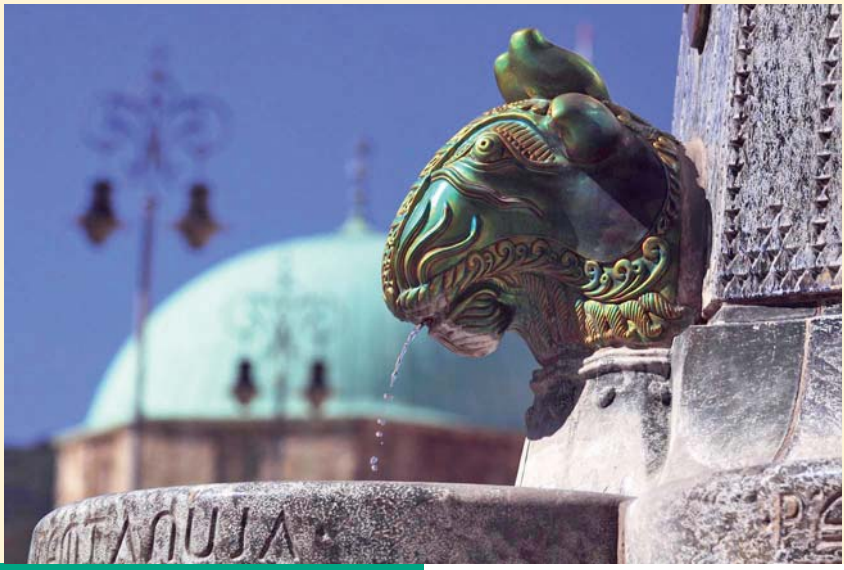
The 11th International Symposium on VIP, PACAP and Related Peptides



27-31 August 2013
PÉCS, HUNGARY

ÚJ SZÉCHENYI TERV

Mintaelőkészítés

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The organizing committee of the 11th International Symposium on VIP, PACAP and Related Peptides is pleased to invite scientists working in the field of VIP, PACAP and related peptides to join us at this exciting event.

This symposium is the 11th time that scientists sharing common interest in this peptide family come together and discuss their research. The main goal of the symposium is to discuss most recent findings on different functional, structural and genomic aspects of the VIP, PACAP family of peptides.

Our goal is also to promote scientific collaborations between different groups. We would like to invite young scientists to join us and get to know the people working in this field as well as the newest results. We believe that such personal encounters could be a driving force in their carriers.

The symposium is held in Pecs, a multicultural city of Central Europe. Pecs is the fifth largest city in Hungary with a cozy Mediterranean atmosphere and a lively environment. Pecs has a more than 2000-year-old history. The city is rich in Roman, Turkish, Hungarian and Slavic cultural heritage and thanks to the position of the city as Cultural Capital of Europe in 2010, many recent modern developments can also be seen.

The host institution is the University of Pecs, which is a university with 10 faculties, with more than 30 thousand students. The medical faculty has international programs, where more than 2000 students from all over the world are enrolled. This international atmosphere is also visible in the scientific activity of the faculty, which has a long tradition of promoting and conducting science.

The conference venue is the Szentagothai Research Centre, which was inaugurated in 2012, in the year of the 100th birthday of one of the greatest Hungarian scientists. Professor Szentagothai was chair of the Anatomy Department here in Pecs, 1946–1963. One of his main research areas was neuroendocrinology, with special interest in the hypothalamo-hypophyseal system. His followers established the collaboration between Pecs University and the US-Japan Biomedical Research Laboratories led by dr. Arimura in New Orleans. Thanks to dr Arimura's efforts to help Hungarian scientists from Pecs, he was elected a honorary doctor of the University of Pecs. The conference organizers are members of the PACAP Research Team of the University of Pecs and Hungarian Academy of Sciences. The team has a history of conducting PACAP research for more than 10 years, following the heritage of dr. Akira Arimura. Thus, hosting the VIP, PACAP symposium in the Szentagothai Research Centre has symbolic meaning for us and is a true honor. We are looking forward to welcoming all of you here in Pecs.

Dora Reglodi
chair of the org.committee

Andrea Tamas
secretary of the org. committee

ORGANIZING COMMITTEE

Dora Reglodi MD.,PhD.,DSc.
chair of the organizing committee

Andrea Tamas MD.,PhD.
secretary of the organizing committee

PTE-MTA „Lendulet" PACAP Research Team

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Katalin Csanaky MD.
Jozsef Farkas MD.
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CONFERENCE VENUE

University of Pecs
Szentagothai Research Centre
H-7624 Pecs, Ifjusag street 20.

The state-of-the-art research centre, also known as Science Building opened its gates for the public on 27th June 2012 in Pecs, Hungary.

Three identical blocks heated with three different alternative energy methods and a conference room shaped as a stone welcomes scientists of the 22 most current fields from microbial biotechnology to smart city technologies. The newest facility of University of Pecs was named after the renowned Hungarian brain scientist, Janos Szentagothai in October 2012.

The aim of the Szentagothai Research Centre is to enhance the basic research potential at the areas of natural, engineering and health sciences, which will enable the University to enter international tenders. The world standard laboratories and equipment also gives our students the opportunity to study and do research in the most modern environment.

The high-tech facilities and the signature stone-shaped conference room which can host classes and sessions up to 300 people make the Research Centre an ideal conference venue.

REGISTRATION AND INFORMATION DESK

Registration will take place at the conference venue.
The registration desk will have the following opening hours:

Tuesday, August 27th	16:00 – 21:00
Wednesday, August 28th	08:00 – 18:00
Thursday, August 29th	08:30 – 15:00
Friday, August 30th	08:30 – 18:00
Saturday, August 31st	08:30 – 14:00

TECHNICAL INFORMATION TO SPEAKERS

The length of oral lectures is 15 min with 5 min discussion. Please hand over your lecture material with your name and the title of your presentation added, to the technical team working in the lecture room, either in the morning or in the break between sessions.

POSTER PRESENTATIONS

Posters should be placed from Wednesday 09:00, but at the latest by the start of the session (Wednesday 14:00). Posters should be removed by Saturday 12:00. For the duration of the poster sessions authors should be standing in front of their poster and should be prepared for presenting their results to interested attendants.

BADGES AND TICKETS

All participants are requested to wear their badges throughout the scientific programmes. We kindly ask you to bring your tickets for social programmes as well.

COFFEE

During the coffee breaks, coffee with snacks will be served in the Lounge.

LUNCH

Lunch is provided as part of the Registration fee and will be served during the lunch break in the Lounge.

