect on 5-HT1A receptor. VIP did not induce 5-HT2A receptor internalization, suggesting that PAC1 is involved in the PACAP-induced 5-HT2A receptor internalization. In addition, we observed that pretreatment with the protein kinase C (PKC) inhibitor sphingosine inhibited the 5-HT2A receptor internalization. These results suggest that PACAP/PAC1 receptor signaling regulates 5-HT2A receptor-mediated functions in a PKC-dependent manner. Although it is still unclear whether this heterologous receptor regulation is involved in psychomotor abnormalities observed in PACAP-/- mice, the underlying mechanism may be of importance to address the pathophysiology of psychiatric diseases.

P05 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE DERIVATIVES AS TOOLS TO DELIVER CARGOES INTO LIVING CELLS

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The ability of the Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) to cross mammalian cell plasma membrane in a receptor-independent manner was recently demonstrated (Doan et al, 2012). This prompted us to evaluate the possibility to use this peptide for the delivery of non-permeable biomolecules into the intracellular compartment. In fact, diverse cell penetrating peptides have already been characterized and used to deliver various cargoes including proteins and DNA. However, such delivery systems must be devoid of an intrinsic activity and unable to trigger an immunological response, drawbacks that have been observed with cell penetrating peptides derived from viruses. Thus, we first demonstrated the propensity of biotinylated forms of native PACAP peptides (PACAP38 and PACAP27) to efficiently deliver a large molecule, i.e. streptavidin, into living cells. Then, based on previous structure-activity relationship studies, we designed PACAP derivatives devoid of any affinity for the cognate receptors, i.e. PAC1, VPAC1 and VPAC2, while conserving the cell penetrating properties of the endogenous peptide. These new PACAP analogs were then evaluated as delivery vectors and proved their usefulness to deliver various cargoes including peptides, proteins and polynucleotides without affecting cell viability. The uptake mechanism was shown to involve direct translocation, caveolae-dependent endocytosis and macropinocytosis. Thus, this study demonstrated that inactive PACAP derivatives could represent new and interesting delivery vectors for *in vitro* as well as *in vivo* applications.

P06 POTENT REDUCED-SIZE ANALOGS OF PACAP

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The Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) exerts a large array of actions via the activation of three different G protein-coupled receptors, *i.e.* PAC1, VPAC1 and VPAC2. Among others, PACAP is a potent anti-apoptotic, anti-inflammatory and vasodilating agent, and these biological activities are mediated through PAC1, VPAC1 and VPAC2, respectively. In particular, by reducing apoptosis, PACAP exhibits potent neuroprotective effects in experimental models including Alzheimer's and Parkinson's diseases, as well as cerebral ischemia and brain injuries. Also, PACAP is able to down-modulate the inflammatory response, a phenomenon associated with many neurodegenerative diseases. Moreover, this peptide possesses the ability to cross the blood-brain barrier, a key property for CNS drug candidates. Therefore, this peptide represents an excellent molecular template for the development of a therapeutic strategy aiming at slowing and even stopping the neuronal death occurring during many brain diseases and injuries. Hence, the ideal compound must exhibit actions limited to PAC1 and VPAC1 receptors, while avoiding activation of the VPAC2 receptor, mostly involved in peripheral actions, such as vasodilation and water retention.
Previous structure-activity relationship studies showed that the biological activity and selectivity of PACAP is dependent on the molecular assembly adopted by the N-terminal segment of the molecule. This is well demonstrated by the PAC1/VPAC2 antagonism exhibited by the fragment PACAP(6-38) [Eur J Biochem 207:239 (1992)], as well as the PAC1/VPAC1 selectivity of the agonists [Ala7]PACAP27 and [Hyp^2]PACAP27 [Biochem Pharmacol 81:552 (2011)]. Therefore, using a binding assay and a calcium mobilization assay, with cell lines stably transfected with the PAC1, VPAC1 and VPAC2 receptors, respectively, we explored the activity and selectivity of PACAP N-terminal fragments and found that PACAP(1-23) retains high potency and affinity towards the PAC1 and VPAC1 receptors (EC_{50} ≈ 40 nM; IC_{50} ≈ 30 nM). Moreover, using the JC-1 dye, a mitochondrial membrane potential probe, we observed that the PACAP(1-23) protected human SH-SY5Y neuroblasts against MPP+, a dopaminergic neurotoxin, as well as glutamate, an excitotoxic compound. In summary, the PAC1/VPAC1 biological activity and specificity of PACAP(1-23) show that this peptide might be a useful template for the development of CNS drugs, intended for neurodegenerative diseases and brain injuries.

P07 PITUITARY ADENYLATE-CYCLASE ACTIVATING POLYPEPTIDE AND ITS ANALOGUES ACTIVATE THE SPECIFIC PAC1 AND VPAC1/VPAC2 RECEPTORS ON THE CELL BODIES OF PRIMARY SENSORY NEURONS AND TRANSFECTED CELL LINES

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Pituitary adenylate-cyclase activating polypeptide (PACAP) acts on G protein-coupled receptors: the specific PAC1 and VPAC1/VPAC2. PACAP6-38 was descibed as a potent PAC1/VPAC2 antagonist in several models. Maxadilan is a selective PAC1 agonist, while its fragment, MAXA65, is a specific antagonist. ^{Ala11,22,28}VIP is a selective VPAC1 agonist, while BAY 55-9837 is a selective VPAC2 agonist. We described previously that both PACAP1-38 and PACAP6-38 are able to decrease the electrical-field stimulation-induced release of the sensory neuropeptide CGRP from sensory nerve endings of the isolated rat trachea. PACAP6-38 did not behave as an antagonist. We aimed to analyse the actions of peptide fragments on sensory neural and cell line responses *in vitro.*

Ratiometric technique of $[Ca^{2+}]i$ measurement with the fluorescent indicator fura-2-AM on primary cultures of trigeminal ganglia neurons and PAC1, VPAC1 and VPAC2 receptor-expressing cell lines were performed.

Results on neurons: Slowly increasing $[Ca^{2+}]i$ was detected both after PACAP1-38 and PACAP6-38 administration. The PAC1 receptor agonist maxadilan, the PAC1 receptor antagonist MAXA65 and the VPAC2 receptor agonist BAY 55-9837 caused similar response. In contrast, the VPAC1 receptor agonist ^{Ala11,22,28}VIP had no significant effect on $[Ca^{2+}]i$.

Results on cell lines: Our data show that PACAP1-38 increased $[Ca^{2+}]i$ on PAC1, VPAC1 and VPAC2 receptor-expressing cell lines. PACAP6-38 had no similar effect on these cell lines. Maxadilan and MAXA65 increased $[Ca^{2+}]i$ on PAC1 receptor-expressing cell lines. The selective VPAC1 agonist ^{Ala11,22,28}VIP and the selective VPAC2 receptor agonist BAY55-9837 activated the VPAC1 and the VPAC2 receptor-expressing cell line, respectively.

Conclusion: We are able to test the PACAP receptor-selective agonists and antagonists by Ca-imaging. Some antagonist of PACAP receptors act as agonists on the sensory neurons, and cell lines. Presently unknown receptors or splice variants linked to distinct signal transduction pathways might explain these differences. The VPAC1 receptor does not play a role in these processes.

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P08 ENVIRONMENTAL ENRICHMENT CHANGES THE LEVELS OF PACAP IN THE CENTRAL NERVOUS SYSTEM

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic and multifunctional neuropeptide is widely distributed throughout the body. It is involved in the regulation of various physiological and pathophysiological processes. Numerous studies have shown that PACAP is involved in the development of the central nervous system, and has neuroprotective effects. Environmental enrichment has also demonstrated to be protective in various injuries. A few studies have suggested that trophic factors are involved in the protective mechanisms exerted by environmental enrichment. The interaction between PACAP levels in the brain and environmental effects has not yet been studied. The aim of the present study was to measure PACAP levels from different brain areas in rats and investigate whether environmental enrichment has any influence on PACAP levels.

Wistar rats were divided into two groups: control group and environmental enrichment group. PACAP27 and 38– like immunoreactivity was measured with a specific and sensitive radioimmunoassay in brain samples. Enriched environment has the most beneficial effects in newborn animals, and these rats have the highest rate of neuroplasticity, so we examined them first. The second part of the experiment was to investigate two groups of adult rats: newborn enriched and adulthood enriched animals, both group were examined in adulthood.

Environmental enrichment started at birth led to decreased levels of PACAP in several areas of the brain (brain stem, cerebellum and different areas of the telencephalon). When animals were kept in enriched environment after birth, then put back under regular circumstances and 3-6 months later checked for their PACAP levels, we found it also decreased. But when the rats were kept under regular circumstances and then in adult age we put them into environmental enrichment for a week, their samples showed higher PACAP levels.

Environmental enrichment causes changes in the PACAP levels of the central nervous system. The perinatal effect of environmental enrichment seems to decrease the level of PACAP, and it shows the same pattern in adulthood as well, but the only adult exposure to enrichment in adulthood leads to increases in PACAP immunoreactivity.

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P09 PACAP PROMOTES BOTH SURVIVAL AND NEURITOGENESIS IN PC12 CELLS THROUGH ACTIVATION OF NF-KB TRANSCRIPTION FACTOR

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The pituitary adenylate cyclase-activating polypeptide (PACAP) is a trophic factor that promotes survival and differentiation of neuronal cells. However, the signaling pathways and the transcriptional mechanisms involved in these processes are not completely elucidated. Our previous studies aimed at characterizing the transcriptome of PACAP-differentiated PC12 cells revealed an increase in the expression of nuclear factor kappa B 2 (NF-kB2) gene coding for p100/p52 subunit of NF-kB transcription factor composed of two subunits from RelA, RelB, c-Rel, p50 and p52 and involved in a wide range of processes including promotion of cell survival of different cells. In the present study, we sought to determine the role of the NF-kB pathway in neuronal differentiation promoted by PACAP. We first showed that PACAP-driven survival and neuritic extension in PC12 cells is inhibited following NF-kB pathway blockade. PACAP stimulated both c-Rel and p52 NF-kB subunit gene expression and nuclear translocation, while c-Rel down-regulation inhibited cell survival and neuritogenesis elicited by the neuropeptide. PACAP-induced c-Rel nuclear translocation was inhibited by ERK1/2 and Ca^{2+} blockers. Furthermore, the neuropeptide stimulated NF-kB p100 subunit processing into p52, indicative of activation of the NF-kB alternative pathway. Taken together, our data show that PACAP promotes both survival and neuritogenesis in PC12 cells by activating the NF-kB pathway, most likely via classical and alternative signaling cascades involving ERK1/2 kinases, Ca^{2+} and c-Rel/p52 dimers.

P10 PACAP AND TPA REGULATE INTERNEURON MIGRATION IN THE DEVELOPING CEREBELLUM

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During post-natal development of the cerebellum, granule neurons (GN) exhibit a centripetal migration to reach the internal granular layer (IGL) while basket/stellate cells (B/SC) migrate centrifugally to achieve their final position in the molecular layer (ML). Interneuron migration is a process which is orchestrated by a number of factors including neuropeptides and enzymes involved in the degradation of the extracellular matrix, but little is known regarding their combination and cortical-layers specificities. In particular, we have previously shown that pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates in vitro the expression and the release of a serine protease called tissue-type plasminogen activator (tPA) from rat GN (Raoult et al., 2011) but the role of PACAP-induced tPA secretion during interneuron migration in the cerebellum has not yet been considered. In the present study, we showed that exogenous PACAP reduces in vitro (microexplants from P2-P4 rats) and ex vivo (organotypic slices from P10 rats) by 70% the migration rate of GN but the inhibitory effect of endogenous PACAP is located in the Purkinje cell layer (PCL) where application of the PACAP antagonist PACAP6-38 increased by 23% the migration speed of GN. tPA, plasminogen activator inhibitor 1 (PAI-1) and plasminogen were devoid of direct effect on GN motility in vitro. Immunohistochemical labeling revealed moderate to intense tPA-like immunoreactivity in the ML, PCL and IGL suggesting multiple sources of endogenous tPA. PAI-1 reduced GN migration in the ML and the PCL by 70% and 27% respectively. In the ML, slow (9.6±0.4 μm/h) and fast (17±0.5 μm/h) B/SC were identified during radial migration. PAI-1 inhibited migration of fast cells while PACAP had no effect on any B/SC. These results suggest that endogenous tPA facilitates, independently of PACAP, the migration of GN and fast B/SC in the ML by degradating the extracellular matrix of the cerebellar cortex. In contrast, endogenous PACAP exerts a direct inhibitory effect which is restricted to GN migration at the level of the PCL. Migration resumption of GN and crossing the border between the PCL and the IGL could be attributed to PACAP-induced tPA release.

P11 INTERACTION BETWEEN THE VIP-RECEPTOR SYSTEM AND THE HEDGEHOG PATHWAY IN THE REGULATION OF GLIOBLASTOMA MIGRATION AND INVASION

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Background: Vasoactive intestinal peptide (VIP) and PACAP (pituitary adenylyl cyclase-activating peptide), are regulatory factors in the central and peripheral nervous systems. They also modulate numerous functions in cancer cells. Recent studies demonstrate that PACAP inhibits proliferation of medulloblastoma cell lines in a PKA-dependent manner, 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). Previous data from our group demonstrate that expression of VIP and VIP receptors (the VIP-receptor system) are associated with a decreased migration in glioblastoma (GBM) cells (Cochaud *et al.,* Neuropeptides, 2010). However, little is known about the mechanisms linking the VIP-receptor system and the Hh pathway in GBM migration.
Results: We found that VIP, PACAP38 and a synthetic antagonist, VIP_{10-28} did not affect proliferation but controlled cell migration and invasion in two GBM cell lines, U87 and C6. VIP and PACAP38 also inhibited invasion of rat C6 GBM cells in rat brain slices cultured *ex vivo.* On the contrary, the VIP receptors antagonist VIP_{10-28} significantly stimulated C6 GBM migration and invasion, a process which was PKA-, Akt- and Hh-dependent. In our studies to elucidate the mechanisms of the contribution of VIP and PACAP38 to the malignant behavior of GBM cells, we found that VIP and PACAP38 strongly inhibited expression of the Gli1 protein in U87 and C6 cells which express the components of the Hh pathway. Accordingly, we also observed that VIP_{10-28} increased the expression of Gli1 in C6 cells. Finally, we found that VIP and PACAP38 down-regulated Akt phosphorylation in C6 cells, in agreement with the role of this signalling kinase in the regulation of migration and Gli1 activity.
Conclusion: Taken together, our observations indicate that the VIP-receptor system regulates invasion via possible crossed interactions between the Hh and the Akt/PTEN pathway in GBM cells.

