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20th International Symposium on Regulatory Peptides (REGPEP2014)

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General information

Symposium Venue
Kyoto Garden Palace
605 Tatsumaecho, Shimochojamachi-agaru,
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Dr. Kenji Kangawa (National Cerebral and Cardiovascular Center Research Institute, Japan)
Dr. Sarah F. Leibowitz (The Rockefeller University, USA)

Program

Tentative Program

	AM	PM	Night
Sep. 7 Sunday		Registration	Welcome Party
Sep. 8 Monday	Opening Plenary Lecture Symposium	Symposium Poster Session	
Sep. 9 Tuesday	Symposium	Plenary Lecture Symposium	Banquet
Sep. 10 Wednesday	Plenary Lecture Symposium	Symposium Closing	

Sessions

Session 1 Search and identification of novel peptides
Evolution, genetic variation and mutation
Session 2 Ligand-Receptor interactions
Session 3 Signal transduction of regulatory peptides
Posttranslational modifications
Session 4 Central nervous system function of regulatory peptides
Circadian system, Blood-brain barrier, Neuroprotection
Session 5 Regulatory peptides in autonomic nervous function and endocrinology
Cardiovascular functions, Gastrointestinal tract
Session 6 Translational research and drug development of regulatory peptides
Session 7 Regulatory peptides in Life-style related diseases
Session 8 Regulatory peptides in Immunology, Inflammation and Cancer

ABSTRACTS

Session 1
Search and identification of novel peptides Evolution, genetic variation and mutation

NON-SULFATED CCK – A NEW HORMONAL SYSTEM IN MAMMALS

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Background: Cholecystokinin (CCK) peptides undergo tyrosyl-O-sulfation within the bioactive region. This post-translational modification is essential for its ability to bind and activate the CCK_A-receptor that is responsible for the classical CCK effects such as gallbladder-contraction and pancreatic enzyme secretion. CCK, however, also activates the CCK_B-receptor independent of the sulfation state of tyrosine. Non-sulfated CCK peptides thus constitute a new endocrine system that only acts through the CCK_B-receptor.
Aim: To quantify the non-sulfated CCK forms in the brain and small intestine of pigs and in the small intestine of rats.
Methods: The concentrations of sulfated and non-sulfated CCKs were estimated through two highly specific RIAs – including a newly developed assay for non-sulfated CCK.
Extracts were obtained from the cerebral cortex of 6 pigs and the entire small intestine of 4 pigs and 6 rats.
Jejunal and cortex extracts from additional 12 pigs were separated by gel chromatography and FPLC and monitored by the RIAs.
Results: The majority of CCK was found within the first third of the small intestine in the rats and pigs. The mucosa of the pigs contained 127±26 pmol/g sulfated CCK and 33±15 pmol/g non-sulfated CCK and the rats 70±7.7 pmol/g and 8.4 ±2.0 pmol/g respectively. The pigs cortex contained 93±21 pmol/g sulfated CCK and 0.8±0.6 pmol/g non-sulfated CCK.
Analysis by gel chromatography and FPLC revealed that CCK-8 was mostly sulfated (88%±4.3) in the pig gut and that the fraction of sulfated CCK peptides was correlated to the length of the peptides; CCK-22 (82±7.4%), CCK-33 (74±6.7%), CCK-58 (64±7.7%). The same tendency was present in the brain, but the fraction of sulfated CCK remained much higher.
Discussion: This study reveals that a substantial part (~20%) of intestinal CCK is non-sulfated, but only little occurs in the brain (<1%). The more complete sulfation in the brain may be due to a longer lasting posttranslational maturation process in neurons.

References:
Agersnap, M. and J. F. Rehfeld. "Measurement of Nonsulfated Cholecystokinins." Scand J Clin Lab Invest, (2014). Rehfeld, J. F. and M. Agersnap. "Unsulfated Cholecystokinin: An Overlooked Hormone?" Regul Pept 173, no. 1-3 (2012): 1-5.

HEMOKININ-1 IS A POTENT INFLAMMATORY AND PRO-NOCICEPTIVE PEPTIDE IN ACUTE AND CHRONIC MOUSE ARTHRITIS MODELS

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*These authors made equal contributions to the field.

The Tac4 gene-derived hemokinin-1 (HK-1) is expressed in the nervous system and inflammatory cells. Despite several similarities to substance P, it is suggested to have different binding site at the NK1 tachykinin receptor, distinct activation mechanism and signal transduction pathways, but a specific own target was also proposed. In this study, its role was investigated in arthritis models of distinct mechanisms.
In acute mechanism models Complete Freund's adjuvant (CFA) or the protease-activated receptor 2 agonist mast cell tryptase (MCT) was injected into the knee joint of C57Bl/6 wildtype (WT) and Tac4 gene-deleted ($Tac4^{-/-}$) mice. Joint diameter was measured by micrometry, the mechanonociceptive threshold by aesthesiometry, synovial perfusion by laser Speckle. In the K/BxN serum-transfer chronic polyarthritis model, arthritogenic or control serum was injected i.p. on days 0 and 3. Paw volume was measured by plethysmometry, touch sensitivity by aesthesiometry, noxious heat threshold on hot plate, cold tolerance by paw withdrawal latency from 0°C water, joint function in the grid test and arthritis severity by weight loss during 2 weeks.
Both CFA and MCT evoked a maximum of 10-15% knee swelling and 30-35% mechanical hyperalgesia 4-24 hours after administration in WT mice with a longer-lasting inflammation after CFA, which were significantly reduced in $Tac4^{-/-}$ animals. MCT-induced acute synovial vasodilation was also remarkably decreased after 15 minutes in the $Tac4^{-/-}$ group. In the chronic immune-arthritis model 90% ankle joint swelling and 40% mechanical hyperalgesia (but no thermal hyperalgesia) developed in the WTs, which were significantly smaller in the $Tac4^{-/-}$ group. Cold allodynia, joint function and weight loss were not different.
Hemokinin-1 induces joint hyperaemia, swelling and hyperalgesia in both chronic and acute joint inflammation.

Identification of its receptors and molecular mechanisms of action might open novel directions in arthritis research.
Support: Astellas Pharma Kft-PTE ÁOK, TÁMOP-4.2.2.A-11/1/KONV-2012-0024

EGG-LAYING REGULATION IN THE CEPHALOPOD *SEPIA OFFICINALIS*: IDENTIFICATION AND MAPPING OF THE NEUROPEPTIDOME OVEREXPRESSED DURING SPAWNING

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Cephalopods are known to exhibit a wide variety of behaviors such as prey catching, communication, camouflage and reproduction, thanks to a complex central nervous system divided into several functional lobes. Diversity of the neuropeptides expressed in their brain is crucial to modulate the behavior and physiological mechanisms associated with the main stages of their life cycle.
In this context, this work focuses on the neuropeptidome expressed during egg-laying, the last step of reproduction. We identified neuropeptide transcripts by a *de novo* construction of the CNS transcriptome using an RNAseq approach (Illumina sequencing). We completed our *in silico* analysis of the transcriptome by the characterization and the tissue mapping of neuropeptides by mass spectrometry. We determined neuropeptide content and expression levels in the neurohemal area, blood and nerve endings in mature females in order to identify the neuropeptides involved in the egg-laying process. Out of the 52 neuropeptide precursors we identified, 44 were described for the first time in *Sepia officinalis*, 8 were described for the first time in the animal kingdom and 14 were over-expressed in egg-laying females compared to mature males.
Mass spectrometry screening of the neuropeptide content of blood and nerve endings allowed us to clarify the status of many neuropeptides, *i.e.* neurotransmitter and/or neurohormone, and revealed unexpected cleavages from precursors. In addition, comparative analysis of the neuropeptide contents of the neurohemal area and blood suggests the occurrence of at least a second neurohemal area as yet never described in cephalopod decapods. Besides the data concerning egg-laying regulation in cephalopods, this work brings very new and important structural and expression data about the neuropeptidome of *S. officinalis*.

IDENTIFICATION OF THE INVERTEBRATE NOVEL BIOACTIVE PEPTIDES FOR DROSOPHILA ORPHAN GPCRS

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In both vertebrates and invertebrates, there are many orphan G protein-coupled receptors (GPCRs), for which ligands have not yet been identified. Identification of their endogenous ligands is very important for understanding the function and regulation of such GPCRs. Indeed, That has enhanced our understanding of many physiological processes including feeding behavior, sleep-awake system, stress reaction, immunological system and reproduction. Here, we identified five Drosophila endogenous ligands, CCHamide-1, CCHamide-2, dRYamide-1, dRYamide-2 and trissin of the Drosophila orphan GPCRs, using functional assays with the reverse pharmacological technique. And, in various invertebrates, we identified orthologous peptides to Drosophila peptides.
CCHamide-1 and -2 were isolated to drosophila bombesin receptor subtype-3 (BRS-3)-like receptor that was the orphan receptor in the mammal for a long. Although CCHamide-1 and -2 were produced from the different proproteins, both CCHamide-1 and -2 contain disulfide bonds. dRYamide-1 and -2 were isolated to drosophila neuropeptide Y (NPY)-like receptor. Both dRYamide-1 and -2 contain a C-terminal RYamide. Trissin was indentified an endogenous ligand of CG34381, and a peptide comprised of 28 amino acids with three intrachain disulfide bonds. dRYamides and CCHamide modulated feeding motivation in fly and prawn.
These results suggest that identification of novel invertebrates bioactive peptide might facilitate the elucidation of various physiological function and have a useful possibility for the ligand searching of mammal orphan GPCRs.

CHICKEN IS A SPECIFIC AND USEFUL ANIMAL MODEL TO STUDY THE FUNCTIONAL ROLE OF GHRELIN AND MOTILIN IN REGULATION OF GASTROINTESTINAL MOTILITY

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Ghrelin and motilin are structurally-related gut peptide hormones synthetized in the mucosa of stomach and duodenum, respectively. Although both peptides stimulate gastrointestinal (GI) motility, there are marked species difference in responsiveness to ghrelin and motilin. Both peptides exist in humans and are functional. We examined the

effects of ghrelin and motilin on contractility of GI tract from several animals to look for species similar with humans. (1) Only in chickens, ghrelin caused contraction of the non-stimulated GI strips among examined species (goldfish, frog, chicken, Japanese quail, guinea-pig and rat). The contraction was remarkable in the crop, proventriculus and colon but low in the middle intestine. Expression of GHS-R1a matched the contraction. (2) Some GI preparations of quail and guinea-pig were sensitive to ghrelin but the contractions were low and fluctuated. (3) GI tracts of goldfish, frog and rat hardly reacted to ghrelin although GHS-R1a mRNA moderately expressed. (4) Motilin contracted the GI tract of chicken in a region-dependent manner that was opposite to responsiveness of ghrelin. But goldfish, frog, rat and guinea-pig intestine were insensitive to motilin. (5) In the chicken proventriculus, the responses to ghrelin and motilin decreased depending on post-hatching days. In conclusion, ghrelin and motilin cause contraction of the chicken gastrointestinal tract in a region-dependent manner. In addition, the reactivities of ghrelin and motilin to GI tracts decline with age. Taken together, the chicken is a unique animal model for studying the functional role of ghrelin and motilin on GI motility in vitro.

EXPRESSION AND LOCALIZATION OF OREXIN A AND OREXIN B AND THEIR RECEPTORS OX1R AND OX2R IN THE GASTROENTEROPANCREATIC (GEP) SYSTEM OF SEA BASS (*DICENTRARCUS LABRAX*) AND GOLDFISH (*CARASSIUS AURATUS*)

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Orexin A and B are neuroregulatory peptides involved in feeding-associate processes, glucidic and energy metabolism, and in arousal maintenance. Recently mRNAs sequences encoding for prepro-orexin are reported in the gastrointestinal tract of some freshwater, seawater and euryaline fish. Moreover only one winter flounder fish orexin GPCR receptor strictly analogous to the mammalian OXR2 has been characterized and sequenced up today. Since teleost species show different organization of the gut, feeding behaviour and habitat, in this study we employed immunohistochemistry and Western Blot analysis to investigate the distribution of preproorexin, orexin A- and B-like peptides and their receptors in the GEP system of two different species, sea bass and goldfish. In seabass, orexin A- and B-like immunoreactive cells were detected in the epithelium along the mucosal folds or at the upper margin of gastric crypts, and in basal regions of intestinal plicae; in goldfish, thin and elongated orexins-A and B-like immunoreactive cells were observed in the epithelium at the basis of mucosal folds in the pseudogaster and in the mid gut. In both species, some orexin-A and B-like, OXR1 and OXR2-like immunoreactive fibres and neurons were detected in the myenteric plexus and thin fibers in the submucosal plexus and in the circular muscular sheet in gut regions. Many rounded or triangular endocrine cells strongly positive to orexin A- and B-like were observed in the marginal zone of endocrine pancreatic islets. Western blot analysis showed a prepro-orexin band of 16 kDa and two bands of 53 and 38 kDa respectively for OXR1 and OXR2. The prepro-orexin Western Blot showed a more intense band in cardial stomach, blind sac and, mid and hind gut; the receptors molecular weight correspond to those obtained in avian species. Our results could suggest an involvement of these peptides in the regulation of fish gastrointestinal activities and pancreatic functions.

THE PACAP/VIP/RECEPTOR PROTEIN SYSTEM IN THE ZEBRAFISH OVARY

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PACAP and VIP are two pleiotropic neuropeptides showing overlapped structure, distribution and function which act through three receptors: PAC1-R, with high affinity to PACAP, and VPAC1-R and VPAC2-R, with equal affinity to both neuropeptides (Harmar et al. 1998; Vaudry et al., 2000, 2009). In vertebrates VIP and PACAP are expressed in the nervous system and in peripheral tissues including the ovary (Scaldaferri et al., 2000; Wang et al., 2003; Barberi et al., 2007; Agnese et al., 2013). We investigated the PACAP/VIP/receptor system in the zebrafish ovary, since the mRNA expression of PACAP and its receptors has been previously investigated (Wang et al., 2003; Fradinger et al., 2005; Zhou et al., 2010) but to date data on their protein localization are lacking. Our immunohistochemical study demonstrates that PACAP has a wide cytoplasmic localization in primordial oocytes, no longer detectable in primary oocytes reappears in previtellogenic oocytes, simultaneously with the cortical alveoli formation; the subcortical localization becomes more widespread in late previtellogenic and in vitellogenic oocytes. Furthermore, it is present in follicle and theca cells and in interstitial tissue. Cytoplasmic localization of VIP is evident in primary oocytes, just before the cortical alveoli formation, and up to full vitellogenic oocytes; VIP signal, absent in follicle

cells, is present in theca cells and strongly occurs in extrafollicular connective tissue, as reported in other teleosts (Calabro et al., 2008). The PAC1 and the VPAC1 receptors are poor represented, in interstitial tissue and faintly in theca cells, exclusively; in contrast VPAC2-R is well localized in a narrow stripe of sub-cortical cytoplasm in previtellogenic and vitellogenic oocytes and in follicle cells. Our results are in line with mRNA expression patterns already reported (Wang et al., 2003) and highlights the system may play fundamental roles in the development and function of the vertebrate ovary.

CHANGES IN THE EXPRESSION OF GALANIN AND SUBSTANCE P IN GASTRIC DESCENDING NEURONS SUPPLYING THE PORCINE PYLORIC SPHINCTER IN ANIMALS WITH EXPERIMENTALLY INDUCED PYLORIC ULCERS – PRELIMINARY STUDIES

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Gastric ulcerations in the region of the pylorus are common gastric disorders occurring in humans and animals. Such localization of ulcers can influence the intrinsic descending nerve supply to the pyloric sphincter and consequently disturbs the pyloric orifice function. Although the involvement of galanin (Gal) and substance P (SP) in the regulation of inflammatory processes is widely accepted, their contribution to intrinsic nerves plasticity under gastric ulcerations is not known. The pig is an animal of a great economic value and increasing significance in biomedical research. The aim of the study was to verify the changes in the expression of Gal and SP in intramural gastric neurons supplying the pyloric sphincter in pigs with experimentally induced pyloric ulcers.

The experiment was performed on 2 groups of pigs: healthy gilts (n=6) and gilts with experimentally induced pyloric ulcers (bilateral injections of 1cm^3 of 40% acetic acid into the pars pylorica of the stomach wall; n=6). Gastric neurons supplying pyloric sphincter were labeled using the retrograde neuronal tracing (20μl of Fast Blue injected into the pyloric sphincter muscle). Double immunocytochemical stainings with primary (rabbit anti-Gal; rat anti-SP) and secondary (AlexaFluor555/488) antibodies were applied on transverse cryosections of the stomach wall. The immunoreactivity against substances studied was analyzed under a fluorescent microscope in, at least, 150 traced neurons per animal. Results were statistically analyzed and presented as percentages.

The number of traced neurons immunoreactive to Gal increased significantly in pigs with ulcers (from about 14.3% to 38.2%). Surprisingly, in both groups of pigs no neurons immunoreactive to SP were observed. However, traced cells were frequently accompanied by SP-immunoreactive perikarya or nerve fibers.

The results obtained suggest the involvement of Gal in the plasticity of intrinsic nerves under gastric ulcerations.

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FEMALE SEX PEPTIDES IN THE CONTROL OF CUTTLEFISH REPRODUCTION

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In the cuttlefish *Sepia officinalis*, neuropeptides are involved in the successive steps of reproduction. Additionally, female sex peptides secreted by the ovaries and the female genital tract also play an important role in the control of genital apparatus activity. The first category of female sex peptides are ovarian regulatory peptides like ILME, Sepovotropin, OJPs (Ovarian Jelly Peptides) and SepCRPs (Sepia Capsule Releasing Peptides). These paracrine peptides are involved in the modulation of the contractile activity of the genital tract that allows oocyte release and egg capsule secretion. Some of them are released by the oocyte into the lumen of the oviduct or by the egg into the seawater; in that latter case, they act as waterborne low-molecular-weight pheromones. Recently performed *de novo* RNAseq revealed that they are cleaved from yolk proteins, suggesting an original dual processing of protein precursors.

The second category is composed of large sex pheromones identified from *de novo* RNAseq, detected by mass spectrometry and cleaved from three protein precursors by prohormone convertases. Expressed and secreted with the internal egg capsule by the oviduct glands, these pheromones stimulate penis, gill (both sexes) and oviduct contraction, and are also suspected to be involved in mate aggregations in the coastal egg-laying areas. Thus, in cuttlefish, ovarian regulatory peptides and sex pheromones play an important role in the control of gamete release, mating, oocyte fertilization and egg-laying.