BANKING AND EXCHANGE FACILITIES

Banks are generally open Monday-Friday 9:00 – 17:00. Exchange offices are found throughout town, they are usually open till late evening on weekdays and also on Saturdays and Sundays. ATM machines are also found everywhere.

CREDIT CARDS

All major international credit cards are accepted in most shops and restaurants.

TAXI

You may hail taxis in the street, but it is probably cheaper to order a taxi by phone from the hotel reception or the registration desk. We recommend VOLAN TAXI + 36 72 333 333

ELECTRICITY

The electrical current in Hungary is 230V 50 Hz. Standard continental European outlets are used. Most hotels also provide 110V outlets for shavers.

SMOKING

Smoking is not allowed inside the building.

INSURANCE

The congress organizers cannot accept any liability for personal injuries sustained, or for loss or damage to progerty belonging to congress participants (or their accompanying persons), either during or as a result of the congress. The registration fee does not include insurance.

COCKTAIL PARTY

Date: **27th August (Tuesday) 19:00**
Venue: **Szentagothai Research Centre** (7624 Pecs, Ifjusag street 20.)
Ceremonial opening of the Conference with a reception.

The Szentagothai Research Centre of the University of Pecs is a new research institute established on the basis of modern international science organizational and management norms. It covers all aspects of education, research and innovation in the fields of biomedical, natural and environmental sciences.

RECEPTION

Date: **28th August (Wednesday) 18:30**
Venue: **Cathedral of Pecs** (7621 Pecs, Szent Istvan square 14.)
Dom Museum (7621 Pecs, Kaptalan street 8.)
Meeting point: **Cathedral of Pecs** at 18:30
Travelling: individually, recommended taxi companies are
Volan Taxi (+3672/333-333) or **Euro Taxi** (+3672/777-777)

Visiting the Cathedral of Pecs and Dom Museum with guidance in English. Then there will be a reception on the roof terrace of Dom Museum. During this time Vivat Bachus Ensemble will provide a pleasant atmosphere. In case of bad weather it will be inside the museum.
The Cathedral of Pecs is one of the most outstanding buildings of Pecs. Its four towers can be seen from far away.
At the exhibition of Dom Museum you can see the main entrance of the old church. The stone work of the museum has more than a thousand stone carvings from the Arpad age.
Vivat Bachus is a 5 piece acapella ensemble founded in 2001. Their repertoire includes a variety of music genres such as jazz, blues, or rock&roll and wine songs, providing a joyful atmosphere.

MEDIEVAL CASTLE IN SIKLOS – DINNER AT TENKES CSÁRDA

Date: **29th August (Thursday) 15:00**
Venue: **Castle in the town Siklos** (7800 Siklos, Vajda Janos square 8.)
Tenkes Csarda (7815 Csarnota, Kultelek 11.)
Meeting point: Buses depart from the venue of the Symposium (Szentagothai Research Centre) at 15:00. From about 22:00 buses will come back to Pecs.

Visiting the medieval castle in Siklos will include a knight show. The Tenkes 'Csarda' (Inn, pronounced as 'tsarda') lies in Csarnota, along one of the most famous wine-making regions in Hungary. Keeping with old Hungarian style and flavours, fresh bread comes right from the woodburning oven. Goulas, the famous Hungarian meal, is quite delicious here. Native Hungarian and Gypsy music makes every course complete. Before your dinner is served, Mecsek Folk Dance Ensemble will dazzle you. Owner of an 'Excellent Group' title, they have had success on Hungarian festivals and abroad.

GALA DINNER AT ZSOLNAY CULTURAL QUARTER

Date: **30th August (Friday) 19:00**
Venue: **Zsolnay Cultural Quarter** (7630 Pecs, Zsolnay Vilmos street 37.)
Meeting point: Buses depart from the venue of the Symposium at 18:45

Welcome to Zsolnay Cultural Quarter, the extraordinary venue of the Gala Dinner! The largest scale investment programme of the Pecs2010 – European Capital of Culture Project was the reconstruction work carried out on the area of the Zsolnay Porcelain Manufacture, turning part of the old factory into the largest cultural quarter of Eastern Europe.

Twenty five buildings of historic value in beautiful surroundings on some 50 thousand square metres filled with exhibitions, cafes, restaurants, shops, a handcraft quarter, university quarter, puppet theatre halls – this is Zsolnay Cultural Quarter, the legend of Pecs reborn, awaiting all those seeking a creative experience and an exciting cultural adventure.

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MAGYARORSZÁG MEGÚJUL

A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.

28th August, Wednesday – Buffet lunch

- Pljeskavica and Csevap

  It is a typical and authentic croatian food, grilled steak made from beef, pork and calf with ajvar, salad and steak fries. This meal is one of the best meal of the summer.

- Dessert: ice cream

29th August, Thursday – Buffet lunch with sandwiches

- Sandwiches (5 pieces/person):
  - Sango radish with eggplant cream (vegetarian)
  - Vegetable tartar with Curry and with caperberry (vegetarian)
  - With baked ham and green peppers
  - Home made smoked sausages with cherry tomatoes
  - Gravlax (raw spiced salmon) with lemony (limett) wasabi cream
- Salads:
  - Greek salad with stewed feta cheese
  - Classic „Károlyi" salad
    boiled potato, pickled cucumber, paprika, tomato, boiled egg, salad all mixed with seasoned mayo dressing.
  - Crispy selected salad with raspberry vinegar dressing and toasted seeds
- Dessert: ice cream

Teleky catering

30th August, Friday – Buffet lunch

- Honeyed-minty fruit soup served ice-cold
- Potato Casserole with sausages, home made bred and with gherkins
- Dessert: ice cream

31st August, Saturday – Buffet lunch

- Bajaian Carp Fish soup
  Is one of our best-known typical hungarian national dishes beside goulash.
- Traditional Cottage cheese with pork greaves and ample sour cream
- Dessert: ice cream

Note:
– Lunch is available every day at the venue of the conference.
– It is available with your badge.

These delicious, stylish and qualitative food specialities are served by Teleky Restaurant.