P12 CYTOKINE MEASUREMENTS AFTER PACAP-38 CONTAINING INTESTINAL TRANSPLANTATION

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Preservation graft injury is a phenomenon associated with each organ transplant procedure. Many preservation solutions were developed along with research efforts to find appropriate solution for small intestine storage. Pituitary adenylate cyclase-activating polypeptide (PACAP) plays a central role in intestinal cytoprotection. The aim of present study was to measure intestinal PACAP-38 concentration during small bowel cold preservation procedure.
Cold ischemia was produced with small bowel preservation in University of Wisconsin (UW) solution at 4°C for 1 hour (GI), for 3 hours (GII), and for 6 hours (GIII) in Wistar rats (n=5 in each group). One hundred ug PACAP-38 was added to 30 ml UW solution and grafts were storaged for 1 hour (GIV), for 3 hours (GV), and for 6 hours (GVI). Small bowel biopsies were collected after laparotomy (control) and at the end of the ischemia periods. To measure cytokines from tissue homogenates, we used rat cytokine array and Luminex Multiplex Immunoassay.
Cytokine array revealed that expression of the soluble intercellular adhesion molecule-1 (CD54) and L-selectin (CD62L/LECAM-1) was increased in GIII. Both 6 h cold storage in PACAP-38-containing UW solution and 3 h reperfusion caused strong reduction in these cytokines activation in GVI. RANTES (CCL5) levels were increased in all groups. Strong activation of the tissue inhibitor of metalloproteinase-1 was in GIII. However, PACAP-38-containing cold storage could decrease its activation in GVI. Furthermore, strong activation of the tissue inhibitor of metalloproteinase-1 was detected in 6 h preserved grafts without PACAP-38 (GIII). PACAP-38-containing cold storage could decrease its activation in GVI.
Our present study showed that PACAP-38 could attenuate tissue cold ischemic injury-induced changes in cytokine expression. (This work was supported by PTE-MTA „Lendulet" Program, Arimura Foundation, OTKA K104984, TAMOP 4.2.2.A-11/1/KONV-2012-0024, Bolyai Scholarship.)

P13 EXAMINATION OF PACAP-LIKE IMMUNOREACTIVITY IN DIFFERENT PATHOLOGICAL CLINICAL SAMPLES

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Recent trends in PACAP research point to the clinical introduction of PACAP or its analogs/fragments possibly in the near future. Therefore, we aim to examine the relation between PACAP level of different human tissue samples and different disorders (tumors, heart disorders, neurological and metabolic diseases). Earlier we found significantly lower level of PACAP38 and PACAP27-like immunoreactivity (LI) in tumor samples compared to normal healthy tissue in both lung and colon cancers, most probably due to the degeneration of the PACAP containing nerve fibers in the tumor. We also showed that PACAP38 and PACAP27-LI are significantly higher in cardiac samples from ischemic heart diseases compared to valvular abnormalities. In the present study we investigated the PACAP38 and PACAP27-LI from human blood and tissue samples of patients with radioimmunoassay examination. We collected tissue samples from different urological disorders (kidney tumor, urinary bladder tumor and prostatic hypertrophy) and breast cancer and we collected blood samples from patients with diabetes, sleep apnea syndrome and ischemic cardiac diseases. We found significantly higher PACAP38 and PACAP27-LI in breast tumor samples compared to normal mammalian tissue samples. Similarly to our earlier results in kidney tumor samples we found significantly lower amount of PACAP38-LI compared with healthy tissue samples. We did not find significant alterations in the PACAP38 and PACAP27-LI between healthy and tumoral urinary bladder and prostate samples. In all of the samples PACAP27-LI were significantly lower compared to PACAP38-LI. Our result showed inverse relationship between the severity of sleep apnea syndrome and PACAP38-LI. We also found negative correlation between PACAP38-LI and proBNP level in patients with ischemic cardiac disorders. ProBNP level in the blood is used for screening, diagnosis of acute congestive heart failure (CHF) and may be useful to establish prognosis in heart failure, because this marker is typically higher in patients with worse outcome. We found higher PACAP38-LI in patients who had lower proBNP-level in the blood indicating better prognosis. PACAP38-LI were markedly higher in two diabetic patients: in both cases patients had long-time type II diabetes with chronic heart and kidney complications. Our results showed significant correlations with PACAP-LI in the human samples and severity of disorders, but further investigations are necessary to describe the exact function of PACAP in different pathological conditions.

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P14 PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE-LIKE IMMUNOREACTIVITY IN HUMAN DENTAL PULP SAMPLES

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic and multifunctional neuropeptide widely distributed throughout the body. Besides its neurotrophic and neuroprotective effect, it is also involved in the regulation of various physiological and pathophysiological processes, and studies have also demonstrated anti-inflammatory and anti-apoptotic functions. PACAP-immunoreactive fibers were found in the odontoblastic and subodontoblastic layers of the dental pulp, but there is no data about its role in pathological changes. The aim of our study was to examine PACAP38-like immunoreactivity (LI) in various pathological conditions of the pulp. The samples were collected from 60 patients (28 female, 32 male). The samples were divided into groups according to the diagnosis of the pulp (healthy, acute irreversible pulpitis, chronic irreversible pulpitis and gangraena) upon clinical examination and radiographic findings. The samples were also separated on the basis of their locations (front, premolar and molar teeth). PACAP38-LI was measured with specific and sensitive radioimmunoassay. Our data revealed significant difference between chronic and acute irreversible pulpitis in the case of premolars. The difference between premolars and molars was also significant in chronic pulpitis. Our results demonstrated that PACAP38-LI increases in chronic inflammation suggesting that it may play an important role in the inflammatory reactions of the pulp. Further molecular and immunohistochemical examinations are needed to understand the exact effect of PACAP in different disorders of the dental pulp.

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P15 EXAMINATION OF THE ROLE OF ENDOGENOUS PACAP IN DIABETIC NEPHROPATHY

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Hypophysis adenylate cyclase activating polypeptide (PACAP) is a neuropeptide exerting cell protecting effects by inhibiting several apoptotic and inflammatory processes. All its three receptors (PAC1, VPAC1/2) are expressed in the kidney. Previous studies proved, that the extent of damage - for example caused by ischemia/reperfusion – is significantly greater in PACAP knockout mice, than in their wild type mates. The aim of the present study was to investigate the role of endogenous PACAP in diabetic nephropathy.

Mice were randomly divided into 4 groups: intact or diabetic PACAP+/+ and intact or diabetic PACAP-/-. Diabetes was induced by a single intraperitoneal injection of streptozotocin (200mg/kg). After 10 weeks survival, histological analysis was carried out on the kidneys. Alterations in the expression of cytokines and angiogenetic factors, which have a remarkable role in the pathogenesis of diabetic nephropathy, were examined by semiquantitive cytokine and angiogenesis array. Western blot analysis was performed to measure the level of the pro- and antiapoptotic factors.

Histological analysis showed changes typical to diabetic nephropathy in kidneys of both PACAP knockout and wild type diabetic animals, however, lesions were significantly more severe in PACAP knockout mice. Increased expression of several cytokines (RANTES, TIMP-1, MCP-1) was observed already in intact knockout kidneys, while others were decreased or remained stable. Diabetes induced the expression of almost all the cytokines, which was further increased in the PACAP knockout animals (IFNγ, TNFα, interleukins). Levels of angiogenetic factors were markedly elevated in diabetic PACAP+/+ animals, while changes observed in diabetic PACAP knockout mice also proves a more progressed disease. Western blot analysis confirmed our previous results. The present study revealed more advanced histological changes, increased expression of proinflammatory cytokines and enhanced apoptosis in PACAP-/- mice compared to the wild type animals. This raises the possible renoprotective role of PACAP in diabetic nephropathy.

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P16 KANAMYCIN INDUCED CHANGES IN CA^{2+} BINDING PROTEIN EXPRESSION IN THE INNER EAR OF WILD TYPE, HETERO-ZYGOUS AND HOMOZYGOUS PACAP-DEFICIENT MICE

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Recently, we have shown that PACAP protects cochlear cells against oxidative stress-induced apoptosis in vitro and PACAP-deficient animals show significantly higher expression of Ca^{2+} binding protein (parvalbumin, calretinin and calbindin) in the hair cells of the inner ear, but there are no data about the consequences of the lack of endogenous PACAP in different ototoxic insults such as aminoglycoside-induced toxicity.
In this study we examined the effect of a single dose of ototoxic kanamycin treatment (1mg/g) on Ca^{2+} binding protein expression in hair cells of wild type, heterozygous (+/-) and homozygous PACAP-deficient(-/-) mice. We treated 5-day-old mice with kanamycin subcutaneously and 2 days later we examined the Ca^{2+} binding protein (parvalbumin, calretinin) expression of the hair cells with immunohistochemistry. Control animals received physiological saline.
We found that the inner and outer hair cells of control homozygous PACAP-deficient mice and outer hair cells of heterozygous PACAP-deficient mice showed more pronounced parvalbumin and calretinin immunopositivity compared to control wild-type mice. Elevated endolymphatic Ca^{2+} is deleterious for the cochlear function, against which the high concentration of Ca^{2+} buffers in hair cells may protect. Meanwhile, the increased immunoreactivity of Ca^{2+} binding proteins in the absence of PACAP provide further evidence for the important protective role of PACAP in hair cells in pathological conditions. Kanamycin induced a significant elevation in Ca^{2+} binding protein expression in hair cells of wild-type and heterozygous PACAP-deficient mice, but the baseline higher expression in homozygous PACAP-deficient mice did not change significantly after the treatment. Our results showed significant differences in the inner ear of wild type, heterozygous and homozygous PACAP-deficient mice after kanamycin treatment, which indicate the important role of PACAP in ototoxicity, but further investigations are necessary to examine the exact role of endogenous PACAP in ototoxic insults.
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P17 ANGIOGENIC FACTORS ARE INFLUENCED UPON HYPEROSMOTIC STRESS BY PACAP IN RETINAL PIGMENT EPITHELIAL CELLS

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In the retina, the integrity of the pigment epithelial cells is critical for the photoreceptor survival and vision. PACAP (pituitary adenylate cyclase activating polypeptide) is known to exert retinoprotective effects, against several types of retinal injuries in vivo, including optic nerve transection, retinal ischemia, excitotoxic injuries, UV-A-induced lesion and diabetic retinopathy. In our previous studies we proved that PACAP activates antiapoptotic pathways and inhibits proapoptotic signaling in retinal lesions in vivo. In a recent study we have proven that PACAP is also protective in oxidative stress-induced injury in human pigment epithelial cells (ARPE cells). Expression of apoptotic and angiogenetic markers was investigated by specific arrays, while the MAP kinases and Akt was studied by Western blot analysis. With angiogenesis array we showed that oxidative stress induced the activation of pro-angiogenic factors like thrombospondin, endothelin and VEGF, while PACAP treatment could decrease most of them.
Our present study shows the influence of PACAP on ARPE cells exposed to hyperosmotic stress. After 100 mM NaCl or 200 mM sucrose treatment we investigated the change of cytokine and angiogenic factor levels with flow cytometry. Our results were in accordance with our former findings, that PACAP could decrease the pro-angiogenetic factors which were elevated upon hyperosmotic stress induce by either NaCl or sucrose treatment. These mechanisms may have clinical importance in several retinopathies.
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P18 INVESTIGATING THE RETINOPROTECTIVE EFFECTS OF PACAP FRAGMENTS IN ISCHEMIC RETINOPATHY

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Pituitary adenylate cyclase activating polypeptide (PACAP) has neuroprotective effects in different neuronal and retinal injuries. Retinal ischemia can be effectively modelled by permanent bilateral common carotid artery occlusion (BCCAO), which causes chronic hypoperfusion-induced degeneration in the entire rat retina. The retinoprotective effect of PACAP38 and VIP is well-established in ischemic retinopathy. However, little is known about the effects of related peptides and PACAP fragments in ischemic retinopathy. The aim of the present study was to investigate the potential retinoprotective effects of different PACAP fragments (PACAP 4-22, 6-15, 11-15, 20-31) and related peptides (secretin, glucagon) in BCCAO-induced ischaemic retinopathy.
Wistar rats (3-4 months old) were used in the experiment. After performing BCCAO, the right eyes of the animals were treated with PACAP fragments or related peptides intravitreal (100 pM), while the left eyes were injected with saline serving as control eyes. Sham-operated (without BCCAO) rats received the same treatment. Routine histology was performed 2 weeks after the surgery, cells were counted and the thickness of retinal layers were compared. Our results did not reveal retinoprotective effect of the PACAP fragments or related peptides. These results suggest that PACAP 1-38 has the greatest effectiveness in the animal model of ischemic reinopathy.
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P19 EFFECT OF SECRETIN SUPERFAMILY PEPTIDES ON THE VIABILITY OF Y79 RETINOBLASTOMA CELLS

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Introduction: Retinoblastoma is a tumor occurring mainly in children, with the highest prevalence between 0-4 years of age. It is mostly composed of undifferentiated anaplastic cells that arise from the retina, and shows similarities in histology with neuroblastoma and medulloblastoma. Standard treatment strategies of retinoblastoma include: enucleation, radiation, cryotherapy, thermotherapy, and chemotherapy. Although systemic chemotherapy is proven to be effective, the addition of local treatment can improve the outcome and reduce side effects. In this study we examined effects of secretin, VIP, PACAP38, PACAP27, PACAP6-38, maxadilan (high affinity PAC1R agonist), on the viability and proliferation of Y79 retinoblastoma cell line.
Materials and methods: Cell viability and mitochondrial function were measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction to formazan by mitochondrial dehydrogenases following 24, 48 and 72 h incubation with the peptides. Effects of PACAPs on cell viability were measured after incubation with the peptide alone or in combination with signal transduction inhibitors such as: H89 - PKA, GF 109203X - PKC, U0126 - MEK1/2, SB 203580 and SKF-86002 - p38 Kinase and SP600125 - JNK. Cell proliferation was assessed by measuring bromodeoxyuridine (BrdU) incorporation into the newly synthesized DNA strand in propagating cells using BrdU detection ELISA kit. Furthermore, using qPCR we investigated the expression of genes for PAC1R (ADCYAP1R1) and Dipeptidyl Peptidase 4 (DPP4), an enzyme responsible for degradation of PACAP, in Y79 retinoblastoma cell line.
Results: We found that PACAP38 and PACAP6-38 (1-5 μM) decreased cell viability and proliferation of Y79 cells in a concentration-dependent manner. Maxadilan itself did not affect cell viability, but enhanced the effect of PACAP. The cytotoxic effect of both form of the peptide on Y79 cells was not abolished by any of the used signal transduction inhibitors. Secretin (0.1-3 μM), VIP (0.1-3 μM), and PACAP27 (0.1-3 μM), did not alter Y79 cell viability or proliferation. Expression of both ADCYAP1R1 and DPP4 genes in Y79 cells was confirmed.
Conclusion: PACAPs exhibit cytotoxic effect on Y79 cells. Although we confirmed the presence of PAC1 receptor in Y79 cells, the fact that both agonist and antagonist of PAC1 exert similar actions suggest the interaction with splice variants of PAC1 receptor in Y79 cells or the peptides' action on another, non PAC1, receptor. As none of the signal transduction inhibitors abolished the cytotoxic effect of PACAP we hypothesize a possibility of a non-specific mechanism of the peptides' action in Y79 cells. Furthermore, an expression of DPP4 gene in Y79 cell line may suggest that PACAP38 and PACAP6-38 are degraded to shorter, active form of peptide.
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P20 THE PACAP-REGULATED GENE SELENOPROTEIN T IS INVOLVED IN THE PROTECTION OF CATECHOLAMINERGIC NEURONS AND ITS ABSENCE LEADS TO A PARKINSONISM-LIKE PHENOTYPE

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The neuropeptide PACAP exerts neurotrophic activities by regulating the expression of various genes and pathways in a coordinated manner. Transcriptomic analyses of the neurotrophic effects of PACAP in PC12 cells revealed the up-regulation of a new member of the selenoprotein family, selenoprotein T (SelT). These proteins are widely involved in the control of redox homeostasis. Our initial studies showed that SelT is strongly expressed in the nervous system during development and following neuronal injury. In order to determine the role of SelT in catecholaminergic neurons in vivo, we used the neurotoxin 1 methyl-4-phenyl 1, 2, 3, 6 tetrahydropyridine (MPTP) that we applied to wild-type (WT) and brain-specific SelT knockout (KO) mice to induce neurodegeneration. Treatment with MPTP induced a strong increase in SelT gene expression in the nigrostriatal pathway of WT mice. Remarkably, treatment of mutant mice with MPTP led to a Parkinsonism like-phenotype with a marked dyskinesia, tremors, etc.., culminating at animal death within few hours. Analysis of the substantia nigra compacta revealed an accumulation of reactive oxygen species in catecholaminergic neurons of KO mice in comparison to WT animals, suggesting that SelT could be involved in the protection of catecholaminergic neurons against oxidative stress. To test this hypothesis, we used the neuroblastoma SY5Y cells in culture. Treatment of SY5Y with 1-methyl-4-phenylpyridinium (MPP+) triggered a strong increase in SelT gene expression as well as protein concentration as assessed by quantitative PCR and IHC or western blotting, respectively. Finally, overexpression of SelT, but not a mutant form, promoted neuronal cell survival after MPP+ treatment. This effect was associated with a reduction in the intracellular levels of ROS in the presence of SelT but not its mutant form. Taken together, these data revealed for the first time that the PACAP-regulated SelT plays a pivotal role in catecholaminergic neuron protection, and that its deficiency is associated with high oxidative stress in these neurons leading to a marked Parkinsonism-like phenotype.