Session 2
Ligand-Receptor interactions

MOLECULAR INTERACTION OF MOUSE SECRETIN AND ANGIOTENSIN II RECEPTORS AND THEIR POTENTIAL IMPLICATIONS IN WATER HOMEOSTASIS

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Osmoregulation is critical to life and is tightly regulated by three major hormones namely secretin (SCT), angiotensin II (ANGII) and vasopressin (VP). Of note, SCT and ANGII share overlapping physiological roles including similar expression pattern within the brain, dipsogenic actions and activation of VP expression and/or release in mice. However, it remains unclear how their receptor pathways may cross-interact to aid osmoregulation. In recent years, GPCR oligomerization has been implicated to play roles in regulating processes. This project aims to explore the molecular association between SCTR and ANGII receptors by bioluminescence resonance energy transfer (BRET) assays that revealed SCTR and ANGII type 1a receptor (AT1aR) to form hetero-complexes. This oligomerization event was found by BRET competition to be contributed predominantly by transmembrane (TM) domain regions 2 and 4 in SCTR, and TM1 and TM4 in AT1aR. Within which, combinational use of mutant TM peptides and SCTR chimeras revealed the importance of lipid-exposed residues, particularly Leu204 and Ser205 in SCTR TM2 as key contact points for formation of the SCTR/AT1aR complex. Morphologically, the heteromers were visualized by confocal and FRET imaging at the cell surface and found have a role in modulating AT1aR trafficking. It was also found that the SCTR/AT1aR complex affected Gαs signaling specifically, reducing maximal response values by 24.3 ± 2.8 % compared to CHO-K1 cells transfected with only SCTR. While, this negative effect could be abolished by co-application of SCT and ANGII peptides, use of constitutively active AT1aR mutants or disruption of the hetero-complex using SCTR mutants. Taken together, the SCTR/AT1aR complex was proposed to impose conformational restraints on the SCTR that could be overcome upon activation of the AT1aR. Physiologically, hyperosmolality isovolemic induced drinking could be attenuated by central administration of TM peptides and the phospholipase C pathway blocker H-89, indicating receptor oligomerization to have a role in neural osmoregulation via a Gαs dependent pathway. This study presents novel findings regarding the receptor oligomerization of SCTR and AT1aR, which may be the molecular basis to the overlapping roles of SCT and ANGII in water homeostasis.

COLLAGEN TRIPEPTIDE FRAGMENT REGULATION OF CELL SPREADING TO DIFFERENT SUBSTRATES

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Collagen molecules are known to have peptide domains providing collagen binding with specific protein modules. These binding sites in collagen domains may be in native (triple-helical form) as GXOGER-containing peptide fragments or in cryptic form as RGD-containing sites exhibiting their activity after denaturation of triple helix in collagen molecules. Besides, collagens have peptide modules that are not involved in interdomain recognition. Their physiological activity is manifested only after their release during partial proteolysis of collagens in ECM remodeling. The effect of multiply repeated tripeptide (TP) in the primary structure of collagen α-chains on the spreading of mouse embryonic fibroblasts STO to different substrates (untreated plastic, fibronectin, gelatin) has been studied. From 10^{-10} to 10^{-5} M TP was added to cell suspension either directly before seeding or cells were incubated with TP for 30 min before plating. TP effect on cell spreading depends on the chemical characteristics of substrate (polymer of styrene, ECM proteins in native (fibronectin) or partly denatured (gelatin) forms) and the mode in which TP was added to cells. Co-incubation of cells with TP stimulated cell spreading. The peptide cell response on gelatin was essentially lower than on fibronectin or untreated plastic. Cell preincubation with TP reduced the number of spread cells on both fibronectin and gelatin. It is suggested that TP affects the initial stages of cell adhesion to the substrate regulating integrin receptor activity through the interaction with the secondary domains of conformation adjustment of receptor binding sites with ligands (TP co-incubation with cells) or directly with integrin ligand binding modules (TP preliminary incubation with cells).

ACTIVATION OF RENAL GLP-1 RECEPTORS LOCATED IN THE AFFERENT ARTERIOLES CAUSES A DILATATION OF THE AFFERENT ARTERIOLE

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It has become evident that glucagon-like peptide-1 (GLP-1) has a range of extra-pancreatic effects, including renal effects. However, the mechanisms behind these actions are poorly understood, but the GLP-1 receptor has been identified in numerous tissues outside the pancreas including the kidney. Therefore, it is possible that these effects are mediated through the GLP-1 receptor. However, the exact cellular localization of the receptor in these extra-pancreatic tissues is poorly described.

The aim of the present study was to investigate the localization of renal GLP-1 receptors and to describe GLP-1 mediated effects on the renal vasculature.

In vivo autoradiography studies using ^{125}I-labelled GLP-1 were carried out in mice in order to localize specific GLP-1 binding and, thereby, identify the localization of the GLP-1 receptor. Vascular effects of GLP-1 were investigated in isolated mouse kidneys perfused with Tyrodes buffer containing 5 % albumin. A longitudinal slice was made to expose the vasculature on the inner cortical surface. Renal perfusion pressure (RPP) was increased in steps of 20 mmHg from 95 mmHg up to 155 mmHg, and the diameter of the afferent arteriole was measured before and after perfusion with GLP-1 (1 nM).

Binding of ^{125}I-GLP-1 was observed in the renal vasculature, predominantly in the afferent arterioles, whereas the glomeruli were negative. This binding could be completely inhibited by simultaneous administration of excess non-radioactive GLP-1.

In the isolated murine kidney, stepwise increases in RPP induced contraction of the afferent arteriole from 22.3±1.0 to 19.3±1.0 μm, $p<0.01$, $n=5$, and this autoregulatory response was inhibited in the presence of GLP-1.

We conclude that the renal GLP-1 receptor is localized in the renal vasculature, with highest density in the afferent arterioles. GLP-1 infusion mediates a dilatation of the afferent arteriole, most likely mediated by specific binding of GLP-1 to its receptors in the afferent arterioles.

DESIGN AND DEVELOPMENT OF A ^{68}GA/^{177}LU-LABELED UNIVERSAL BOMBESIN PEPTIDE LIGAND FOR IMAGING AND THERAPY OF BOMBESIN RECEPTOR-EXPRESSING TUMORS

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Objectives: Small synthetic peptide based molecular imaging probes that specifically target peptide receptors overexpressed on variety of cancer cells have received enormous attention in the field of receptor-mediated tumor targeting. Among the most clinically-relevant peptide receptor systems, bombesin (BN) peptide receptors are of great clinical interest because of the overexpression of their receptors on various important and frequent human cancers including breast and prostate cancer. The high expression of receptors in cancer cells and low density in normal tissues makes these receptors potential molecular targets with radiolabeled BN peptides. The purpose of current study was to develop and evaluate a novel BN peptide analog derived from the universal BN sequence (capable of targeting all four BN receptor subtypes), and radiolabeled it with both diagnostic (^{68}Ga) and therapeutic radionuclide (^{177}Lu) thus making the same peptide useful for both diagnosis and therapy of BN receptor-expressing tumors.

Methods: DOTA-Glu-Gln-Trp-Ala-Val-βAla-His-Phe-Nle-NH_2 was prepared by solid-phase peptide synthesis following Fmoc/HBTU chemistry. Labeling with ^{68}Ga and ^{177}Lu was accomplished in the presence of 2.5M NaOAc and 0.1M NH_4OAc buffer, respectively. In vitro cell-binding study was conducted on BN receptor-positive T47D breast cancer cell line. In vivo biokinetics was performed on Balb/c mice.

Results and discussion: The structure and purity of the DOTA-coupled BN peptide was confirmed by mass spectrometry and HPLC analysis. Radio-HPLC analysis revealed that the BN analog labeled efficiently with both radionuclides with high labeling efficiency (~98%). The radiopeptide showed high radiochemical stability in excess of DTPA and a high metabolic stability (~95%) when incubated with human plasma. In vitro cell-binding indicated the high affinity and specificity of the radiopeptide towards T47D human breast cancer cells (K_d=~5 nM) and also significant internalization (~20%) into the breast cancer cells. In mice, ^{68}Ga/^{177}Lu-labeled peptide displayed a fast clearance from the blood and excretion primarily by the renal route, with some elimination through the hepatobiliary system. The uptake in the major organs (i.e., lungs, stomach, liver, intestines kidneys, etc.) was low (<3% ID/g).

Conclusions: The preliminary data advocate that the BN peptide ligand under investigation possesses certain favorable in vitro/in vivo characteristics that need to be further explored in order to determine the real potential of this receptor-binding peptide for targeting tumors expressing BN receptor types.

THE PHARMACOLOGICAL AND SPECIES-SPECIFIC MODE OF ACTION OF (PRO3)GIP

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Background and aims: Compared to GLP-1, the characterization of the sister incretin hormone GIP is insubstantial. Specific high potent receptor antagonists are valuable tools when evaluating receptor function. Considerable attention has been given to the proclaimed GIP receptor antagonist (Pro3)GIP and its effect in murine studies. However, the prospect of using this ligand in humans has not been addressed adequately. Therefore, we conducted a pharmacological description of the ligand including interspecies differences.
Materials and methods: COS-7 cells were transiently transfected with vector, hGIPR, rGIPR, or mGIPR cDNA. These cells were assessed for cAMP accumulation upon ligand stimulation and assayed in competition binding using ^{125}I-hGIP. The applied ligands were hGIP, rGIP, mGIP, h(Pro3)GIP, r(Pro3)GIP, and m(Pro3)GIP. Using isolated perfused rodent pancreata, the species-specific GIP analogs were evaluated in the corresponding animal in regard to insulin, glucagon, and somatostatin release.
Results: cAMP experiments revealed h(Pro3)GIP to be a full agonist, while both r/m(Pro3)GIP are partial agonists (50% and 36% of r/mGIP, respectively) on their corresponding receptors. Schild plot analysis estimated a K_i of 12.6 nM and 61 nM for r(Pro3)GIP and m(Pro3)GIP, respectively. Interestingly, when tested in perfused pancreata at 7mM glucose, both rodent (Pro3)GIP analogues showed powerful glucagon releasing properties, but only modest increases in insulin and somatostatin secretion.
Conclusion: When evaluating compound properties it is important to consider interspecies differences in receptor and ligand structure. (Pro3)GIP is a full agonist on the human GIP receptor. However, the huge impact on glucagon secretion may allow further understanding of receptor signaling in alpha and beta cells.

DUAL ANTAGONISTIC ACTION OF A SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) INHIBITOR ON TRP ION CHANNELS ON PRIMARY SENSORY NEURONS AND SENSORY NERVE TERMINALS

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Transient Receptor Potential ion channels, such as TRP Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1 and TRPA1) are expressed on nociceptive primary sensory neurons and regulate inflammation. SSAO expressed predominantly in vascular smooth muscle cells catalyzes oxidative deamination of primary amines. A newly developed SSAO inhibitor, SZV-1287 was described to inhibit both acute and chronic inflammation. Since the chemical structure of SZV-1287 is similar to the selective TRPA1 antagonist HC030031, we investigated the effects of SZV-1287 on the TRPV1 and TRPA1 receptor activation of trigeminal ganglion neurons in comparison with SZV-1911, the reference SSAO inhibitor with different structure.
Measurement of intracellular calcium concentration was performed by microfluorimetry in cultured rat trigeminal neurons. Calcitonin gene related peptide (CGRP) release from the stimulated peripheral sensory nerve terminals of the isolated rat trachea was measured by radioimmunoassay.
Neuropeptide release after mustard oil administration was measured. Both SZV-1287 and SZV-1911 were used in three concentrations (100, 500, 1000 nM). Significant decrease in the mustard oil-evoked CGRP release was observed after SZV-1287 administration in concentration dependent manner. SZV-1911 did not decrease the mustard oil-evoked CGRP release.
SSAO inhibitors were tested on capsaicin-, mustard oil- and KCl-evoked Ca-influx. SZV-1287 and SZV-1911 were used in three concentrations (100, 500, 1000 nM). SZV-1287 decreased the capsaicin- and mustard oil-induced calcium influx in trigeminal neurons in concentration dependent manner. SZV-1911 had no effect in either model. Neither compound had any effect on KCl-evoked calcium influx.
This is the first evidence for an antagonistic action of SZV-1287 on TRPV1 and TRPA1 ion channels. This effect is independent of its SSAO inhibitory action.

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Session 3
Signal transduction of regulatory peptides Posttranslational modifications

KEY PROLINE POSITION IN CHEMOKINE AND VASOACTIVE PEPTIDE RECEPTORS ON CHEMOTAXIC BEHAVIOR

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Studies from Chabbert et al., (2012) showed that chemokine GPCR (CHEM) including CXCR4, and somatostatinergic GPCR (SO) including urotensin II (UII) receptor UT, are phylogenetically linked. Both CHEM and SO receptors possess a proline residue at the position 2.58 in the second transmembrane (TM2) domain (P2.58), while in receptors of the ancestral peptide family (PEP) such as Kiss1 peptide receptor (Kiss1R), the proline is positioned in 2.59. Our modelizing studies indicate that ancestral deletion leading to P2.58 provokes a kink of the TM2 and modifies receptor activation toward chemotaxic behavior.

We generated mutants in which the proline at position 2.58 (UT) or 2.59 (Kiss1R) is replaced by an alanine or repositioned in P2.59 and P2.58, respectively. In this study, we evaluated the impact of these mutations on Gq/Ca^{2+} coupling, chemotaxis and cell-matrix adhesion. On HEK cells, we showed that wild-type and mutant receptors are expressed at the plasma membrane. However, recombinant mutated receptors failed to activate Ca^{2+}/PLC couplings. In migration/adhesion assays, wild-type UT stimulated chemotaxic migration and cell-matrix adhesion (activation of β1-intregrin and P-paxillin) whereas UTP2.58A and UTinsP2.59 did not promote adhesive properties, favoring chemotaxic cell migration. Inhibition of $G_{i/o}$ coupling *via* toxin pertussis (PTX) inhibited chemotaxic migration of UT- and UTP2.58A- and only reduced migration of UTins-expressing cells. The wild-type Kiss1R exhibited both promotion of motility/migration and inhibition of cell-matrix adhesion. The mutants Kiss1RP2.59A and Kiss1RdelP2.58 showed reduced motility and novel acquisition of pro-adhesive capacity. PTX only partially inhibited cell-directed migration of HEK expressing wild-type Kiss1R. Altogether, these data suggest that the proline at P2.58 of chemotaxic receptors plays a major role in the Gq/Ca^{2+} coupling and allows signaling switch between migration and adhesion.

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BONE FORMATION IN PACAP KO MICE

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Pituitary Adenylate Cyclase Activating Peptide (PACAP) is a naturally secreted signalling peptide and has important regulatory roles in the differentiation of central nervous system and several peripheral tissues. However, little is known about the connection of PACAP signalling pathways to osteogenesis or bone regeneration. We have been investigated the morphology of the diaphysis of femur of PACAP KO and Wild Type (WT) C57BL6 mice and signalling pathways regulating osteogenesis has also been followed.

Anterior cortical bone of PACAP KO mice was significant thicker than that of WT mice observed after whole limb alizarin staining and with CT. Inorganic extracellular matrix content of the genetically modified mice investigated with von Kossa and Alizarin staining was found lower than the WT. On the contrary, expression of collagen type I increased in the PACAP KO animals. Expression of PAC1 and VPAC2 receptors was demonstrated and via these receptors PACAP may have influence on PKA signalling: phosphorylated form of CREB - classical downstream target of PKA signalling pathway- was decreased but increased Runx2 expression was detected in PACAP KO mice. As sign of enhanced bone formation, increased protein expressions of ALP, osterix, osteocalcin and osteopontin were detected with Western blot. Elements of BMP signaling pathway have also been investigated and increased BMP6 and 7, moreover, elevated Smad1 expression and nuclear presence have been demonstrated in PACAP KO mice. PACAP KO mice also showed increased expression of the elements of Hedgehog signaling that is IHH, SHH and Gli1.

Our results indicate that PACAP-KO mice show various signs of disturbed osteogenesis. To clarify whether the absence of PACAP itself or activation of any compensatory mechanisms are causative in this phenomenon require further experiments.

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A CROSSTALK BETWEEN PACAP AND SEROTONIN 2A SIGNALING

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Objective: Accumulating evidence from human genetic and animal model studies shows that pituitary adenylate cyclase-

activating polypeptide (PACAP) and its specific receptor PAC1 are implicated as a risk factor for psychiatric disorders, including schizophrenia. Previously, we demonstrated that PACAP-deficient mice display notable psychomotor and neurological abnormalities that are ameliorated by blocking a serotonin type 2 receptor (5-HT_2). This finding suggests a possibility that PACAP modulates 5-HT_2 receptor signaling, although the underlying mechanism remains unclear. Since it is known that 5-HT_{2A} receptor functions are regulated by receptor internalization, in this study, we examined whether PACAP modulates internalization of 5-HT_{2A} receptor.

Methods: HaloTag-fused 5-HT_{2A} receptors expressed in HEK293T cells were labeled with a membrane-impermeable AlexaFluor 488 ligand and the membrane protein internalization was quantified upon PACAP stimulation. In addition, surface biotinylation and Western blotting analysis were performed to detect the surface and total levels of PAC1, 5-HT_{2A} and 5-HT_{1A} receptors.

Results: PACAP induced internalization of 5-HT_{2A} receptors but not 5-HT_{1A} receptors. In support, PACAP significantly decreased cell surface expression of 5-HT_{2A} receptors. In addition, pretreatment of the protein kinase C inhibitor sphingosine and beta-arrestin2 siRNA blocked the PACAP-induced internalization of 5-HT_{2A} receptors.

Conclusions: The present results suggest that PACAP signaling regulates 5-HT_{2A} receptor internalization in a PKC- and beta-arrestin2- dependent manner, suggesting a previously uncharacterized role for PACAP in psychiatric disorders accompanied with 5-HT_{2A} signaling alterations.

CHEMOSENSORY SIGNALLING PATHWAYS REGULATING AMINO ACID-INDUCED GHRELIN RELEASE

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Introduction. Taste receptors on gut enteroendocrine cells sense nutrients and transmit signals that control gut peptide secretion. Amino acid (AA) sensing is mediated via 3 receptors: CaSR, GPRC6A and TAS1R1-TAS1R3. It is currently unknown whether the postprandial decline in plasma ghrelin levels is regulated by AA taste receptors on the ghrelin cell.