AUGUST 27TH, TUESDAY	
18:00–19:00	Welcome reception, Memorial remarks
19:00–	Cocktail Party

AUGUST 28TH, WEDNESDAY	
09:30–10:50	**SYMPOSIUM 1.** RECEPTORS OF VIP, PACAP AND RELATED PEPTIDES, DRUG DEVELOPMENT *(Chairs: Atsuro Miyata and Alain Covineau)*
10:50–11:40	Coffee break
11:40–13:00	**SYMPOSIUM 2.** DEVELOPMENTAL ASPECTS OF VIP, PACAP AND RELATED PEPTIDES *(Chairs: Seiji Shioda and David Vaudry)*
13:00–14:00	Lunch
14:00–15:30	**POSTER SESSION**
15:30–17:30	**SYMPOSIUM 3.** CLINICAL ASPECTS OF VIP, PACAP AND RELATED PEPTIDES *(Chairs: Victor May and Tomoya Nakamachi)*
18:30–	Reception

AUGUST 29TH, THURSDAY	
09:00–10:20	**SYMPOSIUM 4.** DEGENERATION AND CYTOPROTECTION I. *(Chairs: James Waschek and Hirokazu Ohtaki)*
10:20–11:00	Coffee break
11:00–12:10	**SYMPOSIUM 5.** DEGENERATION AND CYTOPROTECTION II. *(Chairs: Yousef Tizabi and Yousef Anouar)*
12:10–14:30	**POSTER SESSION WITH SANDWICH LUNCH**
15:00–	Dinner at Tenkes Csarda

AUGUST 30TH, FRIDAY	
09:00–10:40	**SYMPOSIUM 6.** IMMUNOLOGY, PAIN AND STRESS I. *(Chairs: Mario Delgado and Zsuzsanna Helyes)*
10:40–11:00	Coffee break
11:00–12:20	**SYMPOSIUM 7.** IMMUNOLOGY, PAIN AND STRESS II. *(Chairs: Hitashi Hashimoto and Vincent Lelievre)*
12:20–13:20	Lunch
13:20–14:30	**SYMPOSIUM 8.** EVOLUTIONARY ASPECTS OF VIP, PACAP AND RELATED PEPTIDES *(Chairs: Gyorgy Kemenes and Leo Lee)*
14:30–16:00	**POSTER SESSION WITH COFFEE**
16:00–17:50	**SYMPOSIUM 9.** VASOACTIVE INTESTINAL PEPTIDE IN MEMORIAM SAMI I. SAID *(Chairs: Rosa Gomariz and Illana Gozes)*
19:00–	Gala Dinner

AUGUST 31ST, SATURDAY	
09:00–10:40	**SATELLITE SYMPOSIUM ON REPRODUCTIVE ASPECTS OF PACAP** *(Chairs: Dora Reglodi and Jozsef Bodis)*
10:40–11:00	Coffee break
11:00–12:40	**NEUROTOXICITY SOCIETY HUNGARIAN SATELLITE MEETING** *(Chairs: Gilles Guillemin and Richard M. Kostrzewa)*
12:40–	Closing the conference and Lunch

August 27th, Tuesday

16:00–21:00 Registration

18:00 **Welcome reception in János Szentágothai Research Centre (conference venue)**
Dora Reglodi chair of the organizing commitee
Department of Anatomy, University of Pecs, Hungary

18:10 **In memoriam Dr. Sami I. Said**
Anthony Szema (via skype)
Stony Brook University School of Medicine, USA

18.25 **Arimura Akira, Behind and Beside PACAP**
O01 Memorial remark by *Sandor Vigh*
Department of Anatomy, School of Medicine, Ross University, Dominica

18.40 **Cradle of PACAP: Belle Chasse, Herbert Center**
O02 *Katalin Koves*
Department of Human Morphology and Developmental Biology, Semmelweis University, Hungary

19.00 **Cocktail Party**

August 28th, Wednesday

08:00–18:00 Registration

09.00 **Opening of the conference – Arimura Award Ceremony**
Jozsef Bodis Rector of University of Pecs
Dora Reglodi chair of the organizing committee
Andrea Tamas secretary of the organizing committee
Katie Arimura and *Mark Arimura,* Arimura Foundation

09.30–11.10 SYMPOSIUM 1. RECEPTORS OF VIP, PACAP AND RELATED PEPTIDES, DRUG DEVELOPMENT

(**Chairs:** *Atsuro Miyata* and *Alain Covineau*)

09.30 **Cross-linking of Sp1 by transglutaminase2 suppresses PAC1 gene expression in neuronal cells**
O03 *Ayako Miura*
Department of Pharmacology, Graduate School of Medical and Dental Sciences, University of Kagoshima, Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Japan

09.50 **PACAP effects on CRMPs regulation in PC12 cells**
O04 *Roger Cubi*
INSERM Unit 982, Universite de Rouen, Differenciation et Communication Neuronale et Neuroendocrine, Mont-Saint-Aignan, France

10.10 **Acute activation of astrocytes in spinal dorsal horn via PAC1 receptor is involved in PACAP-induced persistent aversive behavior**
O05 *Atsuro Miyata*
Departments of Pharmacology and Anesthesiology, Graduate School of Medical and Dental Sciences, Kagoshima University, Japan

10.30 **The preparation of the recombinant VIP-TAT and its effects on the scopolamine-induced amnesia in mice**
O07 *Rongjie Yu*
Life Science Department of Jinan University, Guangzhou, P. R. of China

10:50–11:40 Coffee break

11.40–13.00 SYMPOSIUM 2. DEVELOPMENTAL ASPECTS OF VIP, PACAP AND RELATED PEPTIDES

(**Chairs:** *Seiji Shioda* and *David Vaudry*)

11.40 **Human stem/progenitor cells from bone marrow improve spinal cord injury via communicating with PACAP**

O08 *Hirokazu Ohtaki*
Department of Anatomy, Showa University School of Medicine, Tokyo, Japan

12.00 **Effects of PACAP on differentiation processes of UMR106 osteoblast cell line**

O09 *Tamas Juhasz*
Department of Anatomy, Debrecen, Hungary

12.20 **Cross-talks between the VIP-receptors system, Akt/PTEN and Hedgehog pathways in the regulation of glioblastoma migration and invasion**

O10 *Jean-Marc Muller*
Team 'Recepteurs Regulations Cellules Tumorales', Universite de Poitiers, Faculte des Sciences, CNRS FRE3511, Poitiers, France

12.40 **Comparison of tooth development in wild type and PACAP-deficient mice**

O11 *Balazs Sandor*
Department of Dentistry, Oral and Maxillofacial Surgery, University of Pecs, Hungary

13.00–14.00 Lunch

14.00–15.30 POSTER SESSION

15.30–17.30 SYMPOSIUM 3. CLINICAL ASPECTS OF VIP, PACAP AND RELATED PEPTIDES

(**Chairs:** *Victor May* and *Tomoya Nakamachi*)

15.30 **VPAC2 receptor, a novel target in the treatment of multiple sclerosis**

O12 *Yossan-Var Tan*
Neuropsychiatric Institute – Semel Institute, University of California Los Angeles, USA

15.50 **PACAP stimulates the corneal healing via lacrimal-mediating and direct pathways in mouse**