P21 NEUROPROTECTIVE EFFECT OF ENDOGENOUS PACAP IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Introduction: Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional neuropeptide, highly abundant in the central and peripheral nervous system. Numerous experiments have shown that PACAP has neurotrophic and neuroprotective effects both in vivo and in vitro studies. We have previously demonstrated that exogenous PACAP ameliorates the behavioral impairments and enhances dopaminergic cell survival after unilateral 6-hydoxydomanine(6-OHDA)-induced lesion of substantia nigra, a rat model of Parkinson's disease. We have also proven earlier that PACAP deficient mice have higher vulnerability in a number of pathological conditions. The aim of the present study was to examine the effect of endogenous PACAP in a mouse model of Parkinson's disease.
Methods: Wild type and PACAP-deficient mice were treated with unilateral injections of 6-OHDA (5 µg/ 1 µl) into the substantia nigra, control animals received 1 µl physiological saline. Behavioral experiments were done preinjury, 1 and 14 days after the operation evaluating hypokinetic and asymmetrical symptoms of the animals. Tyrosine-hydoxylase immunohistochemistry was performed after the behavioral testing to label dopaminergic cells of the substantia nigra.
Results: We observed that PACAP-deficient mice showed more severe hypokinetic symptoms and asymmetrical turning behavior 1 day after the injury compared to wild type and control animals. We found severe dopaminergic cell loss in the substantia nigra in wild type animals, while in PACAP-deficient mice the cell loss was significantly higher.
Conclusion: Our experiments provided evidence for the protective effect of endogenous PACAP, because PACAP-deficient mice showed more severe acute neurological signs and dopaminergic cell loss after 6-OHDA lesion of the substantia nigra compared to wild-type animals.
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P22 CRITICAL ROLE OF ASTROCYTES ON STRIATAL NEUROCHEMISTRY IN VIP TREATED PARKINSONIAN RATS

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Astrocytes detected by glial fibrillary acidic protein (GFAP) immunoreactivity, plays critical and integral roles in mediating physiological and pathological states of neurons. Dual roles of astrocytes depend largely on the molecules that they release into and take up from the extracellular space. Vasoactive intestinal peptide (VIP) has neuroprotective and neurotropic actions, and it modulates a number of astrocyte activities. VIP has been found to be protective in several experimental parkinson models. This study investigated the effects of VIP on motor deficits, striatal GSH, GABA, Glutamate (GLU) and the expression of GFAP in 6-OHDA lesioned rats. Adult Sprague-Dawley rats were separated into sham operated, unilaterally 6-OHDA lesioned and lesioned + i.p. VIP-injected (25 ng/kg) groups. VIP was first injected 1 h after the intrastriatal 6-OHDA microinjection and then every 2 days for 15 days. Extracellular striatal concentration of GSH, GABA and GLU were measured in microdialysate by HPLC. Density measurements of GFAP expressing astrocytes were determined by using Image J analysis program. Our results demonstrated that 6-OHDA microinjection significantly increased astrocytic density in the striatum compared to the sham operated groups. VIP treatment reduced the density of astrocytes, although density measurements were not significantly different than those of sham operated and Parkinsonian groups. Microinjection of 6-OHDA did not change the extracellular concentration of GABA and GLU, but significantly increased GSH levels in the striatum. VIP treatment significantly reduced GSH levels, comparable to those of sham operated groups. On the other hand, VIP treatment significantly increased extracellular concentration of GABA and GLU in the striatum. These results suggest that astrogliosis was accompanied by the modulation of GSH, GABA and GLU levels in the striatum of both parkinsonian and VIP treated rats.

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P23 DELAYED AND LOCAL PACAP DELIVERY IMPROVES FUNCTIONAL RECOVERY AFTER BRAIN STROKE BY PROMOTING MI2 POLARIZED MICROGLIA

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The neuroprotective effect of the neuropeptide Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) has been demonstrated in several brain stroke models. Numerous studies show that PACAP administration before or within few hours after stroke onset protects against ischemic lesions mainly through its anti-apoptotic property. However, the effects of a delayed delivery of the neuropeptide in brain stroke have not been documented yet. As actually the therapeutic window accessible for stroke treatment only targets the first few hours after artery occlusion, we evaluated the potential effects of a delayed delivery of PACAP, 72 h after permanent Middle Cerebral Artery occlusion (pMCAo). To circumvent the limitations associated with systemic PACAP administration due to its poor plasmatic stability and low ability to reach a territory where vascularization is compromised, we generated a PACAP-expressing ES cell line (ES-P cells), which was used as a vehicle to provide sustained local delivery of the neuropeptide after transplantation.

Our results show that the i.c.v injection of ES-P-cells, 3 days after stroke, improves functional recovery in mice 1 and 2 weeks post-ischemia. This observation correlates with a modulation of the local inflammatory response. Transcriptomic analysis showed a decreased expression of pro-inflammatory related genes (TNF-α, TLR4, IL6R, IL17Rb...) associated with a significant increase of the expression of genes involved in the resolution of inflammation (Chi3l3, IL-10, TGF-β1, TGF-βR1...). A bioinformatic study of the transcriptomic signature through IPA software revealed that the delayed and local PACAP delivery in ischemic brains modulates microglia/macrophages functions related to phagocytic activity, chemotaxis and differentiation. Moreover, IPA analysis indicated that the effects of PACAP on these cellular processes could rely on the regulation of the Notch-RBPJ and NF-κB transcription factors, involved in microglial phenotype response. Indeed, morphometric and phenotypic in situ analyses of microglial cells confirmed that local and delayed PACAP delivery after stroke promotes Mi2 polarization of microglia, as indicated by a high number of Arg-1+ cells.

Taken together, our results showed that the local delivery of PACAP in the vicinity of the infarct zone starting 3 days after brain stroke is still able to improve functional recovery by interfering with the activation and differentiation processes of microglial cells, skewing their response from an inflammatory Mi1 phenotype toward a neuroprotective Mi2 phenotype, consequently damping the inflammatory response.

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P24 PACAP AND NGF INCREASE SERPINB1A EXPRESSION THROUGH THE CALCINEURIN AND MAP-KINASE PATHWAYS TO PROTECT PC12 CELLS FROM APOPTOSIS

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PC12 cells are used to study the signaling mechanisms underlying the neurotrophic and neuroprotective activities of pituitary adenylate cyclase-activating polypeptide (PACAP) and nerve growth factor (NGF). Previous microarray experiments indicated that serpinb1a was the most induced gene after 6 h of treatment with PACAP or NGF. The present study confirmed that serpinb1a is strongly activated by PACAP and NGF in a time-dependent manner with a maximum induction (~50-fold over control) observed after 6 h of treatment. Co-incubation with PACAP and NGF resulted in a synergistic up-regulation of serpinb1a expression (200-fold over control), suggesting that PACAP and NGF act through complementary mechanisms. Consistently, PACAP-induced serpinb1a expression was not blocked by TrkA receptor inhibition. Nevertheless, the stimulation of serpinb1a expression by PACAP and NGF was significantly reduced in the presence of ERK, calcineurin, PKA, p38 and PI3K inhibitors, and totally blocked in the presence of both calcineurin and ERK inhibitors, indicating that the two trophic factors share some common pathways in the regulation of serpinb1a. Finally, functional investigations conducted with siRNA revealed that serpinb1a is not involved in the effects of PACAP and NGF on PC12 cell differentiation but mediates their ability to block caspase-3/7 activity and to promote PC12 cell survival.

Keywords: caspase, cell death, cell survival, nerve growth factor, neuroprotection, pituitary adenylate cyclase-activating polypeptide, siRNA.

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P25 PROTEOMICS OF THE PACAP38 INFLUENCED ISCHEMIC BRAIN IN PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION MODEL MICE

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Purpose: PACAP has pleiotropic functions in damaged central nervous system such as neuroprotection, axonal guidance, and glial activation, but the mechanism behind such action remain undefined. In this study, to reveal the mechanisms, proteomics analysis was performed in ischemic mouse brain with or without PACAP injection.
Methods: Effect of PACAP38 (1 μL containing 1 pmol) injection intracerebroventrically in a mouse model of permanent middle cerebral artery occlusion (PMCAO) was investigated along with corresponding SHAM control (0.9% saline injection). Proteomics was used to identify differentially regulated proteins by PACAP38 under ischemic condition. Ischemic and non-ischemic brain tissues were sampled at 6 and 24 hours post-treatment. Following confirmation of ischemia by behavioural analyses and TTC staining, the proteome-wide changes were examined using two-dimensional gel electrophoresis (2-DGE) in conjunction with matrix assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS), and further by Western blotting and immunofluorescent staining.
Results: 2-DGE analysis in conjunction with Flamingo staining clearly revealed newly appeared spots in the ischemic hemisphere at 6 h, in both PACAP38 and saline-treated ischemic brain. This spot was also observed in PACAP38 treated ischemic brain over SHAM PACAP sample. The same spot was also observed at a much stronger intensity in ischemic hemisphere at 24 h but not in SHAM saline sample. The induced spots were not found in the PACAP38 treatment brain over SHAM PACAP sample. MALDI-TOF-MS analysis revealed a highly expressed protein spot in ischemic hemisphere that was identified as dihydropyrimidinase-related protein 2, also known as collapsin response mediator protein (CRMP2) - a marker for axonal growth and nerve development. Western blot analysis revealed a 56 kDa cross-reacting protein band in PMCAO samples only. At 6 h post-ischemia, the 56 kDa protein was increased in abundance over the minus-PACAP sample. At 24 h post-PACAP treatment the 56 kDa protein band was found at very low levels. Immunofluorescent staining using anti-CRMP2 antibody revealed CRMP2 protein is localized to cytoplasm in neuronal cells as seen in healthy region of the ipsilateral hemisphere. In penumbra, CRMP2 protein appears to be more abundant in the 6 h PACAP group. In core region, CRMP2 protein is reduced in abundance, in particular under PACAP38 treatment, especially prominent by almost no presence of CRMP2 at 24 h after PACAP treatment to the ischemic brain. PACAP treatment slightly increased its abundance by 2-DGE and immunostaining at 6 h but not at 24 h in ischemic hemisphere, suggesting PACAP activates neuronal CRMP-2 related mechanism early on.
Conclusions: Results showed the usefulness of omics approaches in screening of potential targets of PACAP-regulated proteins. CRMP2 might be a key factor for neuronal function of PACAP after ischemia.

P26 PACAP STIMULATES FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY THROUGH AXONAL REGENERATION

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Background: Spinal cord injury in human being may cause of loss of motor function and severe reduction in quality of life. Recently, spinal cord decompression and spinal vertebral fixation are adopted as main reliable operative treatments after spinal cord injury and rehabilitation is vital for maintaining rest of motor functions after injury. However, even steroid pulse therapy in early hours following spinal cord injury is the common treatment for patients, its reliability is still controversial. It is known that pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuroprotective peptide expressed central nervous system and also has a neurogenesis effect. However, it is still unclear how PACAP rescue the spinal cord injury, and beneficial treatment of PACAP on injured spinal cord should be developed. Therefore, purpose of this study is to clarify the effect of PACAP administration with sustainable release hydrogel for spinal cord injury.
Method and materials: Male C57BL/6-J wild type mice were used and received moderate contusion on exposed spinal cord at the level of seventh thoracic vertebrae using a Benchmark stereotaxic impactor. Immediately after spinal cord injury, from 10-8 M to 10-12 M of PACAP or saline were administrated directly attached on the injured lesion using a hydrogel which has a sustainable releasing effect. Functional recovery of hindlimb was evaluated using Basso Mouse Scale at 0, 3, 7 and 14 days after injury. The injury volume of spinal cord was analyzed using GFAP immunostaining and gene analysis was carried using Real-Time polymerase chain reaction (PCR) methods. Tetramethylrhodamine-conjugated dextrans (Molecular Probes.) was used as an anterograde tracer to examine the extent of regeneration of lesioned lateral white matter tract axons. The tracer was injected a day after spinal cord injury from the level of cervical spinal cord bilaterally.
Result: In functional recovery of hindlimb, there was a significant difference in 10-12 M of PACAP treatment group comparing to saline control group in 14 days after spinal cord injury. However, the difference of injury volume using GFAP immunostaining was not significant. The gene analysis using Real-time PCR revealed that mRNA of collapsin response mediator protein-2 (CRMP-2), which is one of the factors related to axonal regeneration, was significantly increased in PACAP-treated group in 14 days after injury comparing to saline control group. CRMP-2 immunoreactivities were overlapped with with NeuN, a marker of neuronal cell, and CNPase, a marker of oligodendrocyte, immunoreactivities in spinal cord. The result of antegrade spinal cord tracing showed that much number of neuronal fibers was detected around injured lesion in PACAP treatment group compared with saline control group.
Conclusion: This result suggested that PACAP may have an effect for functional recovery after spinal cord injury through axonal regeneration.