Aim. To study the amino acid sensing mechanisms of the ghrelin cell.

Methods. The effect of AAs on ghrelin secretion was studied by radioimmunoassay in a ghrelinoma cell line, MGN3-1, in the absence or presence of AA taste receptor antagonists. Changes in intracellular Ca^{2+} release were measured in Fluo-4 loaded cells. Mice were gavaged with 8% peptone or 100mM L-Phe or injected intravenously (IV) with 100mM L-Phe.

Results. Peptone, L-Phe, monosodium glutamate (MSG) or L-Ala significantly increased octanoyl ghrelin release in MGN3-1 cells. The effect of L-Phe was blocked by the CaSR antagonist, Calhex-231, while MSG-induced ghrelin release was reduced by the TAS1R1-TAS1R3 antagonist, gurmarin. Two receptors were activated by L-Ala (CaSR and TAS1R1-TAS1R3) and by peptone (CaSR and GPRC6A). The effect of L-Ala and MSG was accompanied by an increase in intracellular Ca^{2+} release. Intragastric administration of 8% peptone decreased plasma ghrelin levels and increased stomach ghrelin content. Both intragastric and IV L-Phe administration decreased total plasma ghrelin levels and affected ghrelin content in the stomach and duodenum respectively.

Conclusions. Amino acids stimulate ghrelin release via direct activation of specific AA taste receptors on the ghrelin cell. The inhibitory effect of AAs on ghrelin release *in vivo* is indirect. The route of administration determines whether antral or duodenal ghrelin cells are targeted.

Session 4
Central nervous system function of regulatory peptides
Circadian system, Blood-brain barrier, Neuroprotection

PACAP ADMINISTRATION CAN AMELIORATE VASCULAR CHANGES IN RETINOPATHY OF PREMATURITY

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Premature birth can be associated with a series of disorders affecting future life quality. One of these conditions is a neurovascular disease of the retina, called retinopathy of prematurity (ROP). Despite striking advances in neonatology ROP still remains the leading cause of vision impairment in childhood. Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors occur throughout the nervous system, including the retina. The in vivo protective effects of PACAP have been shown in several model of retinal degeneration including diabetic or ischemic retinopathy. Therefore we aimed to study PACAP administration in the well-described animal model of ROP, the oxygen induced retinopathy (OIR).

Sprague-Dawley rat pups were kept in alternating oxygen concentration for 2 weeks to provoke OIR. Pups were treated either with intravitreal (3x3ul) or intraperitoneal (7x100ul)

PACAP injections. After 21 days the rats underwent functional examinations and their eyes were processed to flat mount lectin staining and immunohistochemistry.
Quantification of avascular to whole retinal areas showed that PACAP administration decreases the vasoobliterated territory by almost 50 % and there was also a significant reduction in the number of neovascular tufts. These findings were further supported by functional and immunohistochemical examinations. Our results suggest that PACAP can act against retinal vascular changes and reduce the degree of ROP.

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REGULATORY ROLE OF HEMOKININ-1 IN CHRONIC RESTRAINT STRESS MODEL OF MICE

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The Tac1 gene-encoded Substance P (SP) acting at the tachykinin NK1 receptor has been described to be involved in psychiatric disorders, but NK1 antagonists failed in clinical trials. The Tac4 gene-derived hemokinin-1 (HK-1) is present in brain regions related to anxiety, stress and pain, and also activates the NK1 receptor with different binding site and signaling pathways. However, several other mechanisms have also been proposed for its actions. We investigated its involvement in chronic stress-evoked behavioural and pain reactions.
Chronic restrainment was performed in 25 mm diameter plastic tubes for 6 h/day during 4 weeks in C57Bl/6 wildtype (WT) and Tac4 gene-deleted ($Tac4^{-/-}$) mice. Touch sensitivity was assessed with aesthesiometry, cold tolerance by paw withdrawal latency at 0°C, anxiety and locomotor activity in the light-dark box (LDB), open field (OFT), and tail suspension tests (TST). Adrenal and thymus weights were measured at the end of the study.
Non-stressed $Tac4^{-/-}$ mice spent significantly less time in the lit compartment of LDB, showed decreased motility in OFT and longer immobility in TST, their thymus weighed significantly less and adrenal gland more compared to WTs. In WTs stress induced 20% mechanical and 75% cold hyperalgesia related to central and peripheral sensitization, respectively, mice spent more time in the light, thymus weight decreased and adrenal gland increased. Deletion of Tac4 gene significantly reduced the stress-induced mechanical, but not cold hyperalgesia. $Tac4^{-/-}$ mice spent remarkably more time in light and showed increased mobility in the LDB, OFT and TST, the stress-induced thymus and adrenal gland weight alterations were absent compared to non-stressed controls.
These are the first evidence for HK-1-mediated potent anti-anxiety, anti-depressant and central pro-nociceptive mechanisms under normal and chronic stress conditions. Identification of its targets and signaling can open new directions in anxiety and depression research.

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PEPTIDERGIC SENSORY NERVES PLAY AN IMPORTANT REGULATORY ROLE IN MURINE IMMUNE ARTHRITIS

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Capsaicin-sensitive sensory nerves have complex regulatory functions in the joints under physiological and pathophysiological conditions depending on the mechanisms and the involvement of the simultaneously released pro- and anti-inflammatory neuropeptides. In the present study we investigated their role in a mouse model of rheumatoid arthritis.
Peptidergic nerves were defunctionalized by resiniferatoxin in C57Bl/6 mice, intact animals served as controls. Arthritis was induced by K/BxN arthritogenic serum. The mechano- and thermonociceptive thresholds were determined by esthesiometry and hot-plate test, grasping ability by the wire-grid grip test, paw volume by plethysmometry and clinical scoring. Myeloperoxidase (MPO) and matrix metalloproteinase (MMP) activities were measured by in vivo optical

imaging, bone morphology by micro-CT scans and histopathological evaluation.
The ankle edema, clinical severity and grasping ability on the wire were significantly enhanced, but mechanical hyperalgesia was diminished in desensitized mice. Thermal hyperalgesia did not develop in the model. Both MMP and MPO activities were significantly higher in the joints of desensitized animals. Specific bone surface in the ankle joints and periarticular tibia regions measured significantly decreased in desensitized mice, whereas their bone density increased. This phenomenon was absent in the controls.
Activation of peptidergic sensory nerves has a complex regulatory role in arthritis: it induces pain, but inhibits edema formation, grasping failure, bone reorganization and immune cell activity leading to decreased enzyme production presumably via the release of anti-inflammatory sensory neuropeptides (e.g. somatostatin). Sensory fibers and the released neuropeptides have important immune-regulatory actions and a prominent role in bone remodeling besides their well-known pain signaling functions.

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ELECTROPHYSIOLOGICAL EFFECTS OF GHRELIN ON HYPOTHALAMIC TUBEROMAMMILLARY NUCLEUS NEURONS IN RATS

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Ghrelin, known as potent stimulator of growth hormone (GH) secretion and feeding, is produced by stomach, other peripheral organs and some brain regions and acts on GH secretagogue receptors (GHS-Rs) in the some peripheral organs and brain regions. Recent study also indicates that ghrelin contributes to regulation of sleep-wakefulness. Tuberomammillary nucleus (TMN), which involves histaminergic neurons that participates in maintenance of arousal state, also expresses GHS-Rs. However, direct action of ghrelin on TMN neurons is remained unclear. Thus, we examined electrophysiological effects of ghrelin on TMN neurons using rat brain slice preparations and whole-cell patch clamp recording technique. Application of ghrelin depolarized TMN neurons in both absence and presence of tetrodotoxin, and the depolarization was blocked by [D-Lys^3]-GHRP-6, a specific antagonist for GHS-Rs. The ghrelin-induced depolarization was accompanied by increase of membrane resistance, and decreased under high-K^+ extracellular solution and/or presence of KB-R7943, an inhibitor of Na^+/Ca^{2+} Exchanger (NCX). These results suggest that ghrelin depolarizes TMN neurons postsynaptically via GHS-Rs with a dual ionic mechanism including a decrease in K^+ conductance and an activation of NCX, and may contribute to the regulation of sleep-wakefulness via the excitatory effect on TMN neurons.

BIPHALIN PROTECTS AGAINST COGNITIVE DEFICITS IN A MOUSE MODEL OF MILD TRAUMATIC BRAIN INJURY (MTBI)

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Traumatic brain injury (TBIs) concussion is often a result of traffic accidents, contact sports as well as battlefield or terrorist explosions. TBI is classified based on severity. A mild form of traumatic brain injury (mTBI), often results in the post-concussion syndrome (PCS). Unfortunately, PCS is usually underestimated, because the immediate physical symptoms decrease rapidly and conventional neuroimaging studies of the brains of most mTBI victims often do not express any radiological evidence of brain lesions. However, cognitive impairments persist for weeks, months or even years after the incident. A mouse weight drop model mirrors well the mTBI-induced long-lasting learning and memory impairments observed in humans [1]. Recent results indicate that opioids, especially biphalin show promising anti-neurodegenerative properties [2,3]. Therefore, we decided to assess if an immediate post-injury injection of biphalin provided any benefits in mTBI behavioral impairments. After a systemic administration of biphalin we observed an improvement in spatial and recognition memory in the Morris Water Maze and Novel Object Recognition tests 7 and 30 days post-trauma. Our new data suggest that opioid receptor activation may provide neuroprotection of post-traumatic neurodegeneration processes. Further investigations will be carried out in the development of optimal post-accidental therapeutic time-window for efficacious treatment of mTBI.
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ANTICANCER PROPERTIES OF PROSPECTIVE PEPTIDES GENERATED IN NEURONAL PROTEINS TURNOVER

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Proteins turnover plays important role in numerous cellular processes, including cell cycle regulation, differentiation, apoptosis, and signal transduction [A. Ciechanover, Proteolysis: From the lysosome to ubiquitin and the proteasome. Nat. Rev. Mol. Cell Biol. 6, 79 (2005)]. In mammalians protein turnover comprises a well recognized intracellular proteasome system and extracellular proteases located in extracellular matrix. Generation of short peptide fragments is an obvious stage in route of protein degradation to amino acids. These endogenously generated peptides are supplemented with short peptides from food proteins. We hypothesize that the population of short peptides existing in the extracellular space may play important homeostatic functions. Peptide fragments of neuronal proteins should form a major component of short peptides in CNS extracellular space. Cancerogenesis is one of the most vexing pathological processes that should be under control in a properly functioning body. Gliomas and neuroblastomas are the most often observed cancers of the nervous system. Therefore, we decided to analyze the effects of short peptides generated from spinal cord proteins on the proliferation and colony formation by glioma and neuroblastoma cells. Respective mixture of short peptides of spinal cord has been generated by enzymatic digestion of pig spinal cord. The obtained mixture expresses high *in vitro* activity in tests of proliferation and colony formation of glioma T98G and neuroblastoma SH-SY5Y cell lines.

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MINICHAPERON PROPERTIES OF PEPTIDES GENERATED FROM NEURONAL PROTEINS

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Proteins turnover plays important role in numerous cellular processes [A. Ciechanover, Nat. Rev. Mol. Cell Biol. 6, 79 (2005)]. In mammalians protein turnover comprise well recognized intracellular proteasome system and extracellular proteases located in extracellular matrix. Although amino acids are final product of protein turnover, generation of short peptide fragments are obvious stages. These, endogenously generated peptides are supplemented with short peptides from food proteins. We hypothesize that combination of short peptides existing in extracellular space may play important homeostatic functions. Peptide fragments of neuronal proteins should form major component of short peptides in CNS extracellular space. Uncontroled aggregation of amyloid polypeptides in nervous cell membranes is the reason of neurodegenerative processes in various CNS diseases including Alzheimer disease. We hypothesized that in neurohomeostasis amyloid peptides monomeric, helical structures are stabilized by multiple interactions with short peptides in intracellular space. The concentration of peptides is reduced with age as a result of decreased turnover of endogenous proteins as well as reduced peptides from food proteins. We hypothesized that such deficiency could be supplemented by proper peptide nutraceuticals. Respective nutraceutical mixture of short peptides of spinal cord proteins has been generated by enzymatic digestion of pig spinal cord. The influence of such mixture was tested on aggregation of b-amyloid(1-42) peptide in tube. Secondly, the influence of preventional supplementation with the nutraceutical on mouse FVB/APP+ behavior (animal model of Alzheimer disease) has been accomplished. The obtained results evidenced effectiveness of the digested mixture in both direct prevention of amyloid aggregation as well as suppression of AD like behavioral symptoms in Alzheimer disease animal model.

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EXPRESSION, FUNCTION AND SIGNALING OF GPCRS IN MICROGLIA

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Microglia, the immune cells of the central nervous system (CNS), are busy and vigilant housekeepers in the adult brain. They express numerous G-protein coupled receptors (GPCRs) for neurotransmitters, neurohormones, and neuromodulators. One of the characteristic features of microglia is migration toward lesion or inflammatory sites. The main candidate as a chemoattractant for microglia at damaged site is adenosine triphosphate (ATP), however, many other substances can induce immediate change of microglia. Some neuropeptides such as angiotensin II, bradykinin (BK), endothelin, galanin (GAL), as well as chemokines and neurohormone such as thyroid hormone are also chemoattractants for microglia. Among them, BK increased microglial migration via B_1 receptor with different mechanism from that of ATP. BK-induced migration was controlled by a $G_{i/o}$ protein-independent pathway, while ATP-induced migration was via a $G_{i/o}$ protein-dependent and also mitogen-activated protein kinase (MAPK)/exteracellular signal-regulated kinase (ERK)-dependent pathway. On the other hand, GAL is also reported to be up-regulated following pathologic events, such as neuronal axotomy or inflammation, nociception, and Alzheimer's disease, and have similar signal cascade as that of BK. However, only part of the signaling was similar to that of BK-induced migration. For example, BK activates reverse-mode of Na^+/Ca^{2+} exchange mechanism allowing extracllular Ca^{2+} influx, while GAL induces intracellular Ca^{2+} mobilization via increasing inositol-3,4,5-trisphosphate. In addition, GAL activates MAPK/ERK-dependent signaling but BK did not. These results suggest that chemoattractants and their GPCRs for immune cells in the brain including ATP and each peptide may have distinct role and function through their GPCRs under pathophysiological conditions.

ACTIVATION OF TRPA1 ION CHANNEL BY HYDROGEN SULFIDE AND POLYSULFIDES IN TRIGEMINAL SENSORY NEURONS

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Background: Hydrogen sulfide (H_2S) is an endogenous gasotransmitter, modulates various biological functions, including nociception. It is known that H_2S causes neurogenic vasodilation and elicits hyperalgesia. The Transient Receptor Potential Ankyrin repeat domain 1 (TRPA1) channel is expressed in sensory neurons and is involved in inflammatory pain. It responds to a wide variety of stimuli, including cold temperature, pungent natural compounds and environmental irritants. H_2S was described as stimulator of TRPA1 in rat DRG neurons. The aim of this study to determine the molecular targets of H_2S by investigating the effects of H_2S donors (NaHS and Na_2S) and polysulfides (DMTS, K_2S_X) on trigeminal sensory neurons from rats and mice (wild-type and TRPA1(-/-).
Methods: Ratiometric technique of $[Ca^{2+}]_i$ measurement with the fluorescent indicator fura-2-AM on cultured trigeminal cells (rat and mice) was performed.
Results: Rapid increase in $[Ca^{2+}]_i$ was detected in trigeminal neurons indicating ion channel activation after gasotransmitter administration. DMTS (100 μM), K_2S_X (1mM) and NaHS (10 mM) increased $[Ca^{2+}]_i$ in wild-type mice sensory neurons. Most of the NaHS responsive cells were also sensitive to allyl isothiocyanate (200 μM). Na_2S (500 μM, 1mM) evoked $[Ca^{2+}]_i$ increase on TRG cells from wild type mice in concentration dependent manner. Neither of them had any effect on $[Ca^{2+}]_i$ on TRPA1(-/-) mice trigeminal neurons. NaHS were tested on rat trigeminal sensory neurons in two concentrations (1mM, 10mM,) which evoked a robust $[Ca^{2+}]_i$ increase in concentration dependent manner.
Conclusions: Our results indicate that polysulfides and H_2S activates TRPA1 ion channel on the cell bodies of sensory neurons in concentration dependent manner.
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INVOLVEMENT OF THE VAGUS NERVE IN THE ENHANCING EFFECT OF GASTROINTESTINAL MOTILITY BY GHRELIN

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Ghrelin is a hormone that secreted mainly by the stomach. Ghrelin signals from the stomach are transmitted to the brain via the vagus nerve and stimulate feeding. Because ghrelin has a wide variety of physiological roles not only feeding control, it is necessary to examine the involvement of the vagus nerve for these physiological roles of ghrelin. In this study, we have investigated the control of gastrointestinal motility by ghrelin after vagotomy.
Male C57/BL6J mice and ghrelin gene-deficient mice were used. Mice were fasted for 16 hours, then, was orally administrated the test meal, that consist of 0.5% Evans blue and 2.5% carmellose sodium. The mice were euthanized 10 minutes after the test meal administration, and collect stomach and intestinal tract. We measured the ability of gastric emptying (the residue weight in the stomach test meal, GE) and of the transport of gastrointestinal tract (the ratio of the test meal labeled length with respect to the total length, TGT). Further, in order to investigate the role of ghrelin on gastrointestinal

motility, mice were intraperitoneally pre-administered ghrelin or [D-Lys^3]-GHRP6.
Pre-administrated ghrelin was enhanced GE and TGT in C57BL/6J mice. On the other hand, pre-administration of [D-Lys^3]-GHRP6 showed an inhibitory effect on the GE and TGT. Although GE and TGT had been suppressed in ghrelin gene-deficient mice, pre-administrated ghrelin was also enhanced GE and TGT in ghrelin gene-deficient mice in the same way as C57BL/6J mice. These results indicate that ghrelin has an enhancing effect of both GE and TGT. Next, we investigated the involvement of the vagus nerve on the control of gastrointestinal motility by ghrelin. The gastrointestinal motility was normal in vagotomized C57BL/6J mice with the exception of the thickening of the gastric wall. In addition, pre-administration of ghrelin was normally enhanced gastrointestinal motility in vagotomized C57BL/6J mice. These results imply that vagus nerve is not necessary for the enhancing effect of gastrointestinal motility by ghrelin.