O13 *Tomoya Nakamachi*
Department of Anatomy, Center for Biotechnology, Showa University School of Medicine, Shinagawa-Ku, Tokyo, Japan

16.10 **Urokinase plasminogen activator system in synovial fibroblasts from osteoarthritis patients: modulation by inflammatory mediators and neuropeptides**

O14 *Selene Perez-Garcia*
Departamento de Biologia Celular, Facultad de Biologia, Universidad Complutense de Madrid, Madrid, Spain

16.30 **The pathogenic phenotype and the plasticity of Th17 profile from rheumatoid arthritis patients are modulated in vitro by Vasoactive Intestinal Peptide**

O15 *Yasmina Juarranz*
Departamento de Biologia Celular, Facultad de Biologia, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, Spain

16.50 **PACAP/PKA reduces polyglutamine androgen receptor toxicity in cell models of spinal and bulbar muscular atrophy**

O16 *Maria Pennuto*
Department of Neuroscience and Brain Technology, Istituto Italiano di Tecnologia, Genova, Italy

17.10 **Vasoactive neuropeptides in chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): Possible pathomechanisms in a human disease**

O17 *Donald R. Staines*
National Centre for Neuroimmunology and Emerging Diseases, Griffith University and Gold Coast Health, Australia, Gold Coast Public Health Unit, Robina, Australia

18.30 **Evening program**
Visiting Pecs UNESCO World Heritage Site
Visiting Pecs Cathedral and dinner at Dom Museum

August 29th, Thursday

08:30–15:00 Registration

09.00–10.20 SYMPOSIUM 4.
DEGENERATION AND CYTOPROTECTION I.
(**Chairs:** *James Waschek* and *Hirokazu Ohtaki*)

09.00 **Loss of PACAP in mice sensitizes nigrostriatal dopaminergic neurons to paraquat-induced injury and modulates microglia and peripheral T cell activation**
O18 *James Waschek*
University of California, Los Angeles, USA

09.20 **VIP/PACAP-regulated activity-dependent neuroprotective protein (ADNP) and NAP: microtubule protection**
O19 *Illana Gozes*
The Lily and Avraham Gildor Chair for the Investigation of Growth Factors, Adams Super Center for Brain Studies, and Department of Human Molecular Genetics and Biochemistry, Sagol School of Neuroscience, Sackler Faculty of Medicine, Tel Aviv University, Israel

09.40 **Improvement of behavioral deficits and tau phosphorylation by NAP (davunetide) in the Thy1-aSyn model of Parkinson's disease**
O20 *Iddo Magen*
Tel Aviv University, Israel

10.00 **Protective effects of PACAP against salsolinol- and inflammatory-mediated toxicity in SH-SY5Y cells: Implications for Parkinson's disease**
O21 *Yousef Tizabi*
Department of Pharmacology, Howard University College of Medicine, Washington DC

10.20–11.00 Coffee break

11.00–12.10 SYMPOSIUM 5. DEGENERATION AND CYTOPROTECTION II.

(**Chairs:** *Yousef Tizabi* and *Yousef Anouar*)

11.00 **Effect of PACAP on Cerebral Endothelial Cells**
O22 *Imola Wilhelm*
Institute of Biophysics, Biological Research Centre, Szeged, Hungary

11.20 **Identification and characterization of a novel thioredoxin reductase involved in the neuroprotective effect of PACAP**
O23 *Youssef Anouar*
INSERM U982, Laboratoire de Differenciation et Communication Neuronale et Neuroendocrine, Institut de Recherche et d'Innovation Biomedicale, Mont-Saint-Aignan, France

11.40 **Molecular mechanisms underlying the nephroprotective effects of PACAP in diabetes**
O24 *Eszter Banki*
Department of Anatomy, PTE-MTA „Lendulet" PACAP Research Team, Medical School, University of Pecs, Hungary

11.55 **Examination of the protective effects of PACAP in rat diabetic nephropathy**
O25 *Daniel Nagy*
Department of Anatomy, PTE-MTA „Lendulet" PACAP Research Team, Department of Pharmacology and Pharmacotherapy, Medical School, University of Pecs, Hungary

12.10–14.30 POSTER SESSION WITH SANDWICH LUNCH

15.00 **Social program**
Visiting medieval castle in the town Siklos with knight show, dinner in a traditional Hungarian restaurant with folk dance and gipsy music (Tenkes csarda).

August 30th, Friday

08:30–18:00 Registration

09.00–10.40 SYMPOSIUM 6. IMMUNOLOGY, PAIN AND STRESS I.

(**Chairs:** *Mario Delgado* and *Zsuzsanna Helyes*)

09.00 **Vasoactive intestinal peptide deficient mice exhibit ameliorated immune responses in experimental models of inflammation**
O26 *Catalina Abad*
University of California, Los Angeles, USA

09.20 **Roles of Pituitary Adenylate-Cyclase Activating Polypeptide (PACAP) in nociception and inflammation in an immune mediated arthritis model**
O27 *Zsuzsanna Helyes*
University of Pecs, School of Medicine, Department of Pharmacology and Pharmacotherapy, Pecs, Hungary

09.40 **Capsaicin induces skin inflammation via TRPV1-mediated up-regulation of PACAP**
O28 *Tamas Biro*
DE-MTA „Lendulet" Cellular Physiology Research Group, Department of Physiology, Department of Pharmacology and Pharmacotherapy, University of Debrecen, Debrecen, Hungary

10.00 **PACAP treatment ameliorates toxoplasma gondii - induced encephalitis in mice**
O29 *Markus M. Heimesaat*
Department of Microbiology and Hygiene, Charite – University Medicine Berlin, Germany

10.20 **Antimicrobial activity of a stable analog of Vasoactive Intestinal Peptide**
O30 *Elena Gonzalez-Rey*
Department Cell Biology and Immunology, Institute of Parasitology and Biomedicine „Lopez-Neyra",Spanish National Research Council, Granada, Spain

10.40–11.00 Coffee break

11.00–12.20 SYMPOSIUM 7. IMMUNOLOGY, PAIN AND STRESS II.