P27 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE PROTECTS ASTROGLIAL CELLS AGAINST OXIDATIVE STRESS-INDUCED APOPTOSIS

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Oxidative stress, resulting from a massive production of reactive oxygen species (ROS), is involved in several neurodegenerative disorders. The reactive astrocytes can exert neuroprotective effects by producing anti-inflammatory cytokines and anti-oxidative factors such as glutathione. Astrocytes in culture express functional receptors for Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) and several studies indicate that besides its direct antiapoptotic activity on neurons, PACAP could also exert an indirect neuroprotective action via the activation of astrocytes. Thus, the purpose of the present study was to investigate the potential glioprotective effect of PACAP on H_2O_2-induced astrocytes death. Incubation of cultured astrocytes with graded concentrations of H_2O_2 for 1 h provoked a dose-dependent reduction of the number of living cells. The deleterious effect of H_2O_2 was completely inhibited by increasing doses of PACAP (10^{-14} M to 10^{-6} M). The effect of PACAP on astroglial cell survival was abolished by the PACAP receptor antagonist, PACAP6-38. The protective action of PACAP was blocked by the protein kinase A inhibitor H89, the protein kinase C inhibitor chelerythrine and the mitogen-activated protein kinase inhibitor U0126. Furthermore, the neuroprotective activity of PACAP is based on its capacity to stimulate glutathione formation, and to block H_2O_2-evoked ROS accumulation and glutathione content reduction. In addition, PACAP reduces the effect of H_2O_2 on the activation of caspase-3, the reduction of mitochondrial potential and the inhibition of SOD and catalase activities.

Taken together, these data demonstrate that PACAP is a potent protective agent that prevents oxidative stress-induced apoptotic cell death in astrocytes.

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P28 THE ROLE OF ENDOGENOUS PACAP IN THE KIDNEY DURING ISCHAEMIA-REPERFUSION

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Introduction: Several diseases, certain surgical interventions and kidney transplantation are accompanied by ischaemia-reperfusion-induced kidney injury. We have previously described the presence of PACAP (pituitary adenylate-cyclase activating polypeptide) in the kidney and demonstrated its changes following renal ischaemia-reperfusion. The aim of the present study is to investigate whether endogenous PACAP influences the extent of ischaemia-reperfusion-induced injury in the kidney.
Methods: PACAP knockout (homozygous and heterozygous) and wild-type mice underwent 45 or 60 minutes of renal ischemia followed by a two-week reperfusion. Kidneys were processed for histological analysis. Sections stained with PAS-haematoxylin were graded for histological parameters (dilatation of the Bowman's capsule, tubular dilatation, thyreoidisation-like changes, lymphocyte and macrophage infiltration, damage of the glycocalyx layer) on a three-degree scale. In other sets of experiments, tissue cytokine expression and the activity of the endogenous antioxidant superoxide dismutase (SOD) were also determined after 60 minutes ischemia and 24 hours reperfusion.
Results: No significant difference was observed in postoperative mortality between the investigated groups. PACAP knockout mice showed more severe histological outcome compared to wild-type mice, with significantly higher histological scores for most of the tested parameters. Cytokine profile of the kidney was markedly altered in homozygous PACAP knockout mice and the activity of SOD was significantly reduced in these mice after ischemia-reperfusion.
Conclusion: Both the partial and the total lack of PACAP results in increased susceptibility to renal ischaemia-reperfusion, the developed injuries are more severe, suggesting that endogenous PACAP has a protective effect in the kidney. However, further investigations should be carried out to recognize the exact function of PACAP in the kidney.
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P29 INVESTIGATION OF RENOPROTECTIVE EFFECT OF PACAP: IN VITRO STUDIES

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic neuropeptide, occurring throughout the entire body. One of its well-known effects is its protective actions, including renoprotective effects, which has already been shown against myeloma kidney injury, renal ischemia and diabetic nephropathy. Not surprisingly, the lack of endogenous PACAP has various pathological consequences, indicating that endogenous PACAP plays a protective role against different stressors. However, it is not known, whether PACAP deficient mice are more sensitive to kidney injuries. Thus, the first aim of the present study was to investigate the effects of in vitro oxidative stress induced by H_2O_2 on kidney cells derived from wild type and PACAP KO mice. Kidney cells were treated with 0.5, 1.5, 3 mM H_2O_2. For obtaining evidence that the eventually increased susceptibility is due to lack of PACAP, in another set of experiments PACAP was exogenously added to the H_2O_2-treated cells. The second aim of our study was to investigate the protective effect of exogenously given PACAP. In the first set of experiments, effect of PACAP was investigated using primary rat kidney cell cultures exposed to oxidative stress or *in vitro* hypoxia. Furthermore, in order to examine the effect of exogenous PACAP on human cells, similar experimental paradigm was used on HK-2 cell line derived from human kidney. Besides investigating the survival-promoting effect of the peptide in human cells, we have tested whether it influences the levels of stress-related proteins. For investigating the effect of exogenously administered PACAP, cells derived from rat kidney or HK-2 cell line were exposed to 1,3, 6 mM H_2O_2 for 2 and 4 hs or 300 µM H_2O_2 for 24 hs, respectively. *In vitro* hypoxia was induced by 300 µM $CoCl_2$. Cell viability was examined by MTT assay.

We found that the sensitivity of cells from PACAP deficient mice was greatly increased to oxidative stress: cell viability was significantly reduced compared to control wild type mice. This sensitivity could be attenuated by PACAP-treatment. In addition, in case of experiments investigating the effect of exogenous PACAP, we observed that the applied $CoCl_2$- and H_2O_2-treatment significantly decreased the cell viability, but the co-incubation with PACAP resulted in significant increase in cell survival compared to cell groups treated with H_2O_2 or $CoCl_2$ alone. Furthermore, we could have observed the modifying effect of PACAP on several stress-related protein levels. Our results show that both endogenous and exogenous PACAP protects against harmful stimuli in the kidney.

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P30 INVESTIGATION ON CORTICOTROPIN RELEASING FACTOR EXPRESSION OF BED NUCLEUS OF STRIA TERMINALIS IN PITUITARY ADENILATE CYCLASE-ACTIVATING POLYPEPTIDE HETEROZYGOUS MICE

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Introduction: Based on three hit theory genetic predisposition, epigenetic factors and chronic stress lead to manifest depression. The shortage on pituitary adenilate cyclase-activating polypeptide (PACAP) can cause depression-like behavior. The role of corticotropin releasing factor (CRF) in the hypothalamus-pituitary-adrenal axis is well known in stress adaptation response; however the significance of extra-hypothalamic CRF is largely elusive.
We hypothesized that the three hit theory is applicable on mice and extra-hypothalamic CRF neurons in the BNST could be affected.
Methods: 38 male PACAP heterozygous mice were exposed to 15 or 180 minutes maternal separation on postnatal days (PD) 1-14 vs. non-deprived controls. Half of the mice each group was exposed to chronic variable stress on 106-120 PD. The stress paradigm was validated by measuring the adrenal weight. Indirect CRF immunolabeling was performed on the sections of BNST.
Results: Mice carrying all three risk factors showed significantly higher CRF specific signal density and showed 58% higher CRF immunoreactive cell counts in BNSTov. In subjects without maternal deprivation this alteration did not occur. Adrenal weights in mice exposed to both maternal deprivation and stress was significantly elevated.
In conclusion the rise of adrenal weight demonstrates the adaptation to stress, and supports the validity of the model. CRF neurons in the BNST change their functions if all three risk factors of depression coincide. The use of three hit theory of depression in PACAP heterozygous mice seems to be a promising model for mood disorders, and requires further investigation.
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Keywords: PACAP, depression, CRF, stress, BNST, maternal separation

P31 DUAL ROLE OF PACAP IN THE REGULATION OF BODY TEMPERATURE

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In the past decade it has been shown that intrabrain administration of PACAP causes hyperthermia in different species. Despite of its acute hyperthermic effect, in studies using mice with the *Pacap* gene knocked out (KO), there was no difference between the KO mice and their wild-type littermates in the circadian changes of deep body temperature (T_b). It has been also reported that PACAP attenuates the cytokine response to the injection of bacterial lipopolysaccharide (LPS), but how it affects the temperature response in LPS-induced fever has remained unknown.

Male Wistar rats and female PACAP KO and wild type mice were used in our study. All animals were habituated to the experimental conditions. In loosely restrained rats, PACAP1-38 was injected intracerebroventricularly (ICV) and colonic temperature (a form of deep T_b) was recorded. In freely moving PACAP KO and wild-type mice, circadian changes of abdominal temperature and locomotor activity were registered with telemetry. In a different set of experiments, we studied whether the pharmacological (PACAP6-38, ICV) or genetic blockade of PACAP affects the Tb response of loosely restrained animals to intraperitoneally injected LPS.

In harmony with earlier reports, we found that acute ICV administration of PACAP1-38 caused a marked (>1°C) rise of T_b in rats. Interestingly, when we compared the locomotor activity and T_b of the *Pacap* KO mice with those of controls, we found that in the absence of PACAP, the mice maintained a significantly ($p<0.05$) higher daytime T_b. The locomotor activity of the KO mice was significantly higher than that of controls during both the light and dark phases of the day. Administration of LPS to rats resulted in a fever response, which showed a tendency towards an exaggeration of the response when the animals were pretreated with PACAP6-38 ICV. Similarly to our results with the PACAP antagonist, the T_b response of Pacap KO mice to a low-dose (120 μg/kg) of LPS was more pronounced than that of controls.

In summary, we found that acute injection of PACAP increased deep T_b, while the long-term absence of the peptide in KO mice lead to elevated T_b, which can be explained, at least in part, by the hyperactivity of the animals. Both pharmacological and genetic blockade of PACAP potentiated the fever response to LPS. We suggest that PACAP has a complex role in Tb regulation and it serves as an antipyrogenic agent in endotoxin fever.

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P32 THE EFFECT OF SYSTEMIC PACAP TREATMENT TO LOCOMOTOR ACTIVITY IN MALE AND FEMALE RATS

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Introduction: The main research topic of our team is the pituitary adenylate cyclase activating polypeptide (PACAP) a neuropeptide with diverse functions in various organisms. The PACAP has protective effects in animal models of various disease and increasing number of studies have been published about functions in humans. Therefore, it is important to examine how systemic PACAP treatment affects animal behavior. Some previous studies have revealed that the administration of PACAP in the CNS modifies the locomotor behavior of animals. However, there are no details known about the effect of systemic PACAP on general behavior. Moreover, most studies were carried out with male animals, while it is known that certain effects of PACAP treatment are gender-dependent. Our research group found similar results in previous experiments. In rat model of Parkinson disease PACAP treatment significantly decreased the behavioral deficit in male, but not in female rats. The goal of the present experiment is the investigation of behavior of male and female rats after the systemic PACAP treatment.
Methods: Wistar rats were treated intraperitoneally with 50 μg PACAP-38. One day before, one and ten days after PACAP administration open-field tests were carried out. The behavior of the animals was recorded for 5 minutes and then the locomotor parameters (distance, time activity, rearing, centrally time spent) were evaluated.
Results: We have found that the systemic treatment in male rats did not cause significant alterations in behavior. In contrast, after the PACAP treatment the locomotor activity of female rats significantly decreased and their anxiety increased. This difference in behavior after 1 and 10 days of the treatment was also observed.
Conclusion: In conclusion, our results show that a single systemic PACAP administration causes changes in the behavior of female rats. This study also shows the importance of necessity that both sexes should be tested because there could be significant gender difference.
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P33 ROLE OF C-JUN N-TERMINAL KINASE (JNK) ACTIVATION IN MICTURITION REFLEXES IN CYCLOPHOSPHAMIDE (CYP)-INDUCED CYSTITIS IN FEMALE RATS

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c-Jun N-terminal Kinase (JNK) is member of the Mitogen-Activated Protein Kinase (MAPK) family, activated through phosphorylation following cytokine exposure and stress. CYP-induced cystitis is known to increase cytokine and chemokine expression in the urinary bladder. In this study, phosphorylation of JNK was examined in the urinary bladder with CYP-induced cystitis and the effects of JNK phosphorylation blockade with SP600125, a selective inhibitor of phosphorylation of JNK, on urinary bladder function were assessed using conscious, open outlet, cystometry with continuous instillation of intravesical saline.

To examine JNK phosphorylation in the urinary bladder with bladder inflammation, we induced bladder inflammation in adult female Wistar rats (200-300 g) by injecting CYP intraperitoneally at acute (150 mg/kg; 4 h), intermediate (150 mg/kg; 48 h) and chronic (75 mg/kg; every third day for 10 days) time points.

Western blotting of urinary bladder demonstrated a significant ($p \leq 0.01$) increase in JNK activation with 4 h and 48 h CYP-induced cystitis. Immunohistochemistry and image analyses demonstrated a significant ($p \leq 0.01$) increase in JNK activation in the urothelium with 4 h and 48 h CYP-induced cystitis. Blockade of JNK phosphorylation with intravesical SP600125 was evaluated with conscious cystometry in CYP-treated rats. Blockade of JNK phosphorylation significantly ($p \leq 0.01$) increased bladder capacity and intercontraction void intervals in CYP-treated rats (4 h and 48 h). Furthermore, blockade of JNK phosphorylation reduced ($p \leq 0.05$) neuropeptide (substance P, calcitonin gene-related peptide (CGRP) expression in the urinary bladder with CYP-induced cystitis (4 h and 48 h). In contrast, blockade of JNK phosphorylation was without effect on bladder function or neuropeptide expression in urinary bladder in control (no inflammation) rats. Activation of JNK in the urinary bladder following CYP-induced cystitis may contribute to urinary bladder dysfunction and blockade of JNK phosphorylation may represent a novel target for improving urinary bladder function with CYP-induced cystitis.

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P34 THE RELATIONSHIP OF PACAP AND KYNURENIC ACID IN THE ACTIVATED TRIGEMINOVASCULAR SYSTEM

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Introduction: Our experimental and clinical data suggest that pituitary adenylate cyclase-activating polypeptide (PACAP) may have a crucial role in the activation of the trigeminovascular system (TS). Glutamatergic transmissions are also involved in the activation of the TS, which is supported by experimental results concerning to kynurenic acid (KYNA) analogues as potential NMDA receptor antagonists.
Our aim was to investigate the PACAP-38-like immunoreactivity (PACAP-38-LI) in activated TS of the rat pre-treated by an effective KYNA derivative.
Methods: Anaesthetized, adult male SPRD rats were used to evoke activation of the TS with electrical stimulation (ES) of the trigeminal ganglion (TRG) according to the following parameters: 30 min, 1mA, 10 Hz. Animals were treated with intraperitoneally applied KYNA analogue 30 min prior to the ES. 180 minutes after the stimulation, blood samples were taken from the right cranial vena cava into anticoagulant and protease inhibitor containing tubes. Rats were transcardially perfused then the caudal trigeminal nucleus (TNC) and TRGs were excised. Blood samples were kept at 4°C and centrifugated (4°C, 12000 rpm, 10 min) then the plasma samples and nerve tissues were stored at -80°C until the PACAP-38 radioimmunoassay (RIA) measurements.
Results: In response to the ES of the TS, the PACAP-38-LI was significantly elevated both in the plasma and TNC, which was mitigated by KYNA analogue pre-treatment. There were no significant changes detected in the stimulated and the contralateral TRG.
Discussion: It is assumed that the ES can induce a massive PACAP-38 release from the peripheral and central terminals of the primary sensory neurons exerting its vasodilatator and sensitizating effect. The KYNA derivative is partly able to preclude this effect proved by several literatures about signalling crosstalk between PACAP and NMDA receptors.
Conclusion: In the future PACAP may serve as a potential biomarker for migraine and the development of KYNA analogues can provide therapeutical opportunities in the headache diseases.