INHIBITORY EFFECT OF SOMATOSTATIN RECEPTOR SUBTYPE 4 ACTIVATION ON THE ACUTE STRESS-RELATED BEHAVIOURS OF MICE

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Somatostatin is expressed by several inhibitory interneurons in the brain. It exerts anxiolytic and antidepressant-like effects in animal models and the alterations of somatostatinergic neurotransmission in different psychiatric disorders has also been shown in clinical studies. However, little is known about the mechanisms and the involvement its receptors. Since the somatostatin receptor subtype 4 (sst_4) is widely expressed in stress-related brain regions, we investigated its role in anxiety and depression-like behaviours.
Anxiety was examined in the open field test (OFT) and elevated plus maze (EPM). Stress-coping behaviour was assessed in forced swim (FST) and tail suspension tests (TST). Mice were perfused with paraformaldehyde 2 hours after TST and c-Fos immunohistochemistry was performed in a variety of stress-related brain regions to detect early neuronal activation. Genetic deletion of sst_4 ($sst_4^{-/-}$) and its pharmacological activation with a selective synthetic non-peptide agonist, J-2156, were used as experimental tools.
$Sst_4^{-/-}$ mice spent significantly less time with spontaneous locomotor activity in the OFT and in the open arms of the EPM than the wildtypes. They showed significantly longer immobility in the FST, but not in the TST. J-2156 (100 microg/kg i.p.) significantly decreased immobility in the TST, but did not influence immobility in the FST. TST significantly increased c-Fos immonopositivity in several stress-related brain areas and this was further augmented by J-2156 in the nuclei of the extended amygdale, in the nucleus raphe dorsalis, in the periaqueductal grey matter and in the central projecting Edinger–Westphal nucleus, but not in the parvocellular part of the paraventricular nucleus of the hypothalamus.
We provided the first data that sst_4 activation decreases anxiety and depression-like. Therefore, it might be a potential drug target in stress-related disorders.

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REGULATORY ROLE OF CAPSAICIN-SENSITIVE NEURONS IN CHRONIC RESTRAINT STRESS-INDUCED PAIN BEHAVIOURS OF MICE

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Experimental and clinical data suggest that chronic stress enhances pain sensation and plays an etiological role in some conditions characterized by clinically significant pain (e.g. fibromyalgia, irritable bowel syndrome). Changes in central pain processing were extensively examined in patients with stress-related pain disorders, but the mechanisms and the role of the peripheral nervous system is unclear. Thus, we studied the effect of chronic restraint stress (CRS - 6 hours/day, 4 weeks) on different behavioural changes of CD1 mice.
The role of capsaicin-sensitive peptidergic neurons was examined by desensitization with resiniferatoxin (RTX), an ultrapotent capsaicin analogue (30, 70 and 100 microg/kg s.c. on 3 consequtive days). The mechanonociceptive threshold was measured by dynamic plantar aesthesiometry, noxious heat sensitivity on a hot plate, cold tolerance was determined by paw withdrawal latency from 0°C water. Behavioural effects of chronic stress were examined in open field (OFT), light-

dark box (LDB) and tail suspension tests (TST). Thymus and adrenal gland weight were measured at the end of the experiment.
CRS decreased the mechanonociceptive threshold by 20 % and increased cold sensitivity by 50 % in non-desensitized mice. RTX-pretreatment significantly enhanced the stress-induced mechanical, but not the cold hyperalgesia. The noxious heat threshold was higher after RTX desensitization, but it was not affected by CRS. Time spent in the lit compartment of LDB was significantly increased in stressed non-pretreated animals, but this was absent after RTX-desensitization. CRS did not influence behaviours in the OFT and TST. Decreased thymus and increased adrenal gland weight in response to chronic stress were not altered by RTX-pretreatment.
These are the first data for the protective role of capsaicin-sensitive peptidergic neurons in chronic stress-induced pain behaviours and anxiety.

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CHARACTERIZATION OF PACAP NEUROPROTECTIVE EFFECTS AND APPROACHES FOR POSSIBLE THERAPEUTIC APPLICATIONS

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a 38-amino acid C-terminally α-amidated peptide that was first isolated from ovine hypothalamic extracts on the basis of its ability to stimulate adenylyl cyclase from rat anterior pituitary cells. The wide distribution of PACAP and its receptors in the central nervous system (CNS) and in peripheral tissues suggested that the peptide could be involved in a large array of biological activities. Indeed, PACAP exerts numerous effects on the cardiovascular and immune systems, the urogenital and respiratory tracts, the endocrine glands, the gonads and the CNS [1]. In the adult brain, PACAP has beneficial effects in various pathological states including Parkinson's disease, ischemia and traumatic brain injury. These results suggest that PACAP could have therapeutic value for the treatment of several pathological states. However, PACAP is prone to rapid enzymatic degradation, which could preclude its use as a therapeutic agent. In particular, it has been shown that after an intravenous injection, PACAP is rapidly metabolized by dipeptidyl peptidase IV (DPP IV), which leads to the formation of PACAP(3-38) and PACAP(5-38). This conversion of the active peptide into antagonists may compromise therapeutic applications. Furthermore PACAP acts on 3 different receptors which can induce some side effects. These observations prompted us to develop PACAP analogs with higher stability and specificity and to use stem cells for a local delivery of the peptide toward the lesion area. Our analogs and stem cells producing PACAP have been characterized *in vitro* and are now being tested *in vivo* in stroke models.
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PACAP PLAYS A CRUCIAL ROLE IN DIFFERENTIATION OF NEURAL PROGENITOR CELLS INTO GLIAL LINEAGE

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Neural development is controlled by region-specific factors that regulate cell proliferation, migration and differentiation. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that exerts a wide range of effects on different cell types in the brain as early as the fetal stage. Previously, we reported that PACAP induced differentiation of cultured mouse embryonic neural progenitor cells (NPCs) into astrocytes. Here, we report that the effect of PACAP *in vivo* on the glial differentiation.
We investigated the localization of PACAP specific receptor (PAC1-R) during neural development by use of immunohistochemistry. The immunereactivity of PAC1-R and proliferation marker, Ki67, showed similar distribution in E14 mouse embryo. Double-immunostaining showed that immunoreactivity for PAC1-R was co-localized with radial glia marker, vimentin. Astrocyte marker, GFAP-positive cells could be detected from E16. These data suggest that endogenous PACAP leads differentiation of NPCs into astrocytes via radial glia differentiation. Addition of PACAP into primary cultured-NPCs increased the expression of another radial grail marker, GLAST after 4 days culture. On the other hand, the expression of GFAP was up-regulated 8 days after PACAP administration. Furthermore, intracerebroventricular injection of PACAP into the ventricle of telencephalon of E13 embryos *in utero* increased the number of GLAST immunopositive cells. These data suggest that PACAP plays crucial roles in the differentiation of NPCs into astrocytes via radial grail lineage.

PACAP TYPE 1 RECEPTOR EXPRESSION IN HEMATOPOIETIC STEM/PROGENITOR CELLS OF MOUSE BONE MARROW WITH SPECIAL REFERENCE TO SYMPATHETIC INNERVATION

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide and contributes to anti-apoptosis, anti-inflammation, cell proliferation and differentiation. However, the role of PACAP in the hematopoiesis has not elicited in detail. The purpose of this study is to investigate the expression and localization of PACAP and its receptors in the hematopoietic organ BM. Gene expressions of three PACAP receptors (PAC1R, VPAC1R and VPAC2R) were recognized in the BM by RT-PCR, but few expressed of these ligand PACAP. Then, PAC1R was multiple-immunostained with hematopoietic cell markers in BM smear sections. Strong expression of PAC1R was detected in larger size and light chromatin condensation cells which were co-expressed with hematopoietic stem/progenitor cell markers (CD34, CD117 and Sca-1). Meanwhile, the sympathetic nerve fibers were detected in the BM by frozen sections with tyrosine Hydroxylase (TH) and neurofilament 200 antibodies. After injection of Flouro-gold into tibia, ganglions at the lumbar 1-4 level of sympathetic chain were retrograde traced and strongly expressed of PACAP and TH mRNA. In addition, PACAP gene expression were few in the adrenal gland and blood cells. These results suggest that PACAP/PAC1R signaling may contribute to the hematopoietic function.

Session 5
Regulatory peptides in autonomic nervous function and endocrinology
Cardiovascular functions, Gastrointestinal tract

PROTEOMICS OF HEART SECRETORY GRANULES: METHOD DEVELOPMENT AND PRELIMINARY RESULTS

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Introduction: Here we assess a proteomics workflow for analysis of secretory granules collected from pig hearts. We aim to identify candidate biomarkers and therapeutic targets for heart disease, and possibly novel regulatory peptides.
Methods: Homogenate of porcine atrial tissue is fractionated by gradient centrifugation and granule-containing fractions are identified by western blotting for known granule markers, including atrial natriuretic peptide (ANP) and peptidyl-amidating monoxygenase (PAM). Relevant fractions are then analyzed by proteomics. In brief, the proteins (5 μg) are treated by SDS-PAGE and in-gel digestion and the proteolytic peptides are extracted from the gel and analyzed by nanoLC-MS (Ultimate3000 LC system (Thermo)) coupled with a MicrO-TOF QII mass spectrometry (MS) instrument (Bruker). The MS data is interpreted using the Mascot Distiller software (Matrix Science).
Results: So far, our proteomic analysis have identified more than 1500 proteins (FDR<1%) in pig atrial tissue and confirmed increased amounts of ANP and BNP in the granule fractions relative to the total homogenate.
Conclusions: The combination of gradient centrifugation and proteomics is a feasible strategy for identifying novel granular proteins and peptides from pig heart tissue. Future work will include establishment of a quantitative proteomics workflow for comparing the granule fraction collected from pigs with myocardial infarction and from control pigs. In perspective, novel secreted proteins and peptides from the heart should be studied for their clinical potential and biological function.

IDENTIFICATION AND QUANTIFICATION OF GLYCOSYLATED PEPTIDES IN PIG HEART

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Heart failure (HF) is a common condition and a challenge to diagnose despite the aid of plasma biomarkers, e.g. natriuretic peptides. B-type natriuretic peptide (BNP) is released from the cardiomyocytes and recent studies have disclosed that BNP is mainly released to plasma as the immature precursor form in HF. One mechanism in the cardiac processing of proBNP is related to O-glycosylation near the processing site and suggests that the ability to glycosylate secreted proteins from the heart changes in disease.
We developed a chronic porcine model for cardiac dysfunction induced by myocardial ischemia with a postoperative period of 8 weeks. To examine if glycosylation is altered, we wish to establish quantitative O-glycoproteomics on cardiac tissue extracts enriched for O-glycosylated proteins. Preliminary results from qualitative glycoproteome analysis of porcine atrium and ventricle have so far identified 140 and 73

glycosylated proteins, respectively. Within these proteins, 421 and 220 glycosylation sites were identified with 63 proteins and 128 glycosylation sites in common, respectively. 3 sites on chromogranin A (CgA) were identified that have not been identified in humans, and 2 of these sites are well conserved suggesting that CgA may be modified to an extent not previously reported. Interestingly, proBNP was identified as a glycosylated protein suggesting this strategy can be used to elucidate alterations in the endocrine capacity of the failing heart.

In summary, we have developed a porcine model of cardiac dysfunction and applied a strategy for glycoprotein enrichment and proteomic analysis to determine the endocrine capacity of the heart in this syndrome. Preliminary results corroborate that this approach can be used to identify known and novel O-glycosylated proteins and be implemented in a more in-depth quantitative analysis to compare two disease states.

DYSREGULATION OF RESTRAINT-INDUCED EXPRESSION OF UROCORTINS IN THE HEART OF LOW BIRTH WEIGHT RATS

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Introduction: Low birth weight is related to increased incidence of cardiovascular and metabolic disorders later in life. Recent studies suggest that low birth weight caused by maternal malnutrition is one of risk factors of hypertension. Since urocortin (Ucn) 1 and 2, members of corticotropin-releasing factor (CRF) family peptides, have vasodilatory and cardiac inotropic effects, Ucns may have an important role in blood pressure regulation. However, the regulatory mechanisms of expressions of Ucns and their receptor (CRF-R2) in the heart are not well understood. We hypothesized that regulatory mechanism of blood pressure is disrupted in low birth weight rats and that impaired expression of Ucns-CRF-R2 in the heart may be involved in the pathophysiology.

Aims: We tried to clarify whether stress-induced expression of Ucns-CRF-R2 is dysregulated in the heart of rats delivered from calorie-restricted dams.

Methods: Blood pressure (BP) was measured by tail-cuff method in low birth weight offspring (LBW), who had been delivered from calorie-restricted dams, and normal birth weight offspring (NBW), who had been delivered from ad libitum fed dams, and the expressions of Ucn 1, Ucn 2 and CRF receptor type 2 (CRF-R2) mRNA were analyzed in the heart before and during restraint.

Results: We found that LBW showed elevated basal BP. Basal expression levels of Ucn 2 mRNA in the heart was higher in LBW than in NBW, while the expression levels of Ucn 1 and CRF-R2 mRNA were unchanged. Restraint significantly increased Ucn 1, Ucn 2 and CRF-R2 mRNA expressions in the heart of NBW, but those expressions were unaffected in the heart of LBW.

Conclusion: These results suggest that expressions of Ucns-CRF-R2 in the heart were dysregulated in LBW whose basal BP were elevated.

IMMUNOHISTOCHEMICAL LOCALIZATION OF PACAP AND VIP RECEPTORS IN MAJOR SALIVARY GLANDS AND THE EFFECT OF PACAP AND VIP ON SALIVA SECRETION IN MICE

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The control of saliva secretion is mainly under the autonomic nervous control. Pituitary adenylate cyclase-activating polypeptide (PACAP) is now recognized as the multi-functional neuropeptide in various organs. Vasoactive intestinal polypeptide (VIP) shows the similar function to PACAP. In this study, we compared the distribution of PACAP receptor, PAC1R and VIP receptor, VPAC1, in major salivary glands; parotid, submandibular and sublingual glands, and examined the effect of PACAP on saliva secretion in 8 weeks old C57BL/6 mice. In parotid gland, PAC1R and VPAC1 were detected in the cells of striated duct. In the case of submandibular gland, PAC1R and VPAC1 were detected in the tall columnar epithelial cells, called pillar cells, in granular ducts and some of the cells in the striated ducts. In sublingual gland, PAC1R and VPAC1 were expressed mainly in the epithelial cells of striated ducts. Intranasal injection of PACAP enhanced the secretion of saliva. Taken together, these results indicated that the distribution of PAC1R and VPAC1 were similar in three major salivary glands and that PACAP and VIP might be the useful agents for the future dry mouth therapeutics.

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MATERNAL HIGH FAT DIET DURING PREGNANCY AND LACTATION INFLUENCES ON OBESTATIN AND GHRELIN CONCENTRATION IN MILK AND PLASMA OF DAMN WISTAR RATS AND THEIR OFFSPRING

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The aim of this study was to investigate a relationship between maternal diets (differing in fat content) and obestatin (OB) balance during gestation and lactation and association to its plasma level in the offspring in the first 21 days of life. The experiment was conducted on 12 females. On the mating day animals were randomly allocated into one of the fallowing diets: high fat diet (30% of fat; HFD, n=6), or breeding diet (5% fat; BD, n=6), which were continuing till the 21st day of lactation. On 14th day of gestation females were sampling for blood and separated from males. The day when females gave birth was consider day 1 of lactation. On the 1st day of lactation the supernumerary pups were sampling for blood and euthanized (n=7, for each maternal diet). On 14th day of lactation 2 pups from each litter were sampling for blood and then sacrificed (n=12 for each maternal diet). The rest pups (n=12, for each diet) were continuing the experiment till the 21st day of lactation. OB concentration was assayed using radioimmunology.

OB concentration in plasma of BD rats was unchanged in pregnancy and lactation. In contrast, HFD resulted in significant decrease in OB level on the 14th day of lactation and significant elevation on the 21st day both comparing to unpregnant females and lactating BD females. OB concentration in milk during lactation was over to fold higher than in blood plasma of dam rats. In BD dams a concentration of OB in milk did not differ significantly during lactation whereas HFD resulted in significant increase in milk OB along the lactation. The highest OB concentration in blood plasma of control suckling rats was observed on day 1 of lactation and then the significant decrease was observed in the following days. In suckling rats from HF dams, OB concentration was significant higher in tested time points. In conclusion, HFD during pregnancy and lactation changes OB balance in maternal blood and milk and influences on its concentration in neonates.

ENTERAL OBESTATIN INFLUENCES ON INTESTINAL CONTRACTILITY IN NEONATAL WISTAR RATS- IN VITRO STUDIES

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Obestatin is a 23-amino acid peptide encoded by the ghrelin gene. Moreover, a substantial amount of this peptide has been found in colostrum and milk. We have investigated the intestinal contractility of neonatal rats enterally treated with obestatin (125nmol/kg b.wt., LO, ; 250 nmol/kg b.wt, HO) or saline solution (C), (n=12 for each treatment) for 7 days. Duodenal and middle jejunum whole-thickness preparations from 21 day old rats were studied in an organ bath, for isometric recording under treatment with acetylocholine (ACh), atropine and tetradotoxin (TTX). The electrical field stimulation (EFS) was performed (voltage 90 V, duration 10 seconds) at three frequencies: 0.5, 5 and 50 Hz with 1 min intervals between each pulse.

After treatment with higher doses of obestatin (250 nmol/kg b.wt, HO) the significant decrease in amplitude was observed for EFS–stimulated off-contraction, as well as spontaneous contractile activity in both studied intestinal segments.

Administration of obestatin in dose 125nmol/kg b.wt. (LO) resulted in decrease in amplitude both for EFS –stimulated off-contraction, as well as spontaneous contractility but only in the middle jejunum. In all experimental groups the amplitude of EFS –stimulated off-contractions and spontaneous contractility was sensitive to treatment with TTX. In obestatin treated animals injection of atropine did not result in a significant decrease of amplitude of spontaneous contractility of both intestinal segments comparing to untreated with atropine intestinal preparations from LO and HO.