(**Chairs:** *Hitashi Hashimoto* and *Vincent Lelievre*)

11.00 **Alterations in PACAP-38-like immunoreactivity in the plasma during ictal and interictal periods of migraine patients**

O31 *Bernadett Tuka*
Department of Neurology, Faculty of Medicine, University of Szeged, Hungary

11.20 **VIP-deficient mice exhibit severe but reversible allodynia to mechanical and cold stimuli**

O32 *Vincent Lelievre*
University of Strasbourg, CNRS UPR3212 Strasbourg, France

11.40 **Application of the three hit theory in PACAP heterozygous mice: maternal separation and chronic stress influence BNST CRF and cpEW Ucn1 in an inverse manner**

O33 *Balazs Gaszner*
Department of Anatomy, University of Pecs, Pecs, Hungary

12.00 **Localization and immunocytochemical characterization of the dorsal vagal nucleus (DMX) neurons projecting to the porcine stomach prepyloric region in physiological state, following stomach partial resection and after prolonged acetylsalicylic acid supplementation**

O34 *Całka Jarosław*
Department of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

12.20–13.20 Lunch

13.20–14.30 SYMPOSIUM 8. EVOLUTIONARY ASPECTS OF VIP, PACAP AND RELATED PEPTIDES

(**Chairs:** *Gyorgy Kemenes* and *Leo Lee*)

13.20 **Pituitary Adenylate Cyclase-Activating Peptide (PACAP) in the teleost fish immune system: from its discovery to their function**

O35 *Juana Maria Lugo Gonzalez*
Center for Genetic Engineering and Biotechnology, Havana, Cuba

13.40 **Reversal of age related learning deficiency by the vertebrate pituitary adenylate cyclase activating polypeptide (PACAP) in Lymnaea**

O36 *Ildiko Kemenes*
Sussex Neuroscience, School of Life Sciences, University of Sussex, Brighton, United Kingdom

14.00 **Transmembrane domain peptides as a new class of drugs to demonstrate the in vivo function of GPCR hetero-oligomerization in water intake**

O37 *Leo T.O. Lee*
School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong

14.30–16.00 POSTER SESSION WITH COFFEE

16.00–17.50 SYMPOSIUM 9.
VASOACTIVE INTESTINAL PEPTIDE IN MEMORIAM SAMI I. SAID
(**Chairs:** *Rosa Gomariz* and *Illana Gozes*)

16.00 **In memoriam Sami Said**

16.10 **Therapeutic effect of VIP on inflammatory cardiovascular disorders: atherosclerosis and autoimmune myocarditis**

O38 *Mario Delgado*
Institute of Parasitology and Biomedicine „Lopez-Neyra", CSIC, Granada, Spain

16.30 **Low baseline serum level of VIP is a marker of worse prognosis in patients with early arthritis**

O39 *Rosa P. Gomariz*
Departamento de Biologia Celular, Facultad de Biologia, Universidad Complutense de Madrid, Madrid, Spain

16.50 **VIP is a negative regulator of mediators involved in the cross-talk of synovial fibroblasts and Th1/Th17 cells in rheumatic diseases**

O40 *Mar Carrion*
University Complutense of Madrid, Faculty of Biology, Department of Cellular Biology, Madrid, Spain

17.10 **Vasoactive Intestinal Peptide maintains the non-patogenic phenotype of human Th17-polarized cells from naive T cells and decreases their Th1 potential**
O41 *Rebeca Jimeno Lumeras*
University Complutense of Madrid, Faculty of Biology

17.30 **PET Imaging of KRAS2 Activated Lung Cancer in Transgenic Mice using VPAC1 receptor specific Cu-64-TP3805**
O42 *Madhukar Thakur*
Departments of Radiology and Radiation Oncology, Thomas Jefferson University, Philadelphia, USA

19.00 **Evening program**
Visiting the Zsolnay Cultural Center and Gala dinner.

August 31st, Saturday

08:30–14:00 Registration

09.00–10.40
SATELLITE SYMPOSIUM ON REPRODUCTIVE ASPECTS OF PACAP
(**Chairs:** *Dora Reglodi* and *Jozsef Bodis*)

09.00 **Peptidomic/proteomic profiling of human embryo sectretome**
O43 *Laszlo Mark*
Department of Analytical Biochemistry, Institute of Biochemistry and Medical Chemistry, Szentagothai Research Centre, Imaging Center for Life and Material Sciences, University of Pecs, Hungary

09.20 **Effects of pituitary adenylate cyclase activating polypeptide on spermatogenesis**
O44 *Dora Reglodi*
Department of Anatomy, PTE-MTA „Lendulet" PACAP Research Group, University of Pecs, Hungary

09.40 **Radioimmunoassay examination of PACAP38-like immuno-reactivity in different milk and infant formula samples**
O45 *Andrea Tamas*
Department of Anatomy, PTE-MTA „Lendulet" PACAP Research Group, University of Pecs, Hungary

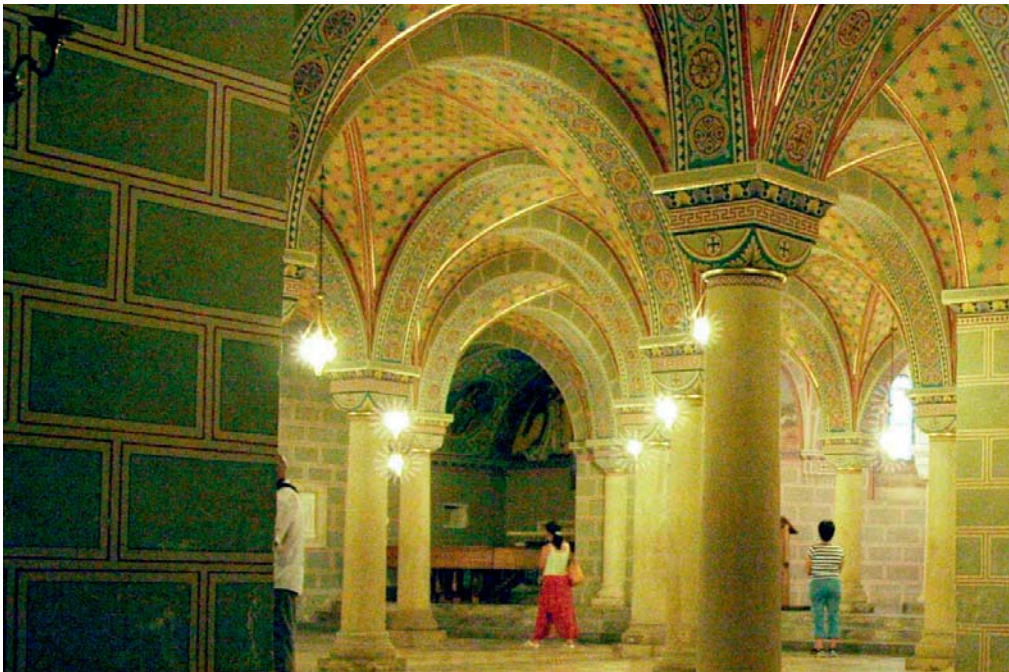
10.00 **Presence of PACAP in human female genital system**
O46 *Miklos Koppán*
Department of Obstetrics and Gynaecology, University of Pecs, Pecs, Hungary