P35 EFFECTS OF MATERNAL DEPRIVATION AND CHRONIC STRESS ON THE CORTICOTROPIN RELEASING FACTOR CONTENT OF THE CENTRAL NUCLEUS OF AMYGDALA IN MICE

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Introduction: According to the three hit theory of depression genetic predisposition, epigenetic changes and stress effects are jointly responsible for the manifestation of the disease. Partial deficiency or lack of pituitary adenylate cyclase-activating peptide (PACAP) causes depression-like behavior, suggesting that these mice could be applied as a suitable model for genetic predisposition for mood disorders. Maternal deprivation (MD) is a widely used tool to study epigenetic effects; furthermore, everyday stress could be studied in mice by applying chronic variable mild stress (CVMS) exposure. The hypothalamus-pituitary-adrenal (HPA) axis plays a pivotal role in stress-adaptation. The significance of corticotropin releasing factor (CRF) producing neurons of the paraventricular nucleus of hypothalamus is well known, however the function of extrahypothalamic CRF containing neurons in stress adaptation such as those in the central nucleus of amygdala (CeA) is practically unknown. The goals of our study were to validate the three hit theory in mice and to perform a semi-quantitation of CRF levels in the CeA. We hypothesized that PACAP heterozygote mice in response to chronic stress with MD history will show bodyweight changes and alterations in the CRF content of the CeA.
Methods: Newborn PACAP heterozygote mice were exposed either to physiological, short term (15 mins) or severe (180 mins) MD on the 1-14th postnatal days vs. non-disturbed controls. Half of these animals each group were subjected to CVMS between postnatal days 106-120. Animal's bodyweights were regularly determined they were perfused and brains were processed for indirect immunofluorescent labeling for CRF.
Results: Bodyweight measurements revealed that mice subjected previously to MD in response to CVMS lost more bodyweight supporting the reliability of the three hit theory. Immunhistological results demonstrate that non-deprived PACAP heterozygotes showed 38% elevation in CRF specific signal density and 30% rise in CRF immunoreactive cell counts upon CVMS in the CeA. In contrast to these in mice with MD history we did not see significant alterations in terms of CRF immunoreactivity in response to CVMS.
Conclusion: In summary, the changes in the CRF content of the CeA suggests that the ability to adapt to CVMS is altered if all three risk factors of depression occur, thus the three hit theory is a promising model for this psychopathology in mice.
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P36 PACAP-REGULATED MICRORNAS IN MICROGLIAL CELLS

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Background: Microglia represents the first line of defense against brain injuries such as stroke. The resident microglial cells are rapidly mobilized to the site of injury and initiate the release of effector molecules and recruitment of other immune cells. Recently, an increasing number of studies agree that microglial are highly plastic cells that can assume diverse phenotypes and different functional profiles in response to specific microenvironmental signals. In particular, *in vitro* stimulation with lipopolysaccharide and interferon-γ (IFNγ) promotes the differentiation of "classically activated" M1 microglia that typically releases potentially damaging proinflammatory mediators. In contrast, interleukin (IL)-4 and IL-10 induce an "alternatively activated" M2 phenotype that possesses neuroprotective properties. The dualistic roles of distinctly polarized microglia/macrophage populations have been reported in several central nervous system diseases, as multiple sclerosis and spinal cord injury. Currently, the concept of microglial M1 and M2 phenotypes has entered the field of stroke research; however, a comprehensive characterization of microglia polarization after ischemic brain injury is still missing. On the other hand, data recently obtained in our laboratory (Brifault et al; *in preparation*) have shown that embryonic stem (ES)-cells expressing PACAP injected in brains of mice subjected to experimental ischemia, promotes functional recovery, that correlates with modulation of inflammatory response, and more specifically with the skewing of microglial response toward a M2 phenotype. These findings suggest that PACAP could orientate microglial cells differentiation to a neuroprotective phenotype.
Objective: As numerous studies report the crucial role of microRNA (miRNA) in the regulation of gene expression and control of differentiation process in peripheral macrophages as well as microglial cells, we focused our study on the PACAP-regulated miRNAs in primary cultured mouse microglial cells submitted to oxygen-glucose deprivation/reoxygenation *in vitro.*

P37 THE ROLE OF PACAP AND TAC1 GENE DERIVED TACHYKININS IN MOUSE MODEL OF TRAUMATIC MONONEUROPATHY

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Pituitary Adenylate-Cyclase Activating Polypeptide (PACAP) and Tac1 gene-encoded tachykinins (substance P: SP, neurokinin A: NKA) are expressed in capsaicin-sensitive peptidergic nerves, but data on their role in nociception, inflammation and vascular responses are contradictory. Therefore, we aimed to investigate the function of these sensory neuropeptides, and the NK1 tachykinin receptor (derived from the Tac1 gene) in the partial sciatic nerve ligation-induced traumatic mononeuropathy model using gene deficient ($PACAP^{-/-}$, $Tac1^{-/-}$, and $Tacr1^{-/-}$) mice. The mechanonociceptive threshold of the paw was measured with dynamic plantar aesthesiometry, the motor coordination on a Rota-Rod device, and cutaneous microcirculation with laser Doppler imaging. Neurogenic vasodilatation was evoked by topical application of the selective Transient Receptor Potential A1 (TRPA1) agonist mustard oil stimulating sensory nerves. In both wildtype groups ($PACAP^{+/+}$, C57Bl/6) 30-40% mechanical hyperalgesia developed one week after nerve ligation, which persisted during the study. This hyperalgesia was not altered in $Tac1^{-/-}$ and $Tacr1^{-/-}$ mice, while it was absent in the $PACAP^{-/-}$ group. Motor coordination of the $PACAP^{-/-}$ and $Tac1^{-/-}$ groups was significantly worse both before and after nerve ligation compared to their wildtypes, but it was not altered in $Tacr1^{-/-}$ mice. Microcirculation on neither the operated nor intact limbs of the $PACAP^{-/-}$ mice differed from the wildtypes during the postoperative control measurements, but it was significantly lower in the $Tac1^{-/-}$ and $Tacr1^{-/-}$ groups. The TRPA1 activation-induced neurogenic vasodilating response was significantly smaller in $PACAP^{-/-}$ mice, but remained unchanged in $Tacr1^{-/-}$ and $Tac1^{-/-}$ animals. As a conclusion, partial sciatic nerve ligation does not induce motor impairment, only sensory neuropathy. Both PACAP and SP/NKA participate in normal motor coordination. In contrast, SP/NKA and the NK1 receptor are not involved in these processes, but play a role in maintaining basal cutaneous blood flow. PACAP is a crucial mediator of neuropathic mechanical hyperalgesia and neurogenic vasodilation. Identifying its target and developing selective, potent antagonists, might open promising new perspectives for the treatment of neuropathic pain and vascular complications.
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P38 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE PLAYS A KEY ROLE IN NITROGLYCEROL-INDUCED TRIGEMINOVASCULAR ACTIVATION IN MICE

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptors (PAC1, VPAC) are present in sensory neurons and vascular smooth muscle. PACAP infusion was found to trigger migraine-like headache in humans and we showed its central pronociceptive function in several mouse pain models. Nitroglycerol (NTG)-induced pathophysiological changes were investigated in this study in PACAP gene-deleted (PACAP–/–) and wildtype (PACAP+/+) mice. Chemical activation of the trigeminovascular system was induced by 10 mg/kg i.p. NTG.

Light-aversive behavior was determined in a light–dark box, meningeal microcirculation by laser Doppler blood perfusion scanning and the early neuronal activation marker c-Fos with immunohistochemistry. NTGinduced photophobia both in the early (0–30 min) and late phases (90–120 min) due to direct vasodilation and trigeminal sensitization, respectively,was significantly reduced in PACAP–/– mice. Meningeal blood flow increased by 30–35% during 4 h in PACAP+/+ mice, but only a 5–10% elevation occurred from the second hour in PACAP–/– ones. The number of c-Fos expressing cells referring to neuronal activation in the trigeminal ganglia and nucleus caudalis significantly increased 4 h after NTG in PACAP+/+, but not in PACAP–/– animals. Similar PAC1 receptor immunostaining was detected in both groups, which did not change 4 h after NTG treatment. PACAP-38 (300 μg/kg, i.p.) produced photophobia similarly to NTG and 30% meningeal vasodilatation for 30 min in PACAP+/+, but not in PACAP–/– mice. It significantly increased neural activation 4 h later in the trigeminal ganglia of both groups, but in the nucleus caudalis of only the PACAP+/+ mice.

We provide the first experimental results that PACAP is a pivotal mediator of trigeminovascular activation/sensitization and meningeal vasodilation related to migraine.

P39 IMMUNOHISTOCHEMICAL LOCALIZATION OF PACAP, VIP, AND THEIR RECEPTORS IN THE GONADS OF THE MUSSEL MYTILUS GALLOPROVINCIALIS

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The mussel *Mytilus galloprovincialis* is a bivalve present in the Mediterranean, Atlantic coast of southern Europe, northern Africa, and the Pacific coast of North America (Gosling 1984, 1992; Koehn 1991; McDonald et al. 1991; Suchanek et al. 1997); gametogenesis occurs, in typical ovarian or spermatic follicles. Between follicles, a connective tissue is present, mainly formed by adipogranular and vesicular cells, which represent storage sites of reserve substances; the ratio of connective tissue to germinal cells modifies considerably throughout the year, according to the gametogenic condition of the mussel (Lowe et al., 1982; Pipe, 1985).We performed an immunohistochemical investigation to demonstrate the presence of neuropeptides PACAP and VIP and their receptors in male and female gonads of *Mytilus galloprovincialis.* In males, in maturing period, PACAP 38 is localized in all the germinal cells, starting from spermatocytes I to spermatozoa, PACAP 27 only in spermatogonia and spermatocytes I. Both neuropeptides are absent in Sertoli cells but are recognizable in adipogranular cells. All the cells present in the gonads, germ and somatic cells, are positive to anti-VIP antibody. The VPAC1 and VPAC2 receptors are present in Sertoli cells, spermatocites II and spermatids; the PAC1 receptors is poor represented, only on adipogranular cells. During spawning period, when the germinal component is reduced compared with the connective tissue, the picture is almost unchanged but PACAP 38 is present also in spermatogonia and PACAP 27 is no longer present in spermatocytes I; VIP is still localized in all the germinal stages and in connective cells.VPAC1 and PAC1 receptors are present only on adipogranular cells, while VPAC2 receptors is no longer detectable.In females, in maturing gonads, PACAP 27 and 38 are localized in full grown oocytes, in follicle cells and in a few previtellogenic oocytes; VIP is present in vitellogenic oocytes assuming a pear form exclusively and in oocytes that have accomplished the growth. The three receptors are localized in previtellogenic and vitellogenic oocytes; VPAC2 receptor also on follicle cells. In spawning period, a few of vitellogenic oocytes, oogonia and early previtellogenic oocytes are present in follicles; the connective tissue fills almost all the gonad. PACAP 38 is localized in all gonadic cellular type, while PACAP 27 is evident only on adipogranular cells; VIP is present in the vitellogenic and previtellogenic oocytes. PAC1 receptor is not detectable, VPAC1 and VPAC2 receptors are localized in the few remaining vitellogenic oocytes and in previtellogenic oocytes.On the whole, the present data indicate for the first time a VIP and PACAP engagement in the mussel gametogenesis, as already demonstrated in vertebrates (Krempels et al., 1995; Gobbetti and Zerani, 2002; Li and Arimura, 2003; Calabro et al., 2008; Agnese et al., 2010; Agnese et al., 2012; Levy and Degani, 2012).

P40 FIRST REPORT OF THE PITUITARY ADENYLATE CYCLASE ACTIVATING PEPTIDE (PACAP) IN CRUSTACEANS: ITS FUNCTION AS GROWTH PROMOTER FACTOR AND IMMUNOMODULATOR IN LITOPENAEUS VANNAMEI

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The high conservation of the pituitary adenylate cyclase activating peptide (PACAP) sequence indicates that this peptide fulfils important biological functions in a broad spectrum of organisms. However, in invertebrates, little is known about its presence and its functions remain unclear. Up to now, in non-mammalian vertebrates, the majority of studies on PACAP have focused mainly on the localization, cloning and structural evolution of this peptide. As yet, little is known about its biological functions as growth factor and immunomodulator in lower vertebrates. Recently, we have shown that PACAP, apart from its neuroendocrine role, influences immune functions in larval and juvenile fish. In this work, we isolated for the first time the cDNA encoding the mature PACAP from a crustacean species, the white shrimp *Litopenaeus vannamei,* corroborating its high degree of sequence conservation, when compared to sequences reported from tunicates to mammalian vertebrates. Based on this, we have evaluated the effects of purified recombinant *Clarias gariepinus* PACAP administrated by immersion baths on white shrimp growth and immunity. We demonstrated that PACAP promotes growth and also increases total protein concentration, hemocyte count, superoxide dismutase, lectins and nitric oxide synthase derived metabolites in treated shrimp. From our results, PACAP acts as a regulator of shrimp growth and immunity, suggesting that in crustaceans, as in vertebrate organisms, PACAP is an important molecule shared by both the endocrine and the immune systems.

P41 PACAP SIGNALING MODULATES ACETYLCHOLINE RELEASE AT NEUROMUSCULAR NICOTINIC CONTACTS OF THE LAND SNAIL

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Pituitary adenylate cyclase activating polypeptide (PACAP) is widely expressed throughout the vertebrate nervous system and can modulate the function of ion channels and synaptic output. It is suggested that in some nerve endings PACAP co-localizes with acetylcholine (ACh) and enhances the nicotinic ACh receptor (nAChR) mediated responses by activating nitric oxide synthase (NOS) and therefore increasing the level of nitric oxide (NO). We have previously found that in flexor muscles of the land snail *(Helix pomatia)* tentacles ACh is the main excitatory neurotransmitter and it acts via α7 nAChRs. The aims of the present study were to investigate the possible enhancing effect of PACAP on the neuromuscular contacts between the tentacular muscle and the olfactory nerve, to find out if this process requires NO production, and to identify the signaling cascade via PACAP may act. Pre-treatment of the muscle for 15 min with 100 nM PACAP increased the amplitude of contractions evoked by stimulating the nerve electrically. In the presence of 100 μM NOS inhibitor L-Nitro-Arginine Methyl Ester (L-NAME), PACAP failed to enhance the muscle contractions. Since PACAP can activate adenylate cylase (AC) and increase the cAMP level, we tested the effect of 10^{-4} M forskolin on nerve evoked contractions. After a 15 min application of forskolin, the nerve stimulation induced contractions increased which was proportional to the enhancement triggered by PACAP. The data suggest that at neuronal nicotinic contacts of the tentacle muscle PACAP acts via the AC-dependent transduction cascade that increase NO production to enhance transmission. The presence of the α4nAChR was immunohistochemically demonstrated in the presynaptic nerves. To obtain further evidences for this assumption, experiments are in progress in order to investigate the possible increasing effect on the muscle contractions of the membrane permeable cAMP analoges and to demonstrate co-localization or co-release of PACAP with ACh.