These results indicate the importance of peripheral obestatin in the programming of intestinal contractility in rats during suckling period. Moreover this effect is dose dependent and independent of cholinergic pathways.

ENTERAL OBESTATIN ADMINISTRATION IN SUCKLING RATS INFLUENCES ON TRAN SCRIPTOMIC PROFILES OF THE SMALL INTESTINE

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The aim of the study was to investigate the effect of enteral obestatin administration on the development of small intestine in neonatal rats. The experiment was carried out on suckling neonatal rats from 4 different litters that reached 14 days of age (n=8). Rats were divided into 2 groups: C- suckling rats, O- suckling rats administered with obestatin (250 nmol/ kg b.wt) via stomach tube once a day for 7 subsequent days.

Mid-jejunum samples were weighed and then the transcriptomic profiles was analysed using Agilent GE 8 x44 whole rat genome microarrays. The results were analysed with Gene Spring Software (Agilent, USA) and Panther Classification System database. No changes were observed in the body weight and the weight of gastrointestinal organs (stomach, pancreas, liver, intestine) between studied groups. However, between both experimental groups the transcriptomic profiles of mid-jejunum differed in n=2365 regulated genes. 1279 genes from this pool are involved in metabolic processes. The analysis of gene profile revealed significant differences in the regulation of genes involved in the signalling pathways for: Wnt, apoptosis, EGF receptor, FGF, integrin, PDGF, GnRH receptor as well as Ras pathway, angiogenesis

and Parkinson disease pathway. Although the role of the most of above interactions is not clear at the moment, the significance of their response after enteral administration of obestatin indicate its involvement in metabolic programming and development of rat small intestine during the early postnatal life.

ELEVATED PLASMA CONCENTRATIONS OF SOLUBLE (PRO)RENIN RECEPTOR IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME

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(Pro)renin receptor is a specific receptor for both renin and its precursor prorenin. (P)RR plays important roles in the pathogenesis of vascular complications of diabetes mellitus and hypertension. Soluble (pro)renin receptor (s(P)RR), which is generated by furin from full length (P)RR, has been shown to be present in blood. Obstructive sleep apnea syndrome (OSAS) is a common disease which affects 2%-4% of total population. Risk factors of OSAS include obesity, hypertension, and diabetes mellitus. The aim of the present study is to clarify the association of plasma s(P)RR concentrations and the severity of OSAS. Plasma samples were obtained from 58 male patients who were diagnosed as OSAS based on polysomnography, and 14 age-matched male control subjects. Plasma concentrations of s(P)RR were measured by ELISA. Plasma s(P)RR levels were significantly higher in patient with OSAS (9.0± 2.0ng/ml, mean±SD) than in control subjects (7.4±1.5ng/ml) (P=0.0026). Plasma s(P)RR levels were in significant positive correlations with %stage1 (r=0.374, p=0.005), arousal index (r=0.341,p=0.01), apnea hypopnea index (AHI) (r=0.352,p=<0.01) and desaturation index (r=0.302,p<0.05), and in a significant negative correlation with %stage rapid eye movement (REM) sleep (r=-0.377,p=0.005). Twelve OSAS patients with AHI>20 were treated with nasal continuous positive airway pressure (nCPAP) treatment for 3 months, and plasma s(P)RR levels were significantly decreased after it. The study has shown for the first time elevated plasma s(P)RR levels in patient with OSAS. Plasma s(P)RR concentrations were associated with the severity of OSAS. Soluble (P)RR may serve as a plasma marker reflecting the severity of OSAS.

OBESTATIN SENSITIZES JEJUNUM BUT NOT DUODENUM TO CONTRACTILE CHOLINERGIC STIMULATION IN NEWBORN PIGS

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Obestatin is produced and secreted in the gastrointestinal tract (GIT). There is little information relevant to impact of obestatin on GIT motility in newborn animals. The study was done on 18 piglets (1-7 d old) housed in the automated sow system. Animals received intragastrically saline solution - 2ml, (n=6, K) or the same volume of saline solution with rat obestatin 10 μg/kg body weight (BW), (n=6, O10) or 15 μg/kg BW (n=6, O15) every 8 h for 7 d. At day 7 animals were sacrificed, duodenal and jejunal segments were removed and placed in organ bath chambers filled with saturated Krebs buffer. Under isotonic condition all segments regained their spontaneous activity within 30 min, then they were subjected to acetylcholine (ACh) stimulation under cumulative doses (10^{-9}-10^{-4} M), washed and relaxed with isoprotenerol 10^{-4}M. Amplitude of spontaneous contraction of duodenum was similar in all groups but in the middle jejunum treatment with obestatin (O10 and O15) increased as compared to K (*p=0.0011*). The effect was dose depended. Acetylcholine stimulation caused a dose-dependent smooth muscle contraction of the duodenum and jejunum. In all groups stimulation of duodenum was effective at Ach 10^{-7}M, but area under the curve (AUC) showed no effect of obestatin on duodenum contraction (*p=0.5972*). In jejunum the effective dose of ACh was 10^{-8}M. The observed differences in responsivness were not significant (*p=0.2381*). In jejunum AUC revealed a highly significant (*p=0.0049*) increase in response to ACh in both experimental groups. In all groups smooth muscle relaxation of the duodenum and jejunum caused by administration of isoproterenol was similar and there was no significant differences.

ANTRAL AND DUODENAL MYOELECTRIC ACTIVITY CHANGES AROUND THE DAY, EFFECT OF GHRELIN IN CONSCIOUS SUCKLING PIGLETS – PRELIMINARY RESULTS

J.Kwiatkowski, M. Słupecka, P. Ochniewicz, J. Woliński

Myoelectrical activity of the stomach and small intestine consists of a periodical presence of migrating myoelectric complexes (MMC), which is susceptible to interruption by feeding - invoked “prandial pattern”. The present study aims to record and examine the preprandial and postprandial patterns of antral and duodenal myoelectric activity potentially altered by intra gastric ghrelin admission (15 μg/kg). Another objective was to estimate the postsurgical time of MMC reemergence – a first sign of the reactivating motility. To this end, conscious suckling pigs (10 days old, n = 2) were fed every hour by artificial sow system and were recorded for minimum 24h with the help of a telemetric measurement system. Anesthetized animals underwent right – side laparotomy during which platinum bipolar electrodes were sutured on the antrum and the proximal duodenum. Electrodes were connected to a telemetry transmitter implant (TL10M3-D70-

EEE, Data Sciences Int., USA) fixed extraperitoneally in a pocket made between the two layers of the abdominal muscles. The resulting surgical wound was sutured with no wires extending through the skin. The piglets were allowed a one day recovery period before the recordings begun. Signal acquisition was carried out by an RMC – 1 receiver (DSI) placed under the cage coupled to a DL10 analogue output (DSI). Each of 3 signal channels was filtered (hi cut – off 50 Hz, low cut – off 10 Hz) and amplified (BioAmp, ADInstruments, Australia). A 6 channel PowerLab/4e (ADInstruments) and a computer outfitted with LabChart software (ADInstruments) were used to record and analyze the data. Raw data composed of mean values of amplitude and frequency described the pre- and postprandial patterns present during intervals lasting respectively 30 minutes before and after feeding. Root mean square and mean power frequency values were also extracted from the data to further describe the signal. Values describing both patterns were tested with the Student's t – test to evaluate any statistically significant differences.

First, full MMC cycle was recorded 28 hours after the surgery. No relevant differences were observed between pre- and postprandial patterns of antral and duodenal myoelectrical activity after the admission of ghrelin.

Session 6
Translational research and drug development of regulatory peptides

MOLECULAR MECHANISM OF VIP CORRECTOR EFFECT FOR THE TREATMENT OF CYSTIC FIBROSIS

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Mutations in the CFTR gene lead to cystic fibrosis (CF), the most common lethal genetic disease in Caucasian populations. CF is characterized by reduced chloride secretion across epithelia, viscous mucus secretions, chronic bacterial infections and inflammation in the airways. F508del, the most common CF causing mutation, induces early degradation and poor trafficking of CFTR chloride channels. Finding molecules capable of correcting F508del-CFTR dysfunctions is the major goal of current therapeutic strategies. Early studies showed a lack of VIP-secreting nerve fibers in CF tissues. In airways epithelial cells we demonstrated that the neuropeptide VIP activates VPAC1 / PKCε signaling cascade and increases CFTR activity by reducing its endocytosis rate through the formation of a stable complex with ERM and NHERF1 at the cell surface. In JME/CF15 human nasal epithelial cells, prolonged stimulation with VIP corrected F508del-CFTR trafficking and function. *In vivo*, features of lung and intestinal disease of VIP-KO mice resembled those reported in CF mice. At the molecular level, CFTR intracellular retention and subsequent loss of chloride current created a CF-like condition. IP injections of VIP corrected tissue abnormalities and CFTR dysfunction. We have tested the F508del-CFTR corrector potential of VIP-ELP biopolymer fusion molecules (PB1120 and PB1046) which are highly resistant to peptidases that rapidly degrade native VIP. In JEM/CF15 cells both drugs induced a functional correction of F508del-CFTR (2-24 hr). The potency of PB1046 was twice that of other known CF correctors and it potentiated F508del-CFTR function to a similar extend as ivacaftor, the only clinically available drug recommended for ~ 4% of CF patients. Our results support the use of VIP derivatives for the treatment of cystic fibrosis at the molecular level for the vast majority of CF patients with the F508del mutation.

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PEPTIDE PROTECTION AGAINST AUTOPHAGY IS COUPLED TO BEHAVIORAL IMPROVEMENTS IN MOUSE MODELS OF SCHIZOPHRENIA

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BECN1/Beclin 1, a key regulator of autophagy, showed a 40% decrease in post-mortem hippocampal tissues of schizophrenia subjects relative to controls. This decrease was coupled to the deregulation of the essential activity-dependent neuroprotective protein (ADNP), a binding partner of microtubule-associated protein 1 light chain 3 (LC3B), another major constituent of autophagy. The drug candidate NAP (davunetide), a peptide fragment from ADNP, enhanced ADNP-LC3B interaction. Together, these studies suggest a key role for autophagy in schizophrenia (Molecular Psychiatry, EPUB). Parallel studies have linked allelic variation in the gene encoding microtubule-associated protein (MAP6) [also known as stable tubule only polypeptide (STOP)] to schizophrenia, along with altered STOP protein expression in the schizophrenic brain and schizophrenic-like behaviors in STOP-deficient mice. Here, we reveal significant decreases in hippocampal BECN1 mRNA and reversal by NAP but not by the anti-psychotic clozapine in STOP-deficient (STOP+/-) mice. Normalization of BECN1 expression by NAP was

coupled to behavioral protection against hyperlocomotion and cognitive deficits measured in the object recognition test. Clozapine reduced hyperlocomotion below control levels and did not significantly affect object recognition. The combination of clozapine and NAP resulted in normalized outcome behaviors. Studies are now extended to a mouse model expressing the mutated disrupted in schizophrenia (DISC1) gene (also associated with microtubule function). Interestingly, ADNP expression was originally discovered as a vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) regulated gene and both VIP and PACAP have been linked to schizophrenia. Phase II clinical studies have shown NAP (davunetide)-dependent augmentation of functional activities of daily living coupled to brain protection. The results provide a novel avenue for drug development.

PROANP AS A PLASMA MARKER OF LEFT VENTRICULAR FUNCTION IN A CHRONIC PIG MODEL OF MYOCARDIAL INFARCTION

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Background: Measurement of natriuretic peptides in plasma is useful in assessing congestive heart failure. In myocardial infarction (MI), the plasma concentrations contain prognostic information of later death. However, the plasma profile following MI is not well established, where the use of medicine may affect the natriuretic peptide response.

Materials and Methods: In 6 adult Göttingen mini-pigs, MI was induced by occluding the second segment of the LAD using a balloon catheter for 60 minutes and compared to 6 controls. Myocardial damage was quantified by serial measurements of cardiac troponin T in plasma. Cardiac function was assessed by 2D echocardiography over a period of 8 weeks from myocardial infarction. Blood was collected every week and plasma proANP was measured by a porcine specific immunoassay.

Results: The troponin T concentration peaked 24 hours post MI (669 ± 156 μg/L versus 3 ± 0 μg/L). During the 8 weeks, MI animals did not develop signs of congestive heart failure, e.g. dyspnea. Echocardiography after 8 weeks revealed a distinct area of hypokinesia in the left ventricular anterior wall. Overall left ventricular systolic function was preserved. Plasma proANP did not change during the 8-week period in the intervention and control groups (pre-intervention mean 362 ± 14.3 pmol/L vs. 551 ± 17 pmol/L ($p = 0.049$), and after 8 weeks; 379 ± 71 pmol/L vs. 516 ± 55 pmol/L ($p = 0.26$)). The mortality rate was 30% on operation day and 0% post-operatively.

Conclusions: Plasma proANP does not change after MI in adult mini-pigs. The data thus challenges the reported increase in patients after myocardial infraction, where the increase may be mediated by a greater loss of pump function or the neurohormonal blockade used in MI patients.

PROTECTIVE EFFECT OF EXOGENOUS PACAP ON ISCHAEMIA/REPERFUSION-INDUCED KIDNEY INJURY OF FEMALE RATS

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Pituitary adenylate-cyclase activating polypeptide (PACAP) is a neuropeptide, its cytoprotective and neuroprotective effects have been shown in several studies. Our team has investigated the role of PACAP in ischaemia/reperfusion-induced kidney injury. We have shown that exogenous PACAP decreases the damage after kidney ischaemia/reperfusion in male rats.

The aim of the present study was to investigate the effect of exogenous PACAP during ischaemia/reperfusion in the kidney of female rats.

Wistar rats were subjected to one-sided renal artery clamping for 60 minutes followed by 24-hour, 48-hour or 14-day reperfusion. PACAP (100 μg) was administered intravenously before arterial clamping in half of the rats in each group. Histological evaluation of the PAS stained sections was performed with Adobe Photoshop and Scion Image programs. In the focus of our investigation was the tubular damage.

The tubular damage was significantly less severe in the PACAP-treated groups after 48-hour and 14-day reperfusion compared to the control groups. The dilatation of the tubular lumen caused by the damage of the tubular epithelium was greater in the control groups. In case of PACAP-treated rats the duration of the reperfusion had no influence on the severity of the damage, but in the control rats the damage was more severe after a longer reperfusion.

Based on our results it can be concluded that exogenous PACAP is protective against ischaemia/reperfusion-induced kidney injury in female rats.

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SERPININ PEPTIDES IN GRANULE BIOGENESIS, NEUROPROTECTION AND CARDIAC FUNCTION

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Chromogranin A (CgA) is a member of the granin family of molecules found in secretory granules of endocrine and neuro-endocrine cells. CgA is processed to several peptides with different functions. Recently we have identified two new peptides processed from the C-terminus of CgA: serpinin and pyroGlu-serpinin (pGlu-serpinin),that is secreted from AtT-20 cells, a pituitary cell line. Immunocytochemistry showed co-localization of serpinin and pGlu-serpinin with adrenocorticotropic hormone (ACTH) in secretory granules of AtT-20 cells, and they are released in an activity dependent manner. Serpinin and pGlu-serpinin were able to augment granule biogenesis in AtT-20 cells or 6T3 cells, by upregulating expression of Protease Nexin-1 (PN-1), a serine protease inhibitor, which then stabilizes granule proteins to increase their levels in the Golgi complex. Serpinin acts to increase PN-1 transcription by binding to a putative G-protein coupled receptor to up-regulate cAMP levels and PKA activity. This in turn causes an increase in translocation of the transcription factor, sp-1 into the nucleus to up-regulate PN-1 expression by binding to the sp-1 promoter. Furthermore, studies demonstrated that serpinin and pGlu-serpinin when added to the cell medium were able to prevent radical oxygen species (ROS, hydrogen peroxide)-induced cell death of AtT-20 cells and cultured rat cerebral cortical neurons. pGlu-serpinin was 100 times more effective than serpinin in inhibiting cell death. These data indicate that serpinin and pGlu-serpinin have anti-apoptotic effects that may be important in neuroprotection of central nervous system (CNS) neurons and pituitary cells. In the heart, three forms of serpinin peptides have been found, serpinin and pGlu-serpinin and serpinin-Arg-Arg-Gly (Ala29Gly). Of these, pGlu-serpinin was the most potent in inducing contractility while the Ala29Gly had no activity. Both these peptides act through a β1-adrenergic receptor /adenylcyclase /cAMP/PKA pathway. In conclusion, the serpinin family of peptides have multiple functions, each having a specific role in different cell types.

ANTIFLAMMIN-1 INHIBITS THE TGF-β1 INDUCED A549 CELLS EPITHELIAL-MESENCHYMAL TRANSITION*

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Epithelial to mesenchymal transition (EMT) is a process by which an epithelial cell alters its phenotype to that of a mesenchymal cell and may play a critical role in the lung fibrosis development. Antiflammin-1 (AF-1, MQMKKVLDS) is a synthetic nonapeptide with a similar sequence to the conserved sequence of CC10 secreted by lung Clara cells. Studies suggest that it has many biological functions. Our previous studies indicated that AF-1 could reduce the collagen production and inhibit the cell proliferation of pulmonary fibroblast NIH3T3 which induced by transforming growth factor-beta1 (TGF-β1). In present study, we observed firstly whether AF-1 can alleviate the progress of TGF-β1 induced A549 cells to mesenchymal transition. Cultured A549 cells were stimulated with 5ng/ml TGF-β1 to induce EMT and treated AF-1 ($1x10^{-4}$mol/L) to observe whether it has inhibitory action or not. The expression of epithelial marker E-cadherin and mesenchymal maker α-smooth muscle actin (α-SMA) were quantified by western blot. The results showed that incubation of A549 cells with TGF-β1 induced an authentic EMT characterized by abundant expression of α-SMA and loss of E-cadherin. However, the progress of EMT in group with AF-1 co-incubated was significantly inhibited. In conclusion, AF-1 can inhibit TGF-β1 induced A549 cells EMT and it may be useful as a adjuvant therapy for the treatment of fibrotic disorders of lung such as idiopathic pulmonary fibrosis.

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ELEVATED GASTRIN LEVELS PROTECT AGAINST HYPOXIA-INDUCED WEIGHT LOSS IN MICE

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Hypoxia, or a low concentration of oxygen, is encountered in humans undertaking activities such as mountain climbing and scuba diving. Although data on the interplay between hypoxia and gastrins are limited, circulating gastrin concentrations rise in rats and newborn calves exposed to experimental hypoxia, and in humans exposed to high altitude. Gastrin expression is upregulated by hypoxia in gastrointestinal cell lines [1], and

gastrins have been shown to stimulate angiogenesis *in vitro* and *in vivo* [2].