10.20 **Personalized examination of VPAC1 biomarker for detecting genitourinary cancer**
O47 *Madhukar Thakur*
Departments of Radiology, Thomas Jefferson University, Philadelphia, USA

10.40–11.00 Coffee break

11.00–12.40
NEUROTOXICITY SOCIETY HUNGARIAN SATELLITE MEETING
(**Chairs:** *Gilles Guillemin* and *Richard M. Kostrzewa*)

11.00 **Survival promotion of cells expressing amyloid-beta and presenilin by nicotine, AMPA and ketamine: Implications for Alzheimer's disease**
O48 *Yousef Tizabi*
Department of Pharmacology, Howard University College of Medicine, Washington DC, USA



11.20 **PACAP27 is neuroprotective against HIV-Tat neurotoxicity**
O49 *Italo Mocchetti*
Department of Neuroscience, Georgetown University Medical Center, Washington DC, USA

11.40 **Evolution of 'Selective' Neurotoxins**
O50 *Richard M. Kostrzewa*
Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, USA

12.00 **Neuroprotective ability of PACAPs against oxidative stress and excitotoxicity in human primary cortical neurons**
O51 *Gilles J. Guillemin*
MND and Neurodegenerative diseases Research Group; Australian School of Advanced Medicine (ASAM); Macquarie University, Peter Duncan Neuroscience Unit, St Vincent's Centre for Applied Medical Research Department of Neuroimmunology, Darlinghurst, Australia

12.20 **Inflammation, glyapse formation and inhibition of microglial motility in parkinsonism**
O52 *Maria Trinidad Herrero*
Department of Medicine, University Jaume I., Castellon, Spain

12.40 Closing the conference and Lunch

"VILLAGE" ECOBUFFET FROM MECSEK GREENWAY

In our service everything is LOCAL, except the coffe and the cinamon which are fair trade products. All our ingredients are originated from local garden, from the meadow, from the forest, from the lake… We offer fresh seasonal fruits – collected either from the garden either from the forest – vegetables, eatable weeds, eatable flowers. We serve traditional hungarian specialities and new healthy dishes: sandwiches with freshly collected wild garlic, traditional cottage cheese, corn pone, forest salad with eatable flowers. Our selected beverages: herb teas, home made refreshing iced teas, and the amaizing "green juice".

Dishes

- Homemade baker's ware: roll, spicy sandwichbread, bread with mixed seeds, semi-brown bread, „striped" bread (made with wild garlic and seasoning red pepper)
- Smoked sausages and hungarian „headcheese"and dripping
- Homemade farmcheese and cheesecreams: traditional hungarian style, (with seasoning red pepper) with bacon and wild garlic, special with forest „weeds"
- Homemade pestos: zukkini pesto flavoured with wild garlic, lentil pesto, garden bean pesto, bean pesto, hungarian pesto with pepper and onion
- Fresh season salads from the garden and the meadow: with Jerusalem artichoke, buttercup, carrot, tomato, chickweed, wild garlic…
- Sandwich decoration: farm eggs, eatable flowers, wild garlic „berry", fresh „weeeds"
- Tasting the fresh season „weeds" from the meadow and forest, homemade garlic pickled in wine, trad, Hungarian hot „cherry"pepper
- Dried friuits, apple, pear, plum, strawberry, peach, walnut
- Jams: grape, strawbery, apple with elder, plum, apricot, blackberry, blackthorn, rosehip
- Traditional hungarian sweets, e.g. corn pone made with plum marmalade and other cakes

Beverages

Mecsek greenway tea: elderblossom, lanceloar rip grass, calendula, verbascum flower, peony petal; Filly tea: milfoil, tutsan, calendula, citronella, larkspur; Fellow tea: mint, celery, cinamon; Mint tea; Iced tea with various flavours: sour-cherry, strawberry, peach; Green juice: seasonal „weeds" from the meadow (nettle, pig-weed, portulaca…) and fresh garden vegetables (sorrel, fennel, orach…) We belive that there was life in here before intreducing the lemmon so we flavour the teas with honey and home made wild-pear vinegar. Of course we offer coffee too, although it is imported, it is important for us to we serve only fair trade products. You can enjoy your coffe with brown sugar, or local honey, with farm milk.

Based on our philosophy we prefer to serv home made, and self grown or collected products.
Our partners: Biobia, Katalin Tóth, Gábor Krómy, Guest house „Vörösdombi" from Cserkút, Barics Farm from Kővágótőttős, Lukovics Farm from Magyarszék.

Be our guest and experience the hungarian cuisine in a new, and green way!

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O02 CRADLE OF PACAP: BELLE CHASSE, HEBERT CENTER

Koves K
Department of Human Morphology and Developmental Biology, Semmelweis University, Hungary

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

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

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

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

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

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

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

O04 PACAP EFFECTS ON CRMPS REGULATION IN PC12 CELLS

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

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

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

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

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

Yu R[1], Zeng Z[1], Zhang H[2]
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Objective: Vasoactive intestinal Peptide (VIP) is a very important signal molecule of neurotransmitter, which participates in information transfer and physiological regulation. So it is a potential therapeutic neuropeptide, but unstable in structure .The 11-amino acid peptide TAT is a cell penetrating peptide, able to deliver protein cargoes across the cell membrane. In this study, the preparation of a recombinant VIP-TAT composed of VIP and TAT was achieved using genetic engineering principle and technology. After its bioactivity of cell penetrating and traversing biological barriers was detected, a mice model with amnesia induced by scopolamine was used to test the function of VIP- TAT and VIP.
Methods: Based on the natural sequence of VIP, VIP-TAT gene was designed and synthesized according to the expression bias of E.coli. VIP-TAT gene was obtained by two steps PCR using four primers and cloned into the expression vector pKYB. Engineering bacteria pKYB-VIP-TAT-ER2566 was constructed, and high-efficient preparation of VIP-TAT was achieved with the IMPACT (Intein Mediated Purification with an Affinity Chitin-binding Tag) protein expressing and purification system. Western blot was used to identify the immunological activity of recombinant VIP-TAT, and the fluorescence labeling technology was used to test its ability to penetrate into the cells and traverse the biological barriers into blood and brain. The inhibitory effects of VIP-TAT medicated by atomization on the food intake were also assayed. At last a mice model with scopolamine-induced amnesia was used to assay the effects of VIP-TAT. After one-time and long-term intervention trial with VIP-TAT and VIP, passive avoidance test was used to test the learning and memory of mice, and the antioxidant activities and oxidative stress indicators including MDA (malondialdehyde) and SOD (superoxide dismutase) in the brain and blood were dimtermined. The mechanism about the VIP-TAT against scopolamine-induced amnesia in mice was also explored from the content of ACHE (acetylcholin esterase) in the brain.
Results: The engineering strain pKYB-VIP-TAT-ER2566 was constructed, and the fermentation conditions acquired for the preparation of VIP-TAT were optimized. The recombinant VIP -TAT was obtained with IMPACT system. SDS-PAGE and western blot were used to characterize the recombinant VIP-TAT. VIP-TAT labeled with fluorescence FITC (fluorescein isothiocyanate) was found to penetrate into the CHO cells more effectively than the VIP labeled with FITC under the fluorescence microscope. VIP-TAT administrated by atomization was shown to get into blood through the respiratory tract and then traversed the blood brain barrier into brain more efficiently than VIP. After the administration by atomization, compared with VIP, VIP-TAT inhibited the food intake more effectively than VIP. These results indicated that VIP-