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P42 FIRST EVIDENCE FOR THE DIFFERENTIAL EXPRESSION OF PACAP AND ITS RECEPTORS IN THE CONTEXT OF VIRAL INFECTION IN FISH

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There are different studies concerning the immune functions of pituitary adenylate cyclase-activating polypeptide (PACAP), however information of its source in lymphoid organs is still scarce. Although the occurrence of the PACAP receptors PAC1, VPAC1 and VPAC2 in the immune system of mammals is known, only limited studies have reported the presence of some of these receptors in lymphoid organs in fish. In order to demonstrate the role of PACAP on the fish antiviral immune responses we have studied both the expression of the two PACAP transcriptional variants (PRP/PACAP and PACAP) together with their receptors in spleen and head kidney leukocytes of the rainbow trout *(Oncorhynchus mykiss)* and in the monocyte cell line RTS11, at different time points after infection with important pathogens for salmonids aquaculture, such as viral hemorrhagic septicemia (VHSV) and infectious pancreatic necrosis (IPNV) viruses. Our results showed for the first time a differential regulation of PACAP transcripts and its receptors after infection, suggesting a direct mechanism of PACAP action to mediate antiviral immune responses in fish.

P43 THE INVOLVEMENT OF THE VERTEBRATE PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) AND INSULIN-LIKE GROWTH FACTOR (IGF-1) IN THE REGULATION OF MOLLUSCAN HEART

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Since their discovery, pituitary adenylate cyclase activating polypeptide (PACAP) and insulin related peptides, such as insulin-like growth factor (IGF-1) or its homologs, bombyxin-A, molluscan insulin-related peptides (MIPs) have been the subject of extensive research successfully locating the peptides and their receptors (PAC1-R, VPAC1, VPAC2 and RTKs, MIPR) in both vertebrates and invertebrates. PACAP and IGF-1 have been well documented to have widespread and fundamental roles in the several physiological functioning of vertebrate systems. The evolutionarily conserved nature of these peptides would suggest that such roles also exist in invertebrate systems. The well characterised role of PACAP and IGF-1 in the vertebrate cardiovascular system is of particular interest to this investigation, in its attempts to identify whether PACAP and IGF-1 have such function in invertebrates. In our experiments we used two different species of invertebrate model animals, the common pond *(Lymnaea stagnalis)* and terrestrial snails *(Helix pomatia)*. We aimed to localize both PACAP and IGF-1 and their receptors in the *Lymnaea* and *Helix* cardiovascular systems by immunohistochemistry (IHC), western blotting and mass spectrometry. A secondary aim was to identify the pharmacological effects of these peptides and their possible signal-transduction pathway on the heart.

PACAP and IGF-1 were found to have a significant physiological effect on both molluscan hearts via the PAC1-R and IGF-1 like receptors - G proteins - AC - cAMP pathway. PACAP increased the amplitude of heart muscle contraction but the heart beat frequency did not change. IGF-1 decreased the amplitude of muscle contraction and the heart beat frequency, but increased the resting tone of the heart musculature. Furthermore, peptides also modified the effect of the cardio-active substances, such as dopamine, serotonin and acetylcholine. Despite these results, we failed to identify a PAC1 receptor-like protein in the *Lymnaea* heart by the anti-PAC1-R antibody used in our IHC experiments. This was in contrast to our findings in the *Helix* heart, where we did detect a putative molluscan PACAP receptor. The IGF-1 and molluscan

insulin-related peptides receptors (MIPR) were observed in the heart muscles of both snails. We cannot therefore rule out the possibility that there could be differences in the structure of PACAP receptors in even closely related molluscan species. The cardioactive nature of PACAP and IGF-1 in invertebrate systems is an entirely novel finding, serving to extend previous knowledge as well as inviting future research to confirm and clarify this outcome.

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P44 EXPRESSION AND FUNCTIONAL ACTIVITY OF PACAP AND ITS RECEPTORS ON CUMULUS CELLS: EFFECTS ON OOCYTE MATURATION

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Pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptor PAC1-R (PACAP type 1 receptor) are transiently expressed in granulosa cells of mouse preovulatory follicles and affect several parameters associated with the ovulatory process. We investigated the expression of PACAP and its receptors in cumulus cells (CCs) after the LH surge and their role on cumulus expansion/apoptosis and oocyte maturation. PACAP and PAC1-R expression increased in CCs isolated at different times after treatment with human chorionic gonadotropin (hCG). Moreover, PACAP was able to reverse the inhibition of oocyte meiotic maturation caused by hypoxantine in cumulus cell-oocyte complexes (COCs) and efficiently promoted male pronuclear formation after fertilisation. PACAP was also able to induce cumulus expansion and prevent CC apoptosis. Our results demonstrated the induction of PACAP and its receptors in CCs by LH and EGF, suggesting that PACAP may play a significant role in the complex interactions of gonadotropin and growth factors during ovulation and fertilisation.

P45 VIP INDUCES AN IMMUNOSUPPRESSANT MICROENVIRONMENT IN THE MATERNAL-PLACENTAL INTERFACE AND IMPROVES PREGNANCY OUTCOME IN MOUSE MODELS OF PREGNANCY COMPLICATIONS

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The maintenance of immune homeostasis at implantation and during the early post-implantation stage involves several immunomodulatory circuits active at the maternal-placental interface. A dynamic response with a predominant anti-inflammatory and tolerogenic profile at the local level is required and various immune cell types and soluble factors are involved. The loss of homeostasis early after implantation can compromise pregnancy in an all-or-none manner with pregnancy loss, or affect its outcome at later stages as in pre-eclampsia. VIP has anti-inflammatory, tolerogenic and relaxing effects so its ability to promote an immunosuppressant microenvironment and improve pregnancy outcome was explored in two mouse models of pregnancy complications, the non obese diabetic (NOD) mouse at the pre-diabetic stage (singeneic mating) and the CBAxDBA abortion prone model.
Pregnant uteri were carefully dissected at day 8-10 of pregnancy and implantation sites either viable or with incipient resorption processes were analyzed to calculate resorption rate, distribution along horns among other gestation outcome signs. Implantation sites were isolated for VIP, VPAC, cytokine and transcription factor assessment by RT-PCR, immunoblotting and immunohistochemistry. To assess the effect of VIP in vitro, implantation site explants were incubated for 6 h with 1-100 nM VIP at 37°C. To study the effect of VIP in vivo, mice were injected ip with 1-10 nmol VIP in PBS at day 6.
Results indicate that VIP and VPAC2 receptor expression was increased in implantation sites at gestation days 8-10. Trophoblast cells were the predominant cell type positive for VIP immunostaining in the viable implantation sites at days 8 and 9, some of them were lying adjacent to maternal blood vessels and at the interface between the placenta and the decidua. Small round scattered cells with VIP immunostaining and negative for cytokeratine were also found in the mesometrial deciduas. VIP treatment of implantation site explants increased VPAC2, TGF-b, Foxp3 and IL-10 expression. Sites with resorption processes presented lower VIP expression, reduced suppressant markers, increased IL-17 and RORgT expression and a reduced response to VIP in Foxp3 protein levels, compared with viable sites. Pregnant NOD or CBA mice treated with VIP at gestational day 6 showed an increased number of implanted embryos, an even distribution of embryos along the horns with increased local expression of IL-10, TGF-b and Foxp3. In addition, VIP treatment promoted a suppressant clearance of apoptotic cells by peritoneal macrophages in both strains.
We conclude that VIP/VPAC system is expressed at the early maternal-placental interface and that VIP induces local anti-inflammatory and tolerogenic signals that partially improve pregnancy outcome in two different murine models of pregnancy complications.

P46 TOLERANCE INDUCTION AT THE EARLY MATERNAL-PLACENTAL INTERFACE THROUGH VIP PRODUCTION BY FIRST TRIMESTER TROPHOBLAST CELLS

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Pregnancy challenges immune cells and immunomodulatory circuits of the mother and the developing fetus to dynamically adapt to each other in an homeostatic and tolerant environment for fetal growth. From an immunological standpoint, pregnancy was proposed to follow a temporal sequence with a predominantly pro-inflammatory first stage, an immunologically more quiescent, fetal growth promoting second period, and a final cut to a prominent inflammatory environment that precedes labor and delivery. In humans, between weeks 3 and 8 of gestation, a variety of cellular processes are encompassed to ensure proper trophoblast growth and invasion, uterine quiescence, vascularization and tissue remodeling in an immunotolerant microenvironment. The transition points imply redundant immunoregulatory mechanisms to tolerance maintenance. In this sense, the inducible regulatory T cells (iTreg) population is essential for maternal tolerance of the conceptus, performs its suppressive actions in the critical peri-implantation phase of pregnancy. On the other hand, the Vasoactive intestinal peptide (VIP) is synthesized and secreted by trophoblast cells and promotes anti-inflammatory and tolerogenic profiles through specific receptors VPAC1 and VPAC2 on immune cells. Here, we evaluated VIP contribution to the differentiation of maternal iTreg after the interaction with trophoblast cell lines obtained from different stages of human pregnany. We used an in vitro model of maternal leukocyte-trophoblast cell interaction represented by cocultures of fertile women PBMC with human trophoblast cell line from first trimestre (Swan71) and from third trimester (JEG3 and BeWo cell lines). We observed that VIP (10-7M) increased the frequency of maternal CD4+CD25+Foxp3+ cells after 48h of coculture with Swan cells (3.9±0.4 vs 8.3%±0.6, $p<0.05$) was prevented by VIP antagonist. This modulation was specific for the first trimester trophoblast cells since neither JEG3 or BeWo cells increased iTregs frequency. In addition, iTreg differentiated upon interaction with Swan cells in the presence of VIP, suppressed the maternal alloresponse and increased the frequency of CD4+IL10+ cells but did not modulate IFNg or IL-17 production. Getting insight into the mechanisms involved in iTreg differentiation, VIP induced the expression of the three isoforms of TGFbeta in Swan cells with a peak at 12h and increased TGFb1 and TGb2 secretion (confirmed by RT-qPCR and Luminex assays). Finally, the increase in iTreg frequency was prevented by an antiTGFb Ab and VIP antagonist. These results suggest that VIP could have an active role in the immunoregulatory processes operating during early stages of the maternal-placental interaction contributing to the induction of iTregs in a TGFb dependent mechanism.

P47 EFFECT OF PACAP ON LACTOGENIC HORMONE INDUCED DIFFERENTIATION OF HC11 MOUSE MAMMARY EPITHELIAL CELLS

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Aims: The process of cell differentiation is regulated by hormones, growth factors, cytokines, chemokines and angiogenesis-related proteins. Pituitary adenylate cyclase activating polypeptide (PACAP) has very potent effects not only on cell survival and proliferation, but on the differentiation as well. The high PACAP-like immunoreactivity in the milk and its changes during the lactating period raise the question of the possible effect of PACAP on the differentiation of mammary epithelial cells.
Materials and methods: HC11 mouse mammary epithelial cell line can serve as an in vitro model of mammary cell differentiation. Lactogenic hormones (DIP: dexamethason, insulin, prolactin) induce β-casein expression, which is characteristic for terminally differentiated mammary epithelial cells. To determine the effect of PACAP on the differentiation on HC11 cells, we measured the changes of β-casein, pAKT, pSTAT5, pp38MAPK expression by Western blot. Furthermore, the impact of PACAP on cytokines and growth factors in differentially regulated non differentiated and differentiated cells was investigated using mouse cytokine and angiogenesis array kits.
Results: PACAP did not modulate the expression of β-casein and the phosphorylation state of the examined pathways. By contrast, secretion of amphiregulin and epidermal growth factor, which were higher in non-differentiated cells, were found to be decreased by PACAP, while on differentiated cells PACAP had no effect.
Conclusions: Our observations show that PACAP has an impact on the differentiation process of mammary cells by changing the pattern of the secreted autocrine growth factors. By this means, PACAP may have physiological functions in the regulation of the mammary gland differentiation.
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P48 PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE (PACAP) INDUCES LOCATION- AND AGE-DEPENDENT CHANGES IN VASOMOTOR RESPONSES ON ISOLATED RAT ARTERIES

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Introduction: PACAP is a potent vasodilator via smooth muscle cell receptors, which then activate adenylate cyclase. It has also been shown that PACAP elicits dilation in isolated cerebral arteries of rats and humans. Less is known regarding the organ specific and age related vasomotor effects of PACAP, which however, would be important to better understand its physiological roles.
Hypothesis: We hypothesized that vasomotor effects of PACAP depend on the origin of vessels and aging substantially modulates them.
Methods: Carotid (CA) and basilar arteries (BA) were isolated from young (2 month: 2m, n=8) and senescent (28 months: 28m, n=8) rats. Their vasomotor responses were measured with an isometric myograph (DMT-610M) in response to cumulative concentrations (after 20 minutes) of PACAP 1-38 (10-9 M - 10-6 M).
Results: In CA, contractions to KCl (60mM) were 2m: 5,27±0.5 mN, 28m: 2,85±0.6 mN, whereas in BA they were: 2m: 3,43±0,8 mN, 28m: 3,49±0,5 mN.
In 2m CA, reduction in tone (elicited by KCl) to increasing concentrations of PACAP were: (ΔF 10-9M: -0,89±0,5mN, ΔF 10-6M: -2,83±0,5 mN; $p<0,05$), whereas in 28m CA, there was only slight reduction in isometric force (ΔF 10-9 M: -0,02±0,01mN, ΔF 10-6M: -0,31±0,04mN; $p<0,05$). In BA, isometric relaxations in response to increases concentration of PACAP was minimal, both in 2m and 28m old rats (2m: ΔF 10-9M: 0±0,08mN, ΔF 10-6M: -0,38±0,15mN; $p<0,05$; and 28m: ΔF 10-9 M: -0,03±0,04mN, ΔF 10-6M: -0,47±0,05mN; $p<0,05$), whereas BA 2m and 28m did not differ from each other.
Conclusions: PACAP elicited dose-dependent relaxations in isolated CA and BA of rats, which were significantly greater in CA than in BA. Aging substantially reduced PACAP-induced relaxations in CA but not in BA. These data confirm previous findings regarding dilator effects of PACAP on cerebral vessels. In addition, they suggest that PACAP-induced relaxation may decline by aging more in vessels outside the central nervous system than in arteries supplying directly the brain, which may favor the idea that PACAP provides a specific vasculoprotection for cerebral vessels.
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P49 PACAP EXERTS PRO-ANGIOGENIC EFFECTS: POSSIBLE ROLE OF DECREASED PACAP EXPRESSION IN IMPAIRED ENDOTHELIAL ANGIOGENIC CAPACITY IN AGING