Aim: The aim of this study was to determine if higher gastrin concentrations were protective against hypoxia.

Methods: Mice (8-10 weeks old) over-expressing gastrin (hGAS) and mice of the corresponding wild-type strain (FVB/N) were subjected to normoxia (21% oxygen; air) or hypoxia (10% oxygen) for 10 days in an enclosed normobaric chamber. Mice were weighed daily and sacrificed on day 10 by inhalational overdose with the anaesthetic isoflurane, and blood and tissues were collected for further analyses.

Results: hGAS mice lost significantly less weight (<10%) than FVB/N mice (>15%) exposed to hypoxia, compared to their normoxic counterparts. hGAS mice also had significantly better health scores than FVB/N mice under hypoxia. Although hypoxic FVB/N mice had a decreased spleen weight and a reduced number of platelets compared to normoxic FVB/N mice, no difference in spleen weight was observed in hGAS mice under hypoxia or normoxia.

Conclusion: The observation that higher concentrations of gastrins decreased the amount of weight loss in animals exposed to hypoxia provides evidence that gastrins may play a protective role in low oxygen conditions.

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PACAP STIMULATES TEAR SERETION THROUGH AQP5 SIGNAL IN MOUSE

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide which is originally isolated from the ovine hypothalamus. PACAP distribute in the eyeball, such as retina, ciliary body and iris. However, the distribution in accessory organs of the eye, and the effect has still unclear. We casually found phenotypes on PACAP null mouse that their eyes were keratinized with aging. The keratinization tended to appear in female mouse rather than male mouse. Moreover, tear secretion level in PACAP null mouse significantly reduced compared with wild-type mouse. However, lacrimal gland of PACAP null mouse was pathologically normal suggesting that PACAP may relate with tear secretion mechanism but not the development.

To reveal the relations between PACAP and tear production, PACAP and PACAP specific receptor (PAC1R) localization in lacrimal gland were observed by immunohistochemistry. PACAP immunoreactivity was co-localized with NeuN (neuronal marker) and ChAT (parasympathetic nerve marker) surrounding acinar cells. PAC1R immunoreactivity was observed within acinar cells. The physiological function of PACAP on tear secretion was examined by administration of PACAP in eye drops applied to the eyes of anesthetized mice. PACAP eye drops stimulated tear production 15 to 45 minutes after application. This effect was inhibited by pre-treatment with PAC1R antagonist, PACAP6-38 or the adenylate cyclase inhibitor, SQ22536. Following the application of PACAP eye drops, levels of cAMP and phosphorylated-protein kinase A increased in lacrimal glands. PACAP stimulated phosphorylation of aquaporin 5, and its translocation from the cytosol to the membrane in lacrimal acinar cells to induce tear secretion. Moreover, aquaporin 5 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, and PACAP is a good candidate for an eye-drop medicine for the dry eye syndrome.

ENDOGENOUS SOMATOSTATIN MEDIATES SYSTEMIC ANTINOCICEPTIVE EFFECT OF TOPICAL CAPSAICINOID THERAPY

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Objectives. The aim of the present study was to evaluate the therapeutic potential of local nonivamide treatment on chronic low back pain in patients with degenerative spine diseases and to investigate the possible mechanism of action of the therapy.

Methods. The qualitative and quantitative analyses of capsaicinoids in EMSPOMA® cream were performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). In the clinical study twenty patients with degenerative spine diseases were involved in a self-controlled examination. During the 21 day therapy they received 30 min daily treatment with 0.01% nonivamide (EMSPOMA® cream) to the lumbar region of the back. The pain (VASs, Oswestry Disability Index) and the mobility of the lumbar region of the spine (Schober's, Domján's L and R test) were detected at baseline and at the end of the 1st, 2nd and 3rd weeks. The plasma level of somatostatin-like immunoreactivity (SST-LI) was measured by RIA before and after the treatment on the first and the last day of the therapy.

Results. Nonivamide (0.01%) was identified as the only capsaicinoid molecule in the cream. In the clinical study the 21 day local nonivamide treatment reduced the pain sensation. Oswestry Disability Index decreased from 39±3.9 % to 32.5 ±4.4 %. VASs showed 37.29%-59.51% improvement. In the plasma level of SST-LI threefold elevation was observed after the first nonivamide treatment.
Conclusions. We conclude that nonivamide treatment exerts analgesic action in chronic low back pain and causes the release of the antinociceptive and anti-inflammatory neuropeptide somatostatin which may play pivotal role in the pain-relieving effect.

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FORMATION OF PYY $_{3-34}$ IN THE PORCINE LIVER

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Background Peptide YY (PYY) is a gastrointestinal hormone released from the enteroendocrine cells. The metabolite PYY_{3-36} exerts an anorexigenic effect, which make PYY a potential target for obesity treatment. However, little is known about the kinetics and degradation products of PYY. Neuropeptide Y, which is 70% homologous with PYY, has been shown to be degraded in vitro from the C-terminal end. In this study we therefore aim to investigate whether PYY is also C-terminally degraded, a degradation that might not be detected by currently used assays.
Methods After infusion of PYY_{3-36} in Göttingen minipigs (supraphysiological doses), a formation of PYY_{3-34} was found in plasma samples by LC-MS. To investigate whether PYY_{3-34} is formed in physiological concentrations, PYY_{3-36} was infused into seven multi-catheterized LYD pigs (2 pmol/kg/min). Plasma samples were analyzed by a radioimmunoassay (RIA) allowing determination of total PYY, as well as a C-terminal specific $PYY_{1/3-34}$ RIA. Activation of the Y2-receptor by PYY_{3-34} was determined by measuring IP3 in COS-7 cells co-transfected with the Y2-receptor and a chimeric Gα-protein.
Results After PYY_{3-36} infusion the peptide was degraded with a T½ of 3.6 ± 0.5 min.
Significant extraction (20.5±8.0%) compatible with glomerular filtration was observed across the kidneys and significant C-terminal degradation (26.5±4.8%) was observed across the liver. Net balances across the hind limb, splanchnic bed, and lungs were not significantly different from zero.
PYY_{3-34} had no agonistic or antagonistic effect on the Y2-receptor.
Conclusions PYY 3-36 is extensively degraded to PYY_{3-34} in the pig and PYY_{3-34} is not active on the Y2 receptor. Current assays cannot detect C-terminal changes, rendering reported levels of PYY_{3-36} inaccurate.

LACTOMEDIN 2, AN OXYTOCIN RECEPTOR AGONIST PEPTIDE DERIVED FROM HUMAN LACTOFERRIN, EXHIBITS ANXIOLYTIC-LIKE ACTIVITY IN MICE

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Oxytocin (OT: CYIQNCPLG-NH_2) has been reported to show central effects such as anxiolytic-like activity besides typical peripheral effects *ie.* uterus-contraction and milk-ejection. In this study, we found that lactomedin 2 (LM-2: CFQWQR), which has been isolated as an ileum-contracting peptide from a tryptic digest of human lactoferrin, exerted an anxiolytic-like activity evaluated by elevated plus-maze test in mice at dose of 0.1 nmol/mice *icv*, 1 mg/kg *ip*, and 30 mg/kg *p*o, respectively. The peptide showed a weak affinity for the oxytocin receptor (OT-R, Ki: 62 μM) in spite of its low structural homology to OT. The anxiolytic-like activity of LM-2 *given icv.* was blocked by L-371,257 (1 nmol/mice *icv*), an antagoinist of OT-R. LM-2 is the first example of OT-R agonist peptide derived from a non-typical precursor protein. Peptides showing affinity for the OT-R were not found in the primary structure of lactoferrins of other species. We found that the anxiolytic-like activities of both and OT and LM-2 were blocked by SCH58261 (1 nmol/mice, *icv.*), an antagonist of A_{2A}-R, suggesting that the adenodsine- A_{2A}-R system is involved downstream of OT-R. Furthermore, the anxiolytic-like activity of LM-2 was also blocked by bicuculline (1 nmol/mice *icv*), an antagonist of $GABA_A$-R (1 nmol/mice *icv*). Thus, we found for the first time that the anxiolytic-like activities of OT-R agonists are mediated by $GABA_A$-R downstream of the A_{2A}-R. LM-2 might be take part in the regulation of mental stress in newborn.

COMPARISON OF 3% NACL AND 20% MANNITOL IN THE TREATMENT OF INCREASED INTRACRANIAL PRESSURE IN NEONATAL PATIENTS *

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Objectives— Hypertonic saline (HS) has been widely used in the treatment adults and older children patients with brain edema and intracranial hypertension resulting from cerebral infarction, hemorrhage or traumatic brain injury. We aimed to compare the effects of 3% sodium chloride (NaCl) and 20% mannitol on increased intracranial pressure (ICP) in neonatal patients.
Design—Prospective, randomized controlled trial.
Setting—Neonatal ICU.
Patients—Newborns with clinical symptoms and signs of intracranial hypertension
Interventions—Patients received 3.5 mL /kg 3% NaCl or 1.25 mL /kg 20% mannitol. 3%NaCl or 20%mannitol were administered according to the ICP of Patients.
Measurements and Main Results—Intracranial pressure was detected in all the patients by lumbar puncture before treatment and 5 days after treatment (12 hours after treatment at the fifth day) and cerebrospinal fluid was collected for chloride detect. At the same time, the venous blood was obtained for testing serum sodium, serum chloride and serum osmolality change. The ICP was significantly decreased after treatment in both groups ($P<0.05$). The curative effect of 3% NaCl was better than 20% mannitol in reducing intracranial hypertension after 5 days treatment; the serum chloride concentration in 20% mannitol group was significantly lower than before treatment ($P<0.05$), while there was no change in 3%NaCl group; serum sodium, serum osmolality and cerebrospinal fluid chloride had no change in both groups after treatment; the final consequence and hospital days were not different between in 20% mannitol group and 3% NaCl group.
Conclusions—Three percent sodium chloride was more effective than 20% mannitol in reducing intracranial hypertension in neonate; 3% NaCl had no effect on the serum sodium, serum chloride or serum osmolality in intracranial hypertension neonates, while 20% mannitol could reduce the serum chloride contents after continue treatment for five days.

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Session 7
Regulatory peptides in Life-style related diseases

ENERGY METABOLISM REGULATION BY INTRA CEREBROVENTRICULAR AND INTRANASAL ADMINISTRATION OF GALP

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Introduction: Galanin-like peptide (GALP), a 60-amino acid neuropeptide that was originally isolated from porcine hypothalamic extracts and is well known as a neuropeptide regulating feeding behavior and energy metabolism. In this study, we examined anti-obesity effect of GALP by the two administration route.
Methods: Mice were i.c.v. injected saline or GALP (2nmol), and removal of the liver and adipose tissue at 100 minutes after the administration of GALP. Then, we studied hepatic and adipose tissue lipid metabolism related gene expression by use of real-time quantitatively PCR analysis. Next, we investigated the effect of GALP in pair fed conditions. Moreover, to investigate the anti-obesity effect of chronic administration of GALP, mice were fed a high fat diet to induce obesity and were intranasal administrated of GALP for 2 week.
Results: The respiratory exchange ratio (RER) of GALP group was lower than that of the saline group at 1 hour after administration. In the GALP-treated group, fatty acid synthesis-related gene mRNA levels were decreased and fatty acid oxidation-related gene mRNA levels were increased in the liver. In the adipose tissue, the mRNA levels of HSL and ATGL, which were involved in lipolysis, were increased in the GALP-treated group compared with the saline group. The hepatic CPT1, MCAD and AOX mRNA levels were increased in the GALP group compared with the saline group in both *ad lib* and pair fed conditions. In chronic infusion study, the body weight gain was decreased by GALP treatment as compared with the control group.
Conclusion: These our studies demonstrate that anti-obesity effect of GALP is evident in both central and intranasal administration. And there beneficial effect was not effect of the feeding and was effect of GALP. It is thought that GALP may be effective for treatment and the prevention for obesity and life-style-related diseases in the near future.

VASODILATORY PROPERTIES OF GHRELIN IN THE RAT

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Ghrelin, an n-octanoyl 28-amino acid peptide, was originally identified, from the stomach, to be an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). Subsequent studies found it in various cells and tissues, such as brain, lung, pancreas, kidney, placenta, thyroid carcinoma cells, and testis. This suggested that the actions of ghrelin might be more diverse than reported physiological roles such as the regulation of feeding behavior, energy metabolism, gastric acid secretion, and motility.
During the course of a study of the renal cytotoxicity, using cultured kidney cells we isolated prepro-ghrelin cDNA from MDCK cells. In this study, therefore, we investigated the physiological role of ghrelin in renal function in the rat. The presence of ghrelin mRNA and its receptor in canine and rat

kidneys was shown by the results of [125I]-ghrelin binding and reverse transcription–polymerase chain reaction (RT-PCR). The displacement curve of [125I]-ghrelin binding competing with GHS-6 was identical to that of unlabeled ghrelin. Rat ghrelin was detected in kidney, heart, and stomach. The intravenous injection of ghrelin into an anaesthetized rat promoted a diuresis. The extent of diuresis by ghrelin was less than that by ANP, a known natriuretic peptide. A 10% decrease in blood pressure was also observed during the administration of ghrelin into a spontaneously hypertensive rat (SHR). The heart rate was not altered by ghrelin during the sampling times. Thus, we show the diuretic and vasodilatory properties of ghrelin in the rat.

LONG-TERM IN VITRO TREATMENT OF RINM5F RAT PANCREATIC β-CELLS BY NMDA CAUSES THE CELL DYSFUNCTION*

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N-methyl-D-aspartate (NMDA) receptors are ionotropic glutamate receptor subtype in the central nervous system (CNS), which are also found in many peripheral non-neural tissues, including pancreatic islet cells. Recently, it has been reported that the excessive activation of NMDA receptor evoked toxic effect on both neural and non-neural tissues such as lungs. Previous research in this Lab. has demonstrated that a prolonged activation of NMDA receptors impaired the function of pancreatic islets of rat in vivo. In the present study, the effect of prolonged activation of NMDA receptor on the function of RINm5f rat pancreatic β-cells in vitro was investigated in order to clarify the underlying mechanism. The results showed that the lactate dehydrogenase (LDH) activity in supernatant of cultured RINm5f β-cells, which was accepted as an index of cell damage, was increased significantly by exposure of cells to NMDA (3 mmol/L) for 48 h ($P<0.01$). Methyl thiazolyl tetrazolium (MTT) assays revealed a dramatic reduction in the cell proliferation of RINm5f β-cells treated with 10 mmol/L of NMDA for 72 h ($P<0.05$). Glucose-stimulated insulin secretion (GSIS) was markedly suppressed by treatments with NMDA in a dose and time-dependent manner (0.1, 0.3, 1, 3 and 10 mmol/L for 24 h, 48 h and 72 h), while basal insulin secretion was no significant altered. In conclusion, present data indicate that prolonged activation of NMDA receptors impair the function of islets β-cells in vitro with reduced cell proliferation and GSIS. The NMDA receptor in β-cells may therefore contribute to the development of type 2 diabetes and be a new target for the treatment of islet β-cells dysfunction.

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THE EXCESSIVE INCREASED GLUTAMATE CONTRIBUTES TO β-CELL DYSFUNCTION IN TYPE 2 DIABETES**

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Object: Although a line of evidence that diabetes affects the metabolism of amino acids, the effect of metabolism of amino acids on diabetes is unclear. Glutamate is an important neurotransmitter. It has been reported that the excessive activation of NMDA receptor by glutamate evoked toxic effect on neural tissues. The glutamate receptors are found in neural tissues and many peripheral non-neural tissues, such as islets. The NMDAR is an important type of glutamate receptor. We investigated whether the metabolism of glutamate changed in elderly type 2 diabetes.

Methods: 32 cases of elderly patients with type 2 diabetes were recruited as the diabetic group, aged 60-75. 20 cases of people who check up healthy were recruited as normal group. No significant difference was noted between the groups in gender, age and body mass index (BMI). Plasma was collected to detect 17 kinds of free amino acid content by high performance liquid chromatography (HPLC). In vitro, MIN6 β-cell was treated with high glucose (25 mM) for 72 h. In the control group, cells were maintained in normal glucose medium.

Results: Plasma glutamate level was elevated in elderly diabetic group compare with the normal group. Interesting, the cysteine level was also increased. In vitro, glutamate content in culture supernatant of MIN6 β-cell was significantly increased after high glucose incubation for 72 h. Meanwhile, high glucose treatment increased the expression of NMDARs in MIN6 β-cell. High glucose also increased apoptosis of MIN6 β-cell.

Conclusions: The excessive increase of glutamate may contribute to the development of elderly type 2 diabetes and the β-cell dysfunction. The NMDAR blockers may attenuate β-cell apoptosis and protect against diabetes.

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FEATURES OF BILE SECRETION IN RATS WITH MODULATION OF ENDOTHELINS SYSTEM IN ACUTE EXPERIMENTS

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Endothelins are synthesized directly in the epithelial lining the arterial and venous vessels and their receptors are identified not only on endothelial cells, but also in fat cells (Ito cells), Kupffer cells, hepatocytes and cholangiocytes. This peculiar distribution of endothelin system components indicates endothelins ability to participate in the regulation of various physiological and biochemical processes in the liver, including energy production and bile secretion.

The aim of the study was to investigate the endothelin system role in bile formation. We explored the endothelin 1 (0,1; 0,2; 0.5; 0,75; 1 μg/kg, single intraportal injection) and blockers of the endothelins ET_A and ET_B receptors BQ-123 (60 μg/kg) and BQ-788 (1 μg/kg) effects on the dynamics of bile secretion and the bile quality in acute experiments on the thiopental anesthetized male rats (200-250 g) with canulated bile duct.

Studies have shown that lower doses of endothelin 1 (0,1 and 0,2 μg/kg) are increased bile flow intensity on 14,1% and 21,6 % ($p < 0.05$) respectively. Endothelin 1in doses 0,75 and 1,0 μg/kg lowered the bile flow level 9,9%, 15,5% ($p < 0.05$) respectively. Exception of regulatory action of endogenous endothelin through blockade ET_A receptors by BQ-123 in the first half-hour after administration reduced the intensity of bile flow by 76,8 % ($p < 0.01$). While the ET_B receptor blockade via BQ-788 led to a gradual increase of bile secretion level. The maximum BQ-788 stimulating effect (16,4 %, $p < 0.05$) was observed at the end of the first hour after blocker administration.