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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O22 EFFECT OF PACAP ON CEREBRAL ENDOTHELIAL CELLS

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Cerebral endothelial cells (CECs) – coming in contact with pericytes and astrocytes – constitute the structural basis of the blood-brain barrier (BBB). The continuous belt of tight junctions (TJs) interconnecting CECs and the presence of specific transport systems, enzymes and receptors in the brain endothelium regulate the molecular and cellular traffic into the central nervous system.
Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide having several cellular protective effects. However, little is known about the effects of PACAP on the cerebral endothelium and BBB functions. Here we investigated the effects of PACAP on the barrier function and survival of CECs.
We have shown that PACAP has a protective effect on the tight and adherens junctions of brain endothelial cells. PACAP induces an increase in the transendothelial electrical resistance (TEER) in control conditions, and ameliorates Ca^{2+}-depletion induced decrease in TEER. Our immunofluorescence studies have shown that PACAP increases the amount of VE-cadherin and ZO-1 at the level of intercellular junctions. We have also observed that PACAP has a protective role against glucose-deprivation induced junctional damage and apoptosis.
In conclusion, our results show that PACAP protects cerebral endothelial cells against junctional disruption and apoptosis.
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O23 IDENTIFICATION AND CHARACTERIZATION OF A NOVEL THIOREDOXIN REDUCTASE INVOLVED IN THE NEUROPROTECTIVE EFFECT OF PACAP

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

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

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

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Introduction: Diabetic nephropathy is the leading cause of end-stage renal failure and accounts for 30-40% of patients entering renal transplant programmes. Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide consisting of 38 amino acids. Its nephroprotective effect was proved in numerous in vivo and in vitro studies. The aim of our study was to investigate the effect of PACAP in diabetic nephropathy.
Methods: Diabetes was induced by a single intravenous injection of streptozotocin (65mg/kg) in male Wistar rats. PACAP-treated animals were administered 20ug PACAP intraperitoneally every second day. Body weight and blood sugar levels were monitored weekly. Kidneys were removed after 8-weeks survival, kidney/body weight ratio was determined and a complex histological analysis was performed. Expression of inflammatory cytokines was evaluated by semiquantitative cytokine array and Luminex assay. Oxidative stress was determined using the colorimetric analysis of stress markers (MDA, GSH and SOD).
Results: There was no difference in the weekly blood sugar level in the intact control and PACAP-treated groups or between the diabetic control and PACAP-treated groups. Diabetic animals showed a significant decrease in their body weight, and PACAP treatment was unable to significantly counteract the weight loss. Histological analysis revealed severe diabetic nephropathy in kidneys of control diabetic animals (glomerular PAS-positive area expansion, tubular damage, Armanni-Ebstein phenomenon, vascular hyalinosis). PACAP-treatment significantly diminished the damage. Diabetic kidneys showed strong cytokine activation compared to their healthy controls. PACAP was effective in counteracting the changed cytokine expression pattern (e.g. L-selectin, TIMP-1, CINC-1), moreover, it elevated the decreased level of GSH in diabetes.
Conclusion: To conclude, PACAP is effective in ameliorating diabetic nephropathy. These results raise the opportunity for the use of PACAP as a possible therapeutic or preventive method in treating renal complications of diabetes.
Support: This work was supported by PTE-MTA „Lendulet" Program, Arimura Foundation, OTKA K104984, TAMOP 4.2.2.A-11/1/KONV-2012-0024, TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program", OTKA PD 109644, Bolyai Scholarship, PTE ÁOK Research Grant KA 34039 04/2013.

O26 VASOACTIVE INTESTINAL PEPTIDE DEFICIENT MICE EXHIBIT AMELIORATED IMMUNE RESPONSES IN EXPERIMENTAL MODELS OF INFLAMMATION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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O44 EFFECTS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE ON SPERMATOGENESIS

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

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

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

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

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

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O46 PRESENCE OF PACAP IN HUMAN FEMALE GENITAL SYSTEM

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

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O47 PERSONALIZED EXAMINATION OF VPAC1 BIOMARKER FOR DETECTING GENITOURINARY CANCER

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Introduction: In 2010, more than 30,000 men succumbed to prostate cancer (PC) and more than 240,000 new PC cases were identified in the USA [1]. Digital rectal examination, MRI, and a blood test for prostate specific antigen (PSA) determination play a significant role in detecting advanced PC. However, they are not considered reliable tools for early warning of PC to detect recurrent cancer or to determine metastatic status of the disease [2-4]. Unreliable diagnosis results in undertreatment or overtreatment of patients with minimal benefit, enormous morbidity, incontinence, and/or impotence. Histology remains the mainstay of PC confirmation. However, out of >750,000 biopsies performed each year in the USA, >65% show benign pathology, and cost hundreds of millions of healthcare dollars. Similarly, in the USA, bladder cancer (BC) kills more than 15,200 and inflicts more than 72,500 new cases each year [5]. Although many types of urinary markers have been explored, none has yet become useful due to the lack of specificity and sensitivity [6]. VPAC1 cell surface receptors, express themselves at the onset of the malignancy, and may be prior to elevation of PSA, and well before cell morphology is altered [7, 8]. We have successfully initiated the use of Cu-64 labeled VPAC1 receptor-specific peptide constructs to image disease specific oncogene products in experimental animal models, and in humans [9-16]. We hypothesized that VPAC1 receptors expressed in high density on PC and BC can be targeted for detection of shed tumor cells (STC) in patient urine, using TP4303, a VPAC1 specific biomolecule labeled with a near infrared fluorophore.
Method: Urine samples (n=42) were collected from normal volunteers (n=23) and from patients with PC (n=7), BC (n=2), elevated PSA (n=1), renal stone (n=1), urethral trauma (n=1), testicular pain (n=1), overactive bladder (n=1), minimal urinary frequency (n=1) and benign hyperplasia (BPH) (n=1). Samples were cytospun onto microscope slides. The immobilized cells were then incubated with TP4303 (1 nM in PBS), washed, incubated with a nucleic acid stain, DAPI (200 nM), and a cover slip was placed. Slides were allowed to dry overnight in a dark room and then observed by confocal fluorescence microscopy (Ex: 633 nm. Em: 754 nm).
Results: Cells could be detected at a concentration of 5 cells per ml. All patients (100%) with PC and BC cells had STC. No STC were detected in the urine samples of normal volunteers or patients with BPH.
Conclusion: The method is simple, noninvasive, rapid and thus far detected STC in patients with a known disease. No STC were found either in normal volunteers or those with biopsy proven BPH.
Support: NIH R01 CA157372-01 and by NuView, Inc. (MLT).