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Introduction: Age-related impairment of angiogenesis is likely to play a central role in cerebromicrovascular rarefaction and development of vascular cognitive impairment, but the underlying mechanisms remain elusive. Pituitary adenylate cyclase activating polypeptide (PACAP) exerts multifaceted cytoprotective effects in the cardiovascular system and recent studies show that its expression decreases with advanced age.
Methods: To test the hypothesis that PACAP regulates endothelial angiogenic capacity, primary cerebromicrovascular endothelial cells (CMVECs) were isolated from young (3 months old) and aged (24 months old) Fischer 344 × Brown Norway rats. Tube formation assay was performed to elucidate the effect of PACAP on the angiogenic capacity of endothelial cells. Expression of PACAP, PAC1R, VPACR1/2, VEGF, VEGFR-1/2, TEK was measured by real-time PCR. Cell adhesion and cell migration capacity were determined by electric cell-substrate impedance sensing technology (ECIS). Mitochondrial ROS production was assessed by flow citometry using MitoSox and C-H2DCFDA.
Results: In CMVECs PACAP treatment (100 nM) significantly increased tube formation, whereas cell adhesion and cell migration were unaffected by PACAP. Downregulation of endogenous PACAP expression by shRNA in CMVECs significantly impaired tube formation capacity. Aged CMVECs showed decreased PACAP expression, which was associated with impaired angiogenic capacity as compared to young cells. Overexpression of PACAP resulted in significantly increased tube formation in aged CMVECs. VEGF expression was not altered by overexpression of PACAP, however, changes in the expression of VEGF receptors were detected. Aged CMVECs exhibited increased apoptosis (caspase 3/7 activity), which was reduced to control levels by PACAP. PACAP did not affect increased production of reactive oxygen species in aged CMVECs.
Conclusion: PACAP exerts pro-angiogenic effects and age-related decline in PACAP expression may contribute to impaired angiogenic capacity of cerebromicrovascular endothelial cells.
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P50 DIFFERENCES BETWEEN WILD TYPE AND PACAP KO MICE IN RETINAL AGING

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a neurotrophic and neuroprotective peptide. PACAP and its receptors are present in the retina. We have provided evidence that PACAP is neuroprotective in metabolically induced retinal degenerations. The role of PACAP has been proposed in aging processes.
The aim of this study was to examine whether histological alterations, ultrastructural and cell type-specific differences or changes in the distribution of PAC1-R expression exist between the retinas of wild type and PACAP deficient mice in adult (3-5-months-old) and aging (1-year-old) animals. Retinas were processed for histology (routine and electronmicroscopical), immunohistochemistry (TH, calretinin, calbindin, parvalbumin, PKCα, GFAP, PNA and PAC1-R) and molecular biology.
Standard histological methods revealed no major differences between the adult retinas of wild type and PACAP deficient mice. Staining for the above markers of adult PACAP KO retinas was similar to that of wild type retinas, with no significant alterations in immunoreactivity patterns except for PAC1-R staining. We observed that fewer cells expressed PAC1-R in adult PACAP KO than in wild type retinas. Among the age-related changes, the number of cone photoreceptor terminals was reduced in both wild type and PACAP KO aging retinas compared to adult controls. Other well-known age-related differences were, however, only observed in the PACAP KO mice. These alterations included: horizontal cell processes sprouted into the photoreceptor layer; bipolar cells showed arbor-specific alterations: their dendrites sprouted but their axons remained stable and Müller glial cells showed elevated GFAP expression compared to the aged wild type retinas. Molecular biological analysis also revealed changes in the pro- and anti-apoptotic pathways (Western blot, TUNEL-positive cells), different PACAP receptors (qRT-PCR and Western blot).
In summary, while there are no major differences in the histological structure and expression of markers between adult wild type and PACAP KO mice, there are marked degenerative changes that appear earlier in aging mice lacking endogenous PACAP. These results support the endogenous protective role of PACAP against aging processes of the nervous system.
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P51 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) PLAYS SIGNIFICANT ROLES IN DENDRITIC SPINE FORMATION AND MORPHOLOGY

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PACAP (pituitary adenylate cyclase-activating polypeptide) exerts multiple activities as a neurotransmitter, neuromodulator, and neurotrophic factor. Previously, we demonstrated that PACAP-deficient (PACAP-/-) mice showed notable psychomotor abnormalities, most of which were reversed by atypical antipsychotics, and that PACAP gene SNPs were associated with schizophrenia. These findings suggest that alterations in PACAP signaling might be involved in the pathogenesis of psychiatric disorders including schizophrenia. However, a pathogenic pathway of PACAP signaling remains unknown. Recent studies implicate dendritic spines as important substrates of pathogenesis in psychiatric disorders. There are genes that are associated with both psychiatric disorders and abnormal spine formation. Mutant mice of these genes sometimes show abnormal behavior and dendritic spine loss. Abnormal spine formation has also been reported in some patients with psychiatric disorders.

In this study, we therefore focused on the effect of PACAP on dendritic spine formation as a possible mechanism for the abnormalities in PACAP-/- mice, and showed that 1) the number of dendritic spines were decreased in hippocampal CA1 neurons but not in the cortex in PACAP-/- mice, 2) the number of PSD-95-labeled synaptic puncta was decreased in primary cultured hippocampal neurons prepared from PACAP-/- mice while it was increased by PACAP in the neurons from wild-type mice, and 3) PACAP increased miR-132 expression and decreased mRNA and protein expression levels of p250GAP which is involved in dendritic spine formation and targeted by miR-132. These results indicated that PACAP is critically implicated in spine formation and miR-132 might be involved therein. In summary, it is suggested that dysfunction of PACAP signaling may contribute to the pathogenesis of psychiatric disorders at least partly through abnormal spine formation.

P52 PEPTIDE AND PROTEIN COMPOSITION OF THE BRAINS OF PACAP-DEFICIENT MICE

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Pituitary adenylate cyclase activating polypeptide (PACAP) has been first isolated from ovine hypothalami. Since its discovery, the distribution of PACAP has been shown to be widespread in vertebrate species. The highest concentration of PACAP has been shown in the central nervous system. PACAP deficient mice display several structural, biochemical and behavioral alterations compared to wild type mice. Mice lacking endogenous PACAP have increased vulnerabiliy to different stressors and toxic insults and they also have accelerated aging. Our aim was to investigate the differences in peptide and protein composition of the brains of PACAP deficient and wild type mice using SDS-PAGE based proteomic analysis. Brains from PACAP deficient mice were removed, and different brain areas (cortex, hippocampus, diencephalon, mesencephalon, brainstem and cerebellum) were separated. Brain pieces were weighed, homogenized and further processed for electrophoretic analysis. Our results revealed several differences in diencephalon and mesencephalon. The protein bands of interest were cut from the gel, samples were digested with trypsin and the tryptic peptides were measured by MALDI TOF MS. Results were analysed by MASCOT Search Engine. Among the altered proteins, several are involved in metabolic processes, energy homeostasis and structural integrity. ATP synthase and tubulin beta 2A were expressed more strongly in PACAP knockout mice. In contrast, the expression of more peptides/proteins markedly decreased in knockout mice, like pyruvate kinase, fructose biphosphate aldolase A, glutathion S transferase, peptidyl propyl cis-trans isomerase A, gamma enolase, aspartate amino transferase. In the presented work we are aiming to find functional correlations regarding the observed changes. For example, the markedly decreased expression of glutathione S transferase might partially account for the decreased antioxidant and detoxifying capacity of PACAP deficient mice. The imbalance in energetic enzymatic machinery may lead to decreased resistance to stressors. Our results provide novel insight into the altered biochemical processes in mice lacking endogenous PACAP.

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P53 IMAGING MASS SPECTROMETRY OF THE BRAIN OF PACAP DEFICIENT AND WILD-TYPE MICE

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Imaging mass spectrometry (IMS) is a rapidly developing technique that uses spatially resolved proteomics/peptidomics and metabolomics techniques to simultaneously trace the distributions of biomolecules directly from tissue samples. IMS is suitable for label-free discovery of multiple classes of biomolecules directly on the surface of a tissue section, and can be combined with other routine imaging and proteomic methods. The most widespread IMS scanning technique is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS). This conventional IMS is a powerful technique that combines the chemical and spatial analysis of dehydrated biological surfaces. High sensitivity and mass accuracy, broad mass range detection, good mass resolving power, high speed, MS/MS capabilities and good spatial resolution make it excellent tool for tissue based imaging purposes. MALDI IMS has been used for discovery of neurodegenerative diseases related lipids, peptides and proteins.
PACAP is one of the neuropeptides implicated in protection against neurodegenerative processes. Its protective effects have already been described in animal models and in vitro experiments in models of Parkinson's disease, Huntington chorea, Alzheimer's disease and motor neuron degeneration. Our aim was to investigate the differences of the local distribution of expressed proteins in the brains of PACAP deficient and wild type mice. Brains were removed from mice, sectioned with cryostat and then the cryosections were coated with MALDI matrix. The samples were analyzed with an Autoflex speed MALDI TOF MS. Our imaging mass spectrometry results show significant differences of the distribution of various proteins in different brain areas, including the hippocampus, mesencephalon and corpus callosum. For example, marked differences could be observed in myelin basic protein, which could explain the differences observed between myelination processes in the two mouse groups. The identified proteins could give an insight into the general protective effect of PACAP and the increased vulnerability to various harmful effects in the nervous system of mice deficient in PACAP.
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P54 PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IN THE AMYGDALA: ORIGIN AND COEXPRESSION

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Pituitary adenylate cyclase activating polypeptide (PACAP) signaling in the central nervous system has been shown to play roles in stress, pain, and other emotion-related processes. PACAP-expressing fibers are abundant in the central nucleus of the amygdala (CeA), a key site of integration for sensory and limbic pathways. Although evidence suggests that these PACAPergic fibers may represent projections from nuclei outside of the amygdala, the locations of these neurons are currently unknown. One potential candidate is the lateral parabrachial nucleus (PBn) as PACAP-expressing cell bodies identified in this region are known to send fibers to the CeA, analogous to PBn calcitonin-gene related peptide (CGRP) projections to the amygdala. These neurons are part of the spino-parabrachial-amygdaloid tract that is implicated in the emotional responses to pain, and thought to converge with fear and anxiety pathways. Following lateral PBn anterograde neuronal tracer injections, a majority of the terminal fibers identified in the lateral capsular division of the CeA also appeared to contain PACAP-immunoreactivity, suggesting that a substantial portion of the CeA PACAP is of PBn origins. Dual immunocytochemical localization studies however suggested that the PACAP- and CGRP-immuno-reactivities are largely distinct representing separate neuronal systems. Hence, multiple peptidergic systems appear to be present in parabrachial-amygdaloid tracts and PBn PACAP projections to the CeA implicate its roles in the integration of emotionally relevant sensory information.
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P55 DISTRIBUTION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) IN THE MEDIAN EMINENCE AND DIFFERENT LOBES OF THE PITUITARY GLAND USING VIP- GREEN FLUORESCENT PEPTIDE (VIP-GFP) TRANSGENIC MICE

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It is well known that vasoactive intestinal peptide (VIP) has a multifaceted role in the regulation of prolactin (PRL) secretion. Its presence has been demonstrated in the hypothalamus including the median eminence (ME) suggesting a neuroendocrine regulatory role for this peptide. VIP is also synthesized within the anterior lobe (AL) of the pituitary gland, more specifically, by the PRL cells which indicates an autocrine regulatory role. The aims of our present studies are to provide further and more direct morphological evidences for the presence of VIP in PRL cells and to obtain more information about the location of VIP terminals in the ME as well as in the neural lobe (NL) of the pituitary gland using transgenic mice expressing a green fluorescent protein (GFP) construct in theVIP gene. Neurons and their axon terminals in the ME and the NL have been examined with a fluorescence microscope as well as with immunhistochemical technique (using anti-GPF antibodies). PRL release by AL cells have been detected by reverse haemolytic plaque assay (RHPA), which is suitable for measuring hormone release of individual cells parallel with the detection of GFP fluorescence in VIP expressing cells. Numerous VIP positive cells have been found in the AL, while no cells and no nerve terminals have been detected in the intermediate lobe (IL) of the pituitary gland. A subpopulation of VIP positive cells also release PRL, thereby providing direct evidence of the existence of PRL secreting cells expressing VIP. However, VIP was also detected in cells, which do not release PRL. A dense network of VIP positive fibers can be seen surrounding blood vessels only in the outer zone of the ME. At the same time, several „boutons en passant" type of terminals could be traced to the stalk of the pituary gland, where they terminate by surrounding the long portal vessels (LPV) of the pituitary stalk. In addition, autofluorescence of GFP-VIP can be clearly localized in the wall of these vessels indicating a vasodilatatory role rather than a neuroendocrine role of this peptide. In summary, our results demonstrate that VIP is present in at least two independent systems, which can have direct regulatory roles and significance in pituitary hormone secretion: (1) in PRL secreting cells presumably involved in the autocrine control of PRL secretion, (2) in nerve terminals of the blood vessels presumably related to a control of blood flow through the ME-LPV system, which is usually not taken into consideration in the hypothalamic control of pituitary function.

P56 PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP)-SIGNALLING PROMOTES CHONDROGENESIS: IMPLICATION OF CALCINEURIN AS A DOWNSTREAM TARGET

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Pituitary adenylate cyclase activating polypeptide (PACAP) is an important neurotrophic factor influencing differentiation of neuronal elements and exerting protecting role during injuries of the central nervous system. Although increasing evidence is available on its presence and protecting function in various peripheral tissues, little is known about the role of PACAP in formation of skeletal elements. To this end, we aimed to map elements of PACAP signalling in developing cartilage under physiological conditions and during oxidative stress. mRNAs of PACAP and its receptors (PAC1,VPAC1, VPAC2) were detectable during differentiation of chicken limb bud-derived chondrogenic cells in micromass cell cultures. Expression of PAC1 protein showed a peak on days of final commitment of chondrogenic cells. Administration of either the PAC1 receptor agonist PACAP 1-38, or PACAP 6-38 that is generally used as a PAC1 antagonist, augmented cartilage formation, stimulated cell proliferation and enhanced PAC1 and Sox9 protein expression. Both variants of PACAP elevated the protein expression of the Ca-calmodulin dependent Ser/Thr protein phosphatase calcineurin. Exogenous PACAPs compensated the chondrogenesis-inhibiting effect of oxidative stress along with partial rescue of calcineurin activity. Taken together, PACAP 1-38 and PACAP 6-38 had the same effects in chicken micromass cultures: both peptides promoted cartilage formation and exerted chondroprotective effect during oxidative stress, although to a different extent. Our results suggest calcineurin as a downstream target of PACAP signalling in differentiating chondrocytes and we propose PACAP as a natural agent that might be applied as a therapeutic substance to protect articular cartilage and/or to promote cartilage regeneration during injuries of joints.

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P57 FUNCTIONAL PROTECTIVE EFFECT OF PACAP ON CHRONIC RETINAL ISCHEMIC INJURY IN RATS – ELECTRORETINOGRAPHIC MEASUREMENTS

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The retinoprotective effects of PACAP are well-known and have been demonstrated in various pathological conditions, such as diabetic retinopathy, excitotoxic retinal injury, UV light-induced degeneration and ischemic retinal lesion. In these models, intravitreal injection of PACAP has been shown to lead to morphological protection: the degeneration observed in the different retinal layers could be decreased by PACAP. However, whether this morphological improvement is also reflected in functional amelioration has remained an important question. Therefore, our purpose was to investigate the protective effect of PACAP on the rat retina after chronic ischemic injury with functional methods and to parallel the functional data with the morphological results. Chronic retinal ischemia was induced by bilateral common carotid artery occlusion (BCCAO). Control eyes received saline treatment and PACAP was injected after the operation into the vitreal space of the other eye immediately after the induction of ischemia. Retinal damage was quantified by the functional changes on the electroretinography (ERG) recordings on the postoperating days 2, 6, 10 and 14. Morphological measurements were conducted by measuring the thickness of the retinal layers and the number of the retinal ganglion cells on day 14. Our results show that BCCAO caused marked damage in both the histological structure and the electrical response recorded by ERG. The protective effect of PACAP was detected in the treated eyes with ERG and histological measurements. These data thus confirm that the morphological protection inudced by PACAP treatment is reflected in functional improvement in ischemic retinal lesions.