The report will present data on the characteristics influence on the various links in the biosynthesis and conversion of bile acids in the liver under the modify endothelin system condution.

SENSING OF CARBOHYDRATES BY THE GHRELIN CELL IS POLARIZED

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Introduction: Long-term administration of sweeteners with prebiotic properties, such as oligofructose (FOS), decreases plasma ghrelin levels and induces body weight loss. Studies have shown that the ghrelin cell is co-localized with the sweet taste receptor, TAS1R2-TAS1R3, and the gustatory G-protein, gustducin, both involved in the sensing of carbohydrates by endocrine cells.

Aim: To study whether sensing of glucose and FOS by the ghrelin cell occurs via the luminal or blood-born direction and whether it involves α-gustducin or not.

Methods: Ghrelinoma cells were stimulated with oligofructose for different time points. In vivo, wildtype (WT) and α-gustducin knockout mice (α-gust-/-) were gavaged with 4g/kg D-glucose or 5.6g/kg FOS. In addition, WT mice were intravenously (IV) injected with 1g/kg D-glucose. Ghrelin levels were determined in culture medium, plasma and gut tissue extracts by radioimmunoassay.

Results: In both WT and α-gust-/- mice gavage of D-glucose decreased plasma octanoyl ghrelin levels (WT: 54%,α-gust-/-: 67%). In both genotypes this was accompanied by an increase ($P<0.05$) in duodenal but not stomach octanoyl ghrelin content. IV administration of D-glucose neither affected plasma octanoyl ghrelin levels nor tissue octanoyl ghrelin content. Acute gavage of FOS tended to decrease plasma octanoyl ghrelin levels in WT mice but not in α-gust-/- mice. In ghrelinoma cells, FOS induced a time-dependent decrease in octanoyl ghrelin release.

Conclusion: The prebiotic sweetener, FOS, is less potent than D-glucose to inhibit ghrelin release after acute administration. The sensing of D-glucose by the ghrelin cell is polarized and occurs via the lumen, where it is sensed by ghrelin cells in the duodenum. α-gustducin is not involved in the sensing of glucose but may play a role in the effect of FOS.

RNA EDITING OF SEROTONIN 2C RECEPTOR IS INVOLVED IN ALCOHOL INTAKE

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Serotonin 2C receptor ($5\text{-}HT_{2C}R$) is a G-protein coupled receptor known to have various actions such as involvements in food intake, emotional behavior and drug addiction. We have recently demonstrated that $5\text{-}HT_{2C}R$ is involved in the increased alcohol intake after chronic alcohol exposure in C57BL/6J strain. $5\text{-}HT_{2C}R$ is also known to undergo mRNA editing that converts genomically encoded adenosine residues to inosines by adenosine deaminases acting on RNA (ADARs). We will present our data in the conference that alcohol preference in mice depends on the degree of $5\text{-}HT_{2C}R$ mRNA editing in the nucleus accumbens (ACC), a crucial region for reward and addiction. We have recently demonstrated that $5\text{-}HT_{2C}R$ in the ACC is involved in the increased alcohol intake after chronic alcohol exposure in C57BL/6J strain. After chronic alcohol vapor exposure for 20 days, C57BL/6J mice grew to take more alcohol voluntarily but C3H/HeJ and DBA/2J mice did not show significant changes. The frequency of $5\text{-}HT_{2C}R$ RNA editing in the ACC of alcohol exposed mice was significantly increased in the C57BL/6J strain accompanied by the increase in the expression of $5\text{-}HT_{2C}R$ mRNA, ADAR1 and ADAR2 but that was

not observed in the C3H/HeJ nor DBA/2J strains. Then, we examined the mutant mice that express exclusively unedited type (INI) of 5-HT_{2C}R mRNA in C57BL/6J strain and found that they did not exhibit the increase of alcohol intake compared with wild type after chronic alcohol exposure. Collectively, these results indicate that the alteration in 5-HT_{2C}R mRNA editing in the ACC underlies the alcohol preference in mice.

INSULIN ACUTELY REDUCES CARDIAC EXPRESSION OF NATRIURETIC PEPTIDES IN A PORCINE MODEL

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Background: Cardiac natriuretic peptides decrease blood pressure and increase natriuresis. A reduction in plasma concentrations of natriuretic peptides has been documented in obese individuals but the underlying mechanism remains to be established.
Methods and results: The effect of hyperinsulinemia (obtained with the euglycemic clamp technique) versus saline infusion was examined in a porcine model. An insulin infusion of 2 mU/min/kg body weight was used; the blood glucose concentration was maintained at 3.3 ± 0.6 mmol/L in the 3 h insulin clamp and at 3.7 ± 0.5 mmol/L in the 3 h insulin + 2 h clamp. The animals were euthanized after 3 h and 5 h respectively and cardiac biopsies were obtained. The transcriptional expression of ANP and BNP were determined with quantitative real-time PCR; plasma proANP was measured with an immunoluminometric assay targeted against the mid-region of the peptide. Right atrial and left atrial ANP mRNA contents were not altered as an effect of insulin, whereas right atrial BNP mRNA contents decreased 21-fold ($p= 0.0002$) and left atrial BNP mRNA contents decreased 2.8-fold ($p=0.04$) in the 3 h insulin + 2 h clamp compared to control animals. Decreased proANP concentrations in plasma ($p = 0.0006$) were noted in the 3 h insulin + 2 h clamp compared to the control.
Conclusion: Our results demonstrate that hyperinsulinemia acutely decreases cardiac BNP but not ANP mRNA expression in a chamber-specific manner. The data supports a role of insulin in obesity-related hypertension, where hyperinsulinemia is associated with increasing body weight.

GLUCAGON RELEASE FROM ISOLATED PORCINE PANCREATA IS NOT ATTENUATED BY MUSCARINIC BLOCKADE

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Background: In a group of healthy participants we observed that glucagon levels were attenuated when atropine was co-infused with glucagon-like peptide 1 (GLP-1) during a hyperglycemic clamp.
We therefore decided to study the direct effects of atropine and GLP-1 on the endocrine pancreas by an ex vivo model – the perfused isolated porcine pancreas with intact vagi.
Methods: Isolated porcine pancreata were perfused continuosly (24mL/min) with an oxygenated medium composed of a buffer and washed bovine erythrocytes (haematocrit 20%, glucose concentration fixed at 3mM). Perfusion pressure was stable during the perfusion period and the characteristics of the medium was monitored using a blood gas analyzer. The organ was allowed a resting period of 30 minutes before sampling commenced.
Vagal stimulations were introduced via a platinum tunnel electrode connected to a stimulator. Pancreatic juice was collected to monitor the efficacy of stimulations. For some experiments atropine (a muscarinic blocker) was added to the medium to attain a final concentration of 10^{-6}M. GLP-1 was delivered via a precision pump for a final concentration of 10^{-10}M. Samples from the portal vein was collected each minute for 120 minutes. Peptides were measured using in-house radioimmunoassays.
Results: Vagal stimulations acutely and briefly increased glucagon secretion while somatostatin secretion was inhibited. Infusion of GLP-1 strongly increased pancreatic somatostatin release while attenuating the release of glucagon,
Over the course of the perfusion experiments glucagon levels gradually decreased. Interestingly, pancreata perfused with atropine reached a final glucagon plateau higher than that of pancreata not perfused with atropine.
Conclusions: Muscarinic blockade in isolated porcine pancreata does not attenuate portal glucagon levels. This suggests that the attenuated glucagon levels observed in humans during atropine infusion is due to blockade of extrapancreatic muscarinic receptors.
Possibly, sympathetic first neurons are inhibited resulting in lowering of sympathetic tone leading to falling glucagon levels. This would explain why the isolated organ is actually reaching even lower levels on no atropine days compared to the human participants. The effect of Atropine on the isolated pancreas is to increase glucagon secretion. Because local ganglion cells are prohibited from lowering glucagon in response to low glucose….

REGULATION OF APPETITE AND OBESITY BY VASOACTIVE INTESTINAL POLYPEPTIDE

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Introduction: The incidence of obesity and its associated chronic disorders has been increasing at a high rate in the last decades. Obesity develops as a result of a disrupted homeostasis between energy intake and energy expenditure. Previous research, which has been focused on obesity development and metabolic pathways, has not successfully elucidated the regulatory mechanisms which are altered in subject affected by overweight and obesity. A better understanding of those mechanisms regulating appetite/satiety, feeding behavior and energy balance is necessary to identify new targets and to develop new clinical protocols for treatment. Vasoactive Intestinal Peptide (VIP) is a 28-amino acid peptide abundantly expressed in the central nervous system as well as in the gastrointestinal tract, where it regulates different physiological functions.

Aims: To establish the overall role of VIP on the regulation of appetite/satiety, feeding behavior, metabolic hormone release and body mass composition.

Methods: VIP deficient and age-matched littermates, WT C57Bl6 mice, were weekly monitored for 22 weeks using a whole body composition EchoMRI analyzer. Food intake and feeding behavior were analyzed using theBioDAQ automated monitoring system. Metabolic hormones: α-ghrelin, GLP-1, leptin PYY, PP, adiponectin and insulin plasmatic levels were measured in fasting as well as in postprandial conditions.

Results: The genetic lack of VIP leaded to a significant reduction of body weight and fat mass and to an increase of lean mass as the mice age. Additionally, VIP-/- mice presented a disrupted pattern of feeding behavior resulting in an abolished regular nocturnal feeding pattern. The lack of VIP affected energy balance through an altered secretion of adiponectin, α-ghrelin, GLP-1, leptin, PYY, and insulin.

Conclusions: Our data show that VIP is involved in the control of appetite/satiety, feeding behavior and in the secretion of some key regulatory metabolic hormones. VIP plays a very important role in the regulation of body weight and mass composition by significantly enhancing body weight and fat mass. Therefore, the VIP pathway could be a crucial target for the regulation of appetite/satiety and body phenotype and it could be a target for the treatment of obesity.

PERIPHERAL REGULATION OF APPETITE AND METABOLISM BY PACAP AND PAC 1 IS MEDIATED BY THE SECRETION OF GHRELIN, GLP-1 AND LEPTIN

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Introduction: PACAP has been found to be expressed within the enteric nervous system and gastric mucosa and has profound physiological effects in the gastrointestinal tract. PACAP plays an important role in the regulation of food intake and thermogenesis by ICV injection. However, the peripheral mechanisms involved in PACAP regulation of appetite and feeding are unknown.

Aims: To explore the role of PACAP and PAC1 in the regulation of appetite and food intake through the analysis of metabolic hormone release, food intake and feeding behavior in WT and PAC1 -/- mice treated by IP injection of PACAP38 and PACAP27.

Methods: PACAP38 and PACAP27 injected (IP) into PAC1 receptor (PAC1-/-) deficient and age-matched WT C57Bl6 mice prior to the dark phase feeding period. Food intake and feeding behavior were analyzed using the BioDAQ automated monitoring system. Metabolic hormones: α-ghrelin, GLP-1, leptin, PYY, PP, and insulin assays were analyzed following PACAP38 administration in overnight fasted WT mice and fasted PAC1-/- mice.

Results: PACAP38 and PACAP27 (100nM, 1μM and 10μM in 200μL) injected by IP in WT mice induced a dose-related decrease in cumulative food intake and significantly reduced bout duration, bout frequency, meal size, time spent in feeding, time spent in meals, total meal time, eating rate and meal duration post injection compared to vehicle injected WT mice. PACAP27 resulted in a longer duration of pharmacodynamics action compared to PACAP38 (12 hrs vs 8 hrs, respectively). PACAP38 IP injected into PAC1-/- mice failed to produce any significant changes in food intake or feeding behavior, thus indicating that the effects of PACAP appear to be PAC1-specific. Furthermore, PACAP38 IP injected into overnight fasted WT mice significantly reduced the plasma levels of α-ghrelin compared to vehicle (306.9 ± 66.8 vs. 533.4 ± 79.8pg/mL). In PAC1-/- mice, fasting levels of α-ghrelin, GLP-1, insulin and leptin, as well as postprandial levels of α-ghrelin and insulin, were significantly altered compared to WT mice.

Conclusion: PAC1 is a novel regulator of appetite/satiety and energy homeostasis. PACAP38 and PACAP27 significantly reduces food intake and appetite through PAC1 and that the pharmacodynamic effects of PACAP27 are longer than PACAP38. Regulation of anorexigenic and orexigenic hormones to control appetite/satiety is abolished in PAC1-/- mice, while α-ghrelin remains elevated even postprandially in contrast to their WT counterparts. PACAP significantly reduced orexigenic α-ghrelin in overnight fasting conditions. These results establish a physiologic role for both PACAP and PAC1 in the peripheral regulation of appetite/satiety and support future studies of the therapeutic use of PAC1 agonists in obesity disorders.

COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT (CART) IMPROVES BETA CELL FUNCTION AND MODULATES INCRETIN HORMONE SECRETION AND EXPRESSION

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Background: CART is expressed in islet- and enteroendocrine cells in humans. Islet CART regulates islet hormone secretion and is upregulated in type 2 diabetes (T2D) patients.
Aim: Here we examined: 1) the impact of upregulated beta cell CART on beta cell function and survival *in vivo* before and after diabetes induction. 2) if endogenous L- and K-cell CART regulates incretin hormone secretion and expression.
Methods: Mice with beta cell specific overexpression of CART (CARTtg) were generated and subjected to streptozotocin (STZ)-treatment to induce T2D, weekly glucose monitoring and intravenous glucose tolerance test (IVGTT). CART was silenced using siRNA in GLUTag and STC-1 cells, used as L- and K-cell models. Gene expression was assessed with qPCR, protein content and secretion with RIA, and cell survival using WST-1 assay. Finally, CART was given i.v. during oral glucose tolerance tests (OGTT) in mice.
Results: CARTtg mice displayed increased insulin secretion ($p<0.02$) and improved glucose elimination during IVGTT ($p<0.03$). Three weeks after STZ-treatment CARTtg mice displayed lower glucose levels ($p<0.001$) and improved insulin secretory capacity and lower glucose levels during IVGTT ($p<0.02$). In GLUTag cells CART silencing increased GLP-1 expression and protein content and provoked 4-fold increased GLP-1 secretion ($p<0.001$). In STC-1 cells CART silencing increased GLP-1 and GIP expression ($p<0.001$). Moreover, CART silencing provoked 2-fold ($P<0.05$) increased cell survival in GLUTag cells. Finally, CARTPT provoked elevated GIP secretion during OGTT in mice ($P<0.001$).
Conclusion: Here we show that overexpression of CART in beta cells restores insulin secretion and glucose homeostasis after T2D induction. We also show that CART expressed in L-cells and K-cells is a regulator of incretin synthesis and secretion. In view upregulated islet CART in T2D patients, our data suggest that CART is upregulated in T2D as a homeostatic response trying to overcome glycemia. The role of CART in enteroendocrine cells during T2D progression needs further investigation.

ATTENUATION OF GASTRIC INHIBITORY POLYPEPTIDE (GIP) SIGNALING WITH GIP/FC-IGG FUSION PROTEINS

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Background and Aims: Despite the importance of GIP as a physiological incretin, postprandial circulating glucose levels in $GIPR^{-/-}$ and wild-type (wt) mice are similar. However, unlike wt mice, $GIPR^{-/-}$ mice do not develop diet-induced obesity. While specific GIPR antagonists have been identified, their short circulating $T_{1/2}$ has hampered their development. We hypothesize that the $T_{1/2}$ of GIPR antagonists can be increased significantly by fusing them to the Fc-fragment of IgG.
Aim: To develop long-acting antagonists to GIPR by fusion to the IgG Fc-fragment.
Methods: PCR and molecular cloning were used to produce chimeric genes encoding GIP(6-42)-Fc and Pro^3-GIP-Fc. After expression in HEK 293 cells, protein A agarose chromatography was used to purify protein from conditioned media. Fc-fusions were identified by Western analysis using a specific GIP antibody, and biological activity was assessed using synthetic GIP(1-42) and a GIP-specific reporter cell line.
Results: Transgenic HEK 293 cells produced protein with the predicted molecular size for the Fc fusion and possessed GIP-specific immunoreactivity. Purified Pro^3-GIP-Fc induced reporter expression with an EC_{50} of 500 nM, whereas GIP(6-42)-Fc did not. Both GIP(6-42)-Fc and Pro^3-GIP-Fc antagonized report gene expression induced by 1 nM GIP.
Summary/Conclusion: Pro^3-GIP-Fc, but not GIP(6-42)-Fc, induced reporter gene expression, while both fusion products inhibited reporter gene expression, indicating that the former is a partial agonist and that the latter only functions as an antagonist. The IC_{50}'s for GIP(6-42)-Fc and Pro^3-GIP-Fc were higher than the corresponding reported values of synthetic GIP(6-42) and Pro^3-GIP, indicating the possibility of partial interference of ligand binding by the Fc fragment. Nevertheless, the increase in $T_{1/2}$ imparted by the fusion of N-terminal truncated GIP peptides by the Fc fragment may thereby provide viable GIPR antagonist candidates for treating obesity and related disorders.

ORAL ADMINISTRATION OF TRANS, TRAN S-2,4-DECADIENAL REDUCES GASTRIC EMPTYING IN RATS

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Introduction: Cholecystokinin (CCK) is a gut hormone which controls gastric emptying, pancreatic enzyme secretion, and appetite. We previously showed that unsaturated aldehydes stimulated for CCK secretion from CCK-producing enteroendocrine cell line, STC-1. Although trans,trans-2,4-decadienal (2,4-C10)

has a strong CCK-releasing activity among unsaturated aldehydes, its CCK-related physiological effects have not been tested.
Aim: To investigate whether oral administration of 2,4-C10 reduces gastric emptying in rats.
Methods: We assessed gastric emptying rate using an absorbable marker {Acetaminophen (APAP)} and a non-absorbable marker {Phenol red (PR)}. APAP test : The solution including APAP was gavaged and blood samples were collected from the tail vein over the following 120 min, and plasma APAP levels were measured. We examined 2,4-C10, Decanal, Decanol, Decanoic acid; they all have the same carbon chain length (C10) but have a different functional group (-CHO, -OH, -COOH) or degree of unsaturation. PR test : Rats were killed under anesthesia at 15 min after oral administration of the test solution containing PR. We collected the luminal content of stomach and small intestine respectively and measured the amount of PR remaining.
Results and Discussion: The concentration of APAP in plasma and gastric emptying rate at 15 min were significantly lower in 2,4-C10-treated rats than that in control rats. PR remained in the stomach content was much higher in 2,4-C10-treated rats than in control rats, while almost no PR was measured in the small intestine in 2,4-C10-treated rats. These results indicate that the oral administration of 2,4-C10 reduces gastric emptying. Furthermore, saturated C10-aldehyde, -alcohol, -fatty acid which do not exhibit CCK-releasing activity in vitro, did not reduce gastric emptying in rats. This suggests that orally administered 2,4-C10 directly acts enteroendocrine cells in the intestine and reduces gastric emptying.