O48 SURVIVAL PROMOTION OF CELLS EXPRESSING AMYLOID-BETA AND PRESENILIN BY NICOTINE, AMPA AND KETAMINE: IMPLICATIONS FOR ALZHEIMER'S DISEASE

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

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

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

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

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

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

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

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

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

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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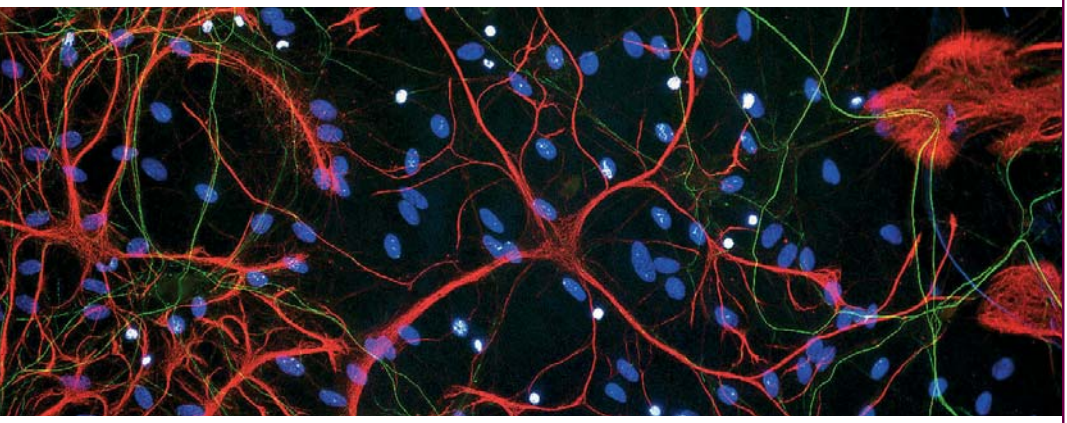

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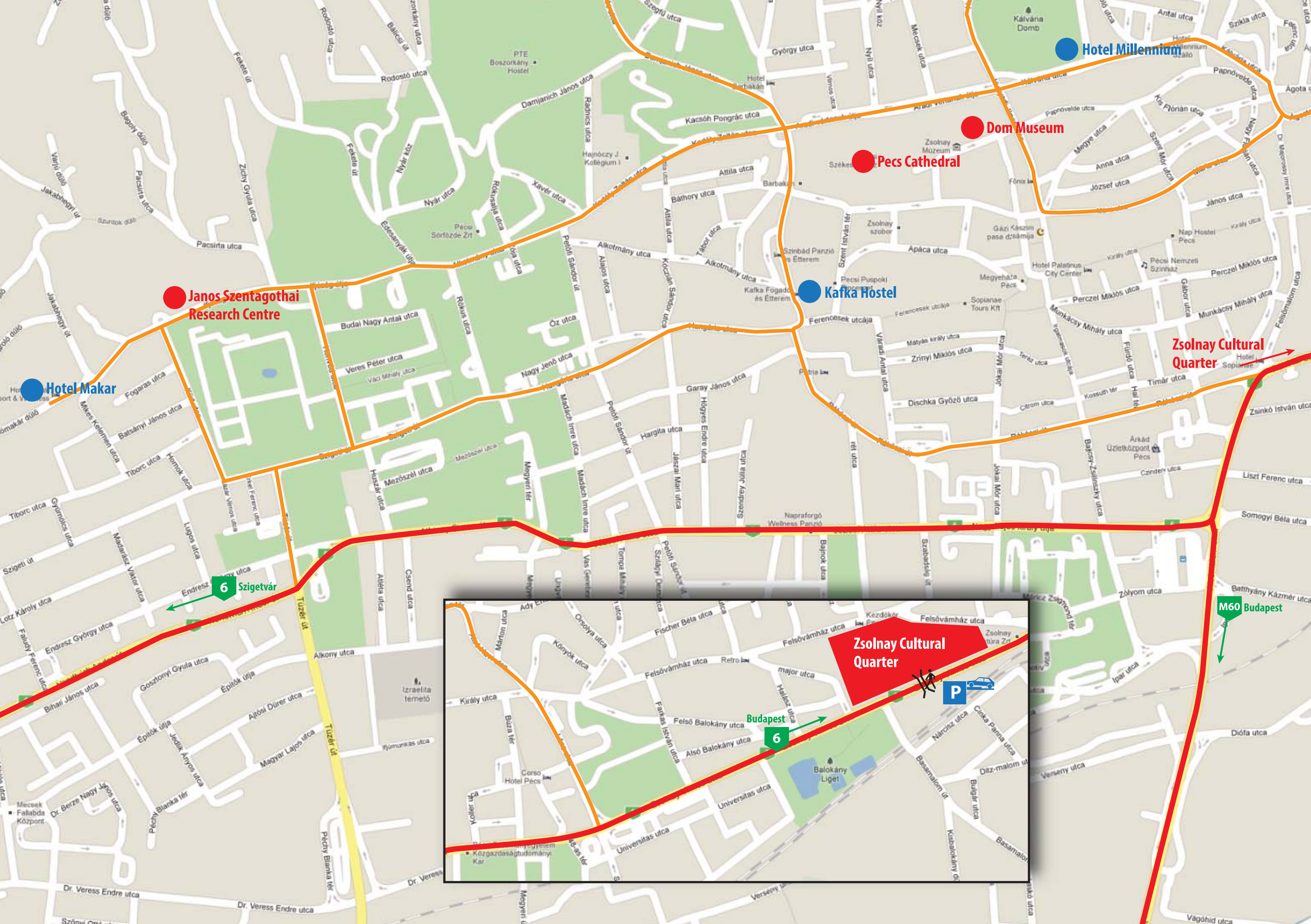
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