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P58 PACAP-DEPENDENT NEURAL REGENERATION IN THE EARTHWORM *EISENIA FETIDA*

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The regeneration of the cerebral ganglion (Cg) was investigated in the earthworm *Eisenia fetida* applying two absolutely different extirpation methods, respectively. In the first group of animals both of the circumpharyngeal connectives (CCs) were transected, in the second group the left CC was transected and the right one was cauterized on the level of the second segmental nerves. During regeneration the concentration gradient of PACAP along the central nervous system and the pattern of the GABAergic landmark structures were investigated. Following Cg extirpation a marked increase of PACAP synthesis was found in the ventral nerve cord. However, a decreasing gradient of PACAP from the subesophageal ganglion (SG) to cross-cut CC was found in regenerating earthworms while in the cauterized CC the PACAP concentration was significantly higher than in the SG suggesting that neural processes transported PACAP and other neuropeptides, transmitters etc. to the site of regeneration. High number of PAC1-receptor expressing cells was located in the regenerating blastema developing close to the cross-cut surface of the CCs. Most of them proved to be stem cells of earthworms. When both CCs were intersected prior to Cg ablation the renewed Cg became identical, both in size and GABA labelling, with the extirpated one in the third week of the regeneration. If one of the CCs was cut through and the other one cauterized during the Cg extirpation, an absolutely asymmetric Cg regenerated: neither its size and shape nor GABA labelling were identical with the excised one. At the cross-cut side approximately a hemiganglion of normal size, while at the cauterized side a significantly smaller one regenerated. In the former one the number and pattern of GABAergic landmark structures were the same as were seen in ablated hemiganglion while in the latter one the reduction of landmark structures, both in the number and position, was observed. In the cauterized CC neither GABA immunoreactive neurons nor migrating undifferentiated cells were found. However, its diameter often increased up to twofold of the original size because of the axon swelling as the consequence of the blocked axonal transport. These findings strongly suggest that the dedifferentiation of neurons and their migration along the ventral nerve cord do not contribute to the Cg regeneration. In contrast the elaboration of neuroactive substances (transmitters, neurohormones like PACAP, growth factors) from the central nervous system via the CCs could mediate the migration and attachment of stem cells to the cut surface of CC and could mediate their differentiation to neuronal, glial, muscular and connective tissue cells resulting in the formation of a new Cg and its capsule. This hypothesis is supported by the results of pharmacological experiments, namely injection of PACAP-antagonist to the site of regeneration strongly inhibited differentiation of neural somata and growing of processes, so the structure of the regenerated Cg was significantly less organized than the original Cg was.

P59 PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) PREVENTS MONOSODIUM GLUTAMATE (MSG) INDUCED FUNCTIONAL DISTURBANCES IN THE MOUSE RETINA *IN VITRO*

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Purpose: MSG binds to glutamate receptors and provokes a chronic activation of postsynaptic neurons, thereby exerting excitotoxic effects. We studied the short term functional consequences of MSG treatment in the mouse retina. We investigated whether administration of PACAP1-38, a neuroprotective peptide, could rescue retinal ganglion cells (RGCs) from MSG-induced excitotoxic effects.
Methods: Spontaneous and light-evoked spikes of RGCs from wild type mice were recorded using a 60-channel multielectrode array. Green (λ = 527nm) light was used to generate full field stimuli. Retinas were treated with MSG (10 mM) or a mixture of MSG + PACAP1-38 (10 μM; 15 min) or MSG + PACAP1-38 antagonist PACAP6-38 (1μM; 15 min) + PACAP1-38. Data were analyzed using the Off-line Sorter/NeuroExplorer software package.
Results: MSG exerted physiologically detectable effects on RGCs only when applied at a concentration >10 mM. These included a characteristic increase of spontaneous spiking 4-5 minutes after drug application. During this time, spike correlations between RGC pairs were reduced. However, after 10-15 minutes of MSG application, the spontaneous activity of most RGCs was dramatically reduced or totally eliminated. Pretreatment with PACAP1-38 prevented the MSG effects as indicated by little or no change in the spontaneous spiking patterns during the course of recordings (up to 60 minutes). In addition, MSG blocked the light-evoked responses of all recorded cells. The light-evoked responses of approximately 40% of RGCs were retained following pretreatment with PACAP1-38.
Conclusions: We found that MSG had clear short term effects on the spontaneous and light-evoked spiking of mouse RGCs. Application of PACAP1-38, well-known for its long term neuroprotective effects, also rescued RGCs from the short term MSG-induced insults. We propose that PACAP1-38 exerts its protective effects either through desensitization of postsynaptic glutamate receptors and/or the extrusion of excess glutamate from the synaptic gap.
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P60 A COMPARATIVE HISTOLOGICAL ANALYSIS OF VARIOUS INTERNAL ORGANS IN WILD AND PACAP KO MICE

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide present in two forms with 27 and 38 amino acid residues. PACAP exerts various biological effects in the nervous system and peripheral organs. PACAP knockout mice provide an excellent opportunity to study the endogenous actions of PACAP. One of the most studied effects of PACAP are its cytoprotective actions. Data available so far indicate that in most cases, the structure and function of organs under intact conditions resemble those of normal animals, but under stressed or challenged conditions, PACAP knockout mice show increased vulnerability, In the present study we decided to examine the histology of various organs in the cardiovascular, gastrointestinal, renal, respiratory, immune and reproductive systems of PACAP deficient mice. The objective of this study is to compare identical tissue samples from wild and PACAP deficient mice and evaluate the similarities or differences, if any. Male and female wild and PACAP knockout mice in the same age group reared under same conditions were used for this study. The animals were sacrificed to collect tissue samples from various organ systems. The tissue samples were processed for routine microscopical analysis and slides were prepared with H & E stains for light microscopy. Photomicrographs were obtained from identical tissue samples and areas under varying magnifications for further comparison and evaluation. Our results show that under unchallenged conditions there are no basic morphological differences between wild type and PACAP KO animals. However, analyzing tissues with electron microscopy, immunohistochemistry or protein composition, various differences can be observed, most of which cause an imbalance in survival/death pathways and cause an increased vulnerability of PACAP KO mice towards different stressors.

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P61 INVESTIGATION OF THE POSSIBLE ROLE OF PACAP ON HUMAN TROPHOBLAST FUNCTIONS

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Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors have been shown in the placenta but its functions in placental growth have not been elucidated yet. The aim of our study was to investigate the effects of PACAP on invasion, proliferation, cell survival and angiogenesis of trophoblast cells. Furthermore, cytokine production was investigated in human decidual and peripheral blood mononuclear cells. For in vitro studies, human invasive proliferative extravillous cytotrophoblast (HIPEC) cells were used for the proliferation assay, and HTR-8/SVneo human trophoblast cells were used for the rest of the studies. Invasion was studied by standardized invasion assay, where we found no difference between control, PACAP38- and the PACAP antagonist PACAP6-38-treated cells. Angiogenic molecules were determined both in the supernatant and the cell lysate by angiogenesis array. In the supernatant we found that PACAP decreased the secretion of various angiogenic markers, such as angiopoetin, angiogenin, activin, endoglin, ADAMTS-1 and VEGF. Cell survival was studied under oxidative stress conditions induced by hydrogen peroxide. PACAP co-treatment had no effects, however, using PACAP as pre-treatment significantly increased the number of surviving HTR8 cells. Using HIPEC cells, we found that PACAP increased proliferation. For the cytokine assay, human decidual and peripheral blood lymphocytes were separated and treated with PACAP. Th1 and Th2 cytokines were analyzed with CBA assay and the results showed that there were no significant differences in control and PACAP-treated cells. In summary, PACAP seems to play a various roles in human trophoblast cells, the details of which need to be further investigated.

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P62 PACAP LEVEL MEASUREMENT IN THE CEREBROSPINAL FLUID AND PLASMA OF SEVERELY HEAD-INJURED PATIENTS: A NEUROPROTECTIVE AGENT, A POTENTIAL NEW BIOMARKER OR JUST A CONSEQUENCE OF THE BLOOD-BRAIN BARRIER DAMAGE?

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PACAP has well-known neuroprotective potential including traumatic brain injury. Its level is up-regulated following various insults of the CNS in animal models. A few studies have documented alterations of PACAP levels in human serum. The time course of post-ictal PACAP levels show correlation with migraine severity and we have also shown changes of PACAP levels in the serum during pregnancy. The presence of PACAP has not yet been detected in human cerebrospinal fluid. Moreover very little is known about the course of PACAP levels following CNS injury in humans. The aim of the present study was to determine whether PACAP occurs in the cerebrospinal fluid (CSF) and the plasma (Pl) in patients suffered severe traumatic brain injury (TBI) and to establish a time course of its levels in the CSF and Pl in 38 patients from the Pecs Severe Head Injury Database (post-resuscitation GCS ≤ 8). Samples were taken daily, until the time of death or for maximum 10 days. Control samples were taken from patients with negative diagnosis for neurological disease. Our results demonstrated that PACAP was detectable in the CSF, with higher concentrations in patients with TBI. PACAP concentrations markedly increased in both Pl and CSF in the majority of patients 24-48 hours after the injury stayed high thereafter. In cases of surviving patients, Pl and CSF levels displayed parallel patterns, which may imply the damage of the blood-brain barrier. However, in patients, who died within the first week, Pl levels were markedly higher than CSF levels, possibly indicating the prognostic value of high Pl PACAP levels.

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P63 EFFECTS OF EARLY HYPERGLYCAEMIA, INSULIN AND PACAP TREATMENT ON THE RETINAL STRUCTURE OF RAT PUPS

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Aims: In spite of major advances in understanding of the pathogenesis, retinopathy of prematurity (ROP) is still one of the leading cause of childhood blindness in developed countries. ROP shares several common features with diabetic retinopathy (DR), in which pathogenic role of hyperglycaemia is well established. Rat pups are applicable to investigate specific role of the factors which are implicated in the pathogenesis of ROP including hyperglycaemia and insulin treatment. The aim of our study was to investigate specific effect of streptozotocin-induced hyperglycaemia, insulin-treatment and intravitreal injection of a potential retinoprotective agent, pituitary adenylate cyclase activating polypeptide (PACAP) on the rat pups' retina.
Methods: We made a comparative analysis between the following treatment-groups: controls (Stz-/Ins-), insulin-treated (Stz-/Ins+), hyperglycemic (Stz+/Ins-), insulin-treated hyperglycaemic (Stz+/Inz+); all animals were treated with intravitreal PACAP or vehicle. Blood glucose levels were monitored. After decapitation (P21) retinas were processed for routine histology and immunohistochemistry for glial fibrillary acidic protein (GFAP), GLUT1 and tyrosine hydroxylase (TH).
Results: Standard histological methods revealed no major differences between the groups. Elevated expression of GFAP - as an aspecific marker of metabolic insults in the retina- was detected from the inner retina in the Stz-/Ins+ group, although hypoglycaemia did not develop. Similar alteration of the GFAP staining was found in the hyperglycaemic (Stz+/Ins-) and insulin-treated hyperglycaemic (Stz+/Inz+) groups. Intravitreal PACAP resulted in suppression of the elevated GFAP expression in the Stz-/Ins+ group, but not in the Stz+/Ins-, and Stz+/Inz+ ones. None of the groups showed alteration in the anti-TH immunoreactivity (dopaminergic amacrine cells) or GLUT1 expression of pigment epithelial cells.
Conclusion: In our model hyperglycaemia or insulin did not induce ROP, however, sign of metabolic insult was detected in the neural retina, which was partly prevented by intravitreal PACAP application.
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P64 THE ROLE OF PACAP IN THE AVIAN CIRCADIAN CLOCK: PHASE RESETTING OF TRANSCRIPTIONAL OSCILLATIONS?

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In the mammalian hypothalamic circadian clock, PACAP is an important player which transmits phase resetting stimuli via the retinohypothalamic tract. In birds, the pineal gland may possess oscillator properties similar to that of the suprachiasmatic nucleus (SCN) of mammals. However, neuronal transmission of daylight information from the retina is indirect, and PACAP positive axon terminals in the gland originate from neurones localized not in the retina. Furthermore, in vitro treatments with PACAP1-38 were previously ineffective in resetting the phase of the circadian melatonin rhythm in the avian pineal gland. On the other hand, PACAP treatments in nanomolar concentrations were shown to alter the mRNA expression of clock genes in the chicken embryonic pineal gland, similarly to that seen in the mammalian SCN. In our present study we measured in vitro the 24 h patterns of melatonin release and the expression of AANAT, Hiomt, VIP, Dio2, Ndrg2, RORb, Clock, Cry1, Dusp and Purpurin mRNAs, which were known to show >2 fold amplitude circadian oscillations in the chicken pineal gland both in vivo and in vitro. We compared the effects of PACAP1-38 treatments of micromolar vs. nanomolar concentrations in superfusion experiments, in 1h administrations at ZT14 vs. ZT22 (i.e. 2 hours after lights off vs. 2 hours before lights on). We have been succesful to confirm in the embryonic chicken pineal model that these treatments show time and dose dependent alterations of transcriptional oscillations. Our data suggest that treatments with PACAP1-38 may induce various signalling mechanisms with detectable transcriptional effects for which a potential in phase resetting of this non-mammalian clock model needs to be tested in further experiments.

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P65 EXPRESSION OF VIP MRNA IN AN AVIAN MODEL OF THE CIRCADIAN CLOCK

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In the mammalian hypothalamic circadian clock, VIP is not only a key neuropeptide but also an important paracrine signal to synchronize neuronal oscillators within the suprachiasmatic nucleus. In lower vertebrates, the pineal gland posseses oscillator properties similar to that of the suprachiasmatic nucleus of mammals: the pinealocytes keep running synchronized to each other for several days under in vitro cultured conditions. However, factors which maintain the coherent synchrony among oscillator units of the pineal gland were not known earlier. In our study we have investigated the 24 hour expression profile of VIP transcription in the chicken pineal gland both in vivo and in vitro. Peak levels of VIP mRNAs were detected at the same time with that of AANAT mRNA, the key enzyme of melatonin synthesis. VIP mRNAs were about 100 folds more abundant than AANAT mRNA during the time of peak melatonin release (i.e. at late subjective night). Since VIP is known to be expressed in the suprachiasmatic nulceus of mammals at the same time (subjective night), our finding suggests that VIP produced in the chicken pineal gland may be a key paracrine signal to maintain intrinsically synchronized oscillations of rhythmic pinealocyte functions. Previous research has demonstrated the presence of VIP in axon terminals and mRNA expression of VPAC1 receptors in the pineal gland of vertebrates, but to our knowledge this is the first demonstration of VIP mRNA expression within the avian pineal gland.
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