IMPORTANT ROLE OF NEUROPEPTIDE FF RECEPTOR-2 SIGNALING IN THE REGULATION OF ENERGY HOMEOSTASIS AND DIET-INDUCED THERMOGENESIS

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The neuropeptide FF receptor 2 (NPFFR2) is highly expressed in the hypothalamus where it is activated by a set of RFamide peptides. However, its physiological function is unclear. Here we show that lack of NPFFR2 in mice results in significantly altered body composition and increases in energy expenditure, body temperature and physical activity. On the other hand, food intake was not significantly altered in NPFFR2-/- versus wild-type (WT) mice. The metabolic phenotypes of NPFFR2-/- mice are associated with significantly lower neuropeptide Y (NPY) mRNA expression and higher pro-opiomelanocortin mRNA expression in the arcuate nucleus of the hypothalamus. Interestingly, when fed on a high-fat diet (HFD), NPFFR2-/- mice showed greater weight gain and fat gain, and significantly reduced energy expenditure compared to WT mice. When energy expenditure from HFD and chow studies are compared, there are significant interactions between genotype and diet effects, i.e. HFD increases energy expenditure in both NPFFR2-/- and WT mice, however this increase is significantly less in NPFFR2-/- than that in WT mice, suggesting an impaired diet-induced adaptive thermogenesis by lack of NPFFR2 signaling. In support, HFD significantly increased UCP-1 and PGC-1α levels in the brown adipose tissue of WT mice but not in that of NPFFR2-/- mice. The mechanism behind NPFFR2 control of energy expenditure and adaptive thermogenesis is likely to involve hypothalamic neuropeptide Y pathways, since the HFD-induced decrease in hypothalamic NPY expression observed in WT is absent in NPFFR2-/- mice. Taken together, these data demonstrate that NPFFR2 signaling plays important roles in the regulation of energy homeostasis and diet-adaptive thermogenesis, which may involve hypothalamic NPY pathways.

Session 8
Regulatory peptides in Immunology, Inflammation and Cancer

POOR PROGNOSIS IN GASRTINOMA WHEN ADDITIONAL HORMONES AND PEPTIDES ARE SECRETED BY THE TUMOUR

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Introduction: Gastrinomas (ZEs) occur 0.5-3/million/year. They may be familial (MEN1) or spontaneous, benign or malignant. Patients with MEN1 and spontaneous ZEs may progress to produce other peptides and hormones affecting prognosis.
Methods and results: We have investigated patients with ZEs attending our clinic over 40 years. Fifty seven patients were studied, 13 with MEN1 (22.85) and 44 with spontaneous tumours. Of the spontaneous group 7 had benign and 37 malignant tumours. Thirteen of those with malignant tumours later secreted additional hormones.
The MEN1 patients presented with a median gastrin of 900 (range 125-3,200) ng/l. Eight had tumours secreting other peptides and hormones including PTH, pro-lactin, insulin, glucagon, PP, somatostatin, VIP, calcitonin or NKA. Several patients had multi-hormone secretion. Median survival was 17 (3-34) years.
The 7 patients with benign tumours presented with gastrin 260(130-1,050) ng/l all were cured by surgery. Four remain alive and survival in all was >17Y.
Twenty four patients with metastatic ZEs presented with gastrin 500 (145-15,800) ng/l, seven remain alive and median survival was 8.2 (0.5-26)Y. None of these

patients secreted additional peptides with the exception of PP <2,500ng/l.
Thirteen patients with metastatic ZEs presented with gastrin 750 (255-70,000) ng/l. All in this group later secreted additional hormones, PTH, pro-lactin, PP, insulin, glucagon, VIP, NKA, somatostatin or calcitonin. Several patients secreted 2 or 3 additional hormones. None remain alive and median survival was 1.9 (0.1-9.9) Y. No patient survived more than 2Y after multi-hormone secretion. Those producing ACTH, PP (>20,000ng/l) or insulin had poor survival.
Discussion: Thirty five percent of patients with spontaneous metastatic gastrinoma go on to secrete additional hormones resulting in poor prognosis. ZE patients should therefore be screened regularly using relevant biomarkers, making earlier treatment intervention possible.

THE IMMUNOREGULATORY ROLE OF GALANIN RECEPTOR 3 IN EXPERIMENTAL ARTHRITIS

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Neurogenic inflammatory components mediated by peptidergic sensory nerves have a crucial impact on the symptoms of arthritis, regarding not only pain perception, but also the formation of the inflammatory microenvironment and immune cell recruitment. Galanin is a regulatory sensory neuropeptide, which was shown to attenuate neurogenic inflammation, but its targets and mechanisms have not been elucidated. Since there are very few *in vivo* data on the role the Gal3 receptor (Gal3R) in inflammation, we analyzed its involvement in a complex model of rheumatoid arthritis (RA). Polyarthritis was induced by K/BxN arthritogenic serum in male C57Bl/6 wildtype and Gal3R gene-deficient mice. The mechanonociceptive threshold was determined by esthesiometry, grasping ability by the wire-grid grip test, paw volume by plethysmometry and clinical scoring. Myeloperoxidase (MPO) activity was measured by luminol-based *in vivo* bioluminescence imaging, histopathological evaluation was performed on the ankle joints at the end of the study. Gal3R gene-deficient mice demonstrated significantly increased and earlier ankle edema and clinical disease severity than wildtypes. Their grasping impairment was also more severe, whereas mechanical hyperalgesia did not differ in the early, but became greater in the late phase. Neutrophil-derived MPO-activity in the ankle joints was similar in the two groups in the acute phase, but it was significantly lower in the knockouts by day 5.
Gal3R activation results in potent anti-inflammatory functions in immune arthritis, as it inhibits early vascular reactions, such as edema formation and grasping disability. Meanwhile, Gal3R-mediated late immune cell activation indicates a potent immune-regulatory role, which does not support the overall functional results and needs further investigations. Selective Gal3R agonists or monoclonal antibodies might provide a novel approach for anti-inflammatory drug therapy in RA.

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THE VASOACTIVE PEPTIDE UROTENSIN II: A NEW CHEMOKINE EXHIBITING MIGRATION/ADHESION PROPERTIES IN GLIOMA

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One of the most potent vasoactive peptides, urotensin II (UII), is involved in endothelial cell proliferation and migration, by activating a G protein-coupled receptor, the UT receptor. We showed a high expression of UII/UT in human glioblastomas (GBM) compared to oligodendrogliomas. In GBM, a strong staining in vascular and peri-necrotic area and a systematic co-expression of UII/UT with SDF1α/CXCR4 were observed. In glioma and endothelial cells, gradient concentrations of UII induced chemoattracting migratory effects and tube formation. This effect was blocked by UT antagonists and mainly involved the G_{13}/Rho/ROCK pathway while partially requiring $G_{i/o}$/PI3K components. In contrast, we observed that homogeneous concentrations of UII blocked cell motility and stimulated cell-matrix adhesions through a UT/$G_{i/o}$ signaling cascade, partially involving PI3K. Finally, homogeneous concentration of UII allowed translocation of $G_{\alpha 13}$ to the UT receptor at the plasma membrane and increased actin stress fibers, lamellipodia formation and vinculin-stained focal adhesions. UII also induced relocalization of UT pre-coupled to $G_{\alpha i}$ in filipodia and initiated integrin-stained focal points. In heterotopic GBM

xenografted in Nude mice, intratumoral injection of UII accelerated tumor growth and necrosis, and stimulated neo-angiogenesis through metalloprotease activation. UT Antagonists/biased ligands inhibited tumor growth, neo-angiogenesis and prolonged mice survival. Micro-SPECT imaging showed increased integrin expression, correlated with large necrotic area in tumors treated with UII. Thus, UII promotes the recruitment of pro-angiogenic cells, induces cell adhesions and stimulates necrosis and neo-angiogenesis involved in glioblastoma growth. The specific blockage of UT signalings by using antagonists or biased ligands would constitute a new route for the treatment of GBM.

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THE NEUROPEPTIDE CORTISTATIN INHIBITS NEOVASCULARIZATION, NORMALIZES TUMOR VESSELS AND SENSITIZES BREAST CANCER TO CHEMOTHERAPY

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Tumor vessels are dilated, leaky and devoid of mural cells. This abnormal structure impairs tumor perfusion, promotes tumor malignancy and impedes the delivery and action of chemotherapeutic agents. Launched a decade ago as an alternative to the anti-angiogenic therapy, the strategy of "vessel normalization" focuses on the improvement of vessel function in the tumor for further treatment with chemotherapeutics. Here, we describe that cortistatin, a cyclic-neuropeptide that exerts potent inhibitory actions on neurons, endocrine and immune cells, regulated the neovascularization process and impaired growth and metastasis of breast tumor by inducing the "normalization" of tumor vessels. Cortistatin exerted its effects at multiple levels. Cortistatin inhibited the proliferation and migration of endothelial cells induced by the vascular endothelial growth factor by interfering with its signaling. Cortistatin reduced the density of microvessels in breast tumors by impairing the production of angiogenic factors and impaired the infiltration of vessel-associated macrophages in the tumor. Moreover, cortistatin increased pericyte coverage of endothelial cells and vessel perfusion and reduced vascular permeability and hypoxia in breast tumors. Consequently, preemptive treatment with cortistatin improved chemotherapy with cisplatin and doxorubicin. Therefore, cortistatin emerges as a "normalizing" factor of tumor vasculature that offers a new approach to manage breast tumors in combined therapies.

TARGETING A G-PROTEIN COUPLED RECEPTOR OVEREXPRESSED IN ENDOCRINE TUMORS BY MAGNETIC NANOPARTICLES TO INDUCE CELL DEATH

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Nanotherapy using targeted magnetic nanoparticles grafted with peptidic ligands of receptors over-expressed in cancers is a promising therapeutic strategy. However, nanoconjugation of peptides can dramatically affect their properties with respect to receptor recognition, mechanism of internalization, intracellular trafficking and fate. Furthermore, investigations are needed to better understand the mechanism whereby application of an alternating magnetic field to cells containing targeted nanoparticles induces cell death. Here, we designed a nanoplatform (termed MG-IONP-DY647) composed of an iron oxide nanocrystal decorated with a ligand of a G-protein coupled receptor, the cholecystokinin-2 receptor (CCK2R) that is over-expressed in several malignant cancers. MG-IONP-DY647 did not stimulate inflammasome of Raw 264.7 macrophages. They recognized cells expressing CCK2R with a high specificity, subsequently internalized *via* a mechanism involving recruitment of β-arrestins, clathrin-coated pits and dynamin, and were directed to lysosomes. Binding and internalization of MG-IONP-DY647 were dependent on the density of the ligand at the nanoparticle surface and were slowed down relative to free ligand. Trafficking of CCK2R internalized with the nanoparticles was slightly modified relative to CCK2R internalized in response to free ligand. Application of an alternating magnetic field to cells containing MG-IONP-DY647 induced apoptosis and cell death through a lysosomal death pathway, demonstrating that cell death is triggered even though nanoparticules of low thermal power are internalized in minute amounts by the cells. These data represent a solid basis for future studies aiming at establishing the proof-of-concept of nanotherapy of cancers using ligand-grafted magnetic nanoparticles specifically internalized *via* cell surface receptors.

PARADOXICAL EFFECT OF THE NEUROPEPTIDE CORTISTATIN IN INFLAMMATION AND AUTOIMMUNITY

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Cortistatin (CST) is a cyclic-neuropeptide related to somatostatin and produced by cortical and hippocampal interneurons and also released by macrophages and T cells in response to

inflammatory and immune stimulation. Although it shares many functions with somatostatin, especially concerning the regulation of hormone secretion and neuronal activities, CST exerts unique functions in the nervous and immune systems. Previously, we described the potent anti-inflammatory activity of CST and its therapeutic effect in many experimental models such as collagen-induced rheumatoid artritis (CIA) or experimental autoinmune encephalomyelitis (EAE). In these disorders, the administration of CST drastically reduced the two deleterious components of the disease, the inflammatory and autoinmune responses, and restored long-lasting immune tolerance. Following these results, we wondered about the role of endogenous CST. Evidence suggests that CST might provide protection against autoinmune pathologies. As expected, immune cells (macrophages, T cells, microglia) isolated from cortistatin-deficient mice showed an exacerbated immune response when stimulated. However, we found that lack of cortistatin surprisingly conferred resistance to systemic inflammatory diseases. Cortistatin-deficient mice were partially resistant to CIA, EAE and other inflammatory disorders, despite showing competent inflammatory/autoreactive responses. This unexpected phenotype was associated with elevated circulating glucocorticoids and an anxiety-like behavior in cortistatin-deficient mice. Our findings demonstrate that cortistatin from the immune source plays a critical role in the tuning of the immune responses in health and disease and identify cortistatin as a key player in the bidirectional communication that exists between the neuroendocrine and immune systems.

ROLE OF N-METHYL-D-ASPARTATE RECEPTOR IN INTRAUTERINE HYPOXIA-INDUCED PULMONARY HYPOPLASIA

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N-methyl-D-aspartate receptor (NMDAR) is present in the lungs, which plays important roles in lung development. Hypoxia is associated with increases in glutamate levels, which coincides with changes in NMDAR activity expression. MK-801, as the NMDAR antagonist, attenuates oxidant lung injury. We hypothesized that intrauterine hypoxia can result in lung injury in the fetuses, and this injury may lead to pulmonary hypoplasia and persist to adulthood. Glu excitotoxicity may participate in this pathogenesis. To determine possible pulmonary protective effects, we administered 0.05 mg/kg MK-801 or saline intraperitoneally daily to pregnant rats on day 19 of pregnancy before exposing to 10.5% oxygen in air for 2 days. On day 21 of gestation, it showed significant increase of NMDAR2B, NMDAR2C, NMDAR2D mRNA expression in the lung tissue of the intrauterine hypoxic fetuses. Significant decrease was seen in the body weight, lung wet weight, lung wet weight /body weight ratios, and RAC in the fetuses. This same pattern is also observed in the offspring pups till postnatal day of 21. On postnatal day 30, there were marked decreases in tidal volume, minute ventilation volume, and dynamic lung compliance but strongly increase in pulmonary resistance in intrauterine hypoxic offspring pups. MK-801 decreased these hypoxia-associated changes. We conclude that intrauterine hypoxia can lead to pulmonary hypoplasia in adult. Glu may play an important role by activation of NMDAR.

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ROLE OF TRPV1 AND TRPA1 ION CHANNELS IN CIGARETTE SMOKE-INDUCED CHRONIC AIRWAY INFLAMMATION MODEL OF THE MOUSE

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Airways are densely innervated by capsaicin-sensitive sensory nerves playing regulatory role in inflammation via the release of pro- and anti-inflammatory neuropeptides. Transient Receptor Potential Vanilloid 1 and Ankyrin 1 ion channels (TRPV1, TRPA1) are expressed on these sensory nerves and immune cells in the lung, and several irritants in the cigarette smoke can activate them. We have previously shown a protective role for TRPV1 in endotoxin-induced acute pneumonitis. Therefore, we investigated the involvement of TRPV1 and TRPA1 in a predictive mouse model of chronic bronchitis.

Gene-deleted mice ($TRPV1^{-/-}$, $TRPA1^{-/-}$) and their C57Bl/6 wildtype counterparts were exposed to tobacco smoke (Kentucky research cigarette) twice a day for 3 months. Bronchial responsiveness (enhanced pause: Penh) was measured with whole body plethysmography. Inflammatory cell profile was determined from the bronchoalveolar lavage fluid (BALF) with flow cytometry. Semiquantitative histopathological scoring of the lung was performed, myeloperoxidase (MPO) activity and inflammatory cytokines (IL-12, M-CSF, MIG, RANTES) were measured with spectrophotometry and Luminex, respectively.

In wildtype mice bronchial hyperresponsiveness developed in the 1st month. The greatest number of granulocytes, macrophages and lymphocytes were observed in the 2nd month, which correlates with the histological picture. Hyperreactivity developed and significantly increased in $TRPV1^{-/-}$ mice, more severe inflammatory histopathological alterations, higher BALF cell counts, MPO activity and cytokine production

were observed in TRPV1$^{-/-}$, but not in TRPA1$^{-/-}$ animals compared to wildtypes.
Our results indicate that TRPV1 receptor activation plays a protective role in smoking-induced chronic bronchitis presumably through the release of anti-inflammatory neuropeptides (e.g. somatostatin), but TRPA1 ion channel does not play a crucial role.

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CIGARETTE SMOKE EXPOSURE UPREGULATES TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 ION CHANNELS IN THE MOUSE LUNG AND IN A HUMAN PULMONARY TISSUE 3-DIMENSIONAL MODEL

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The Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel is present on capsaicin-sensitive peptidergic sensory nerves and non-neural cells, e. g. fibroblasts, smooth muscle and epithelial cells in the lung, but its function is contradictory. Chemical irritants, such as unsaturated aldehydes and nicotine in cigarette smoke activate TRPA1. This points to its role in the pathogenesis of airway diseases, such as chronic obstructive pulmonary disease (COPD). We investigated the expression and cigarette smoke-induced alterations of TRPA1 in the mouse lung and human pulmonary cell culture.
We evoked chronic bronchitis by 2x1 hour/day whole-body cigarette smoke exposure for 3 months in C57Bl/6 mice. TRPA1 mRNA was detected with qPCR in whole lung tissue and Fluorescence-Activated Cell Sorting (FACS)-isolated epithelial cells, fibroblasts and leukocytes. 3-dimensional (3D) human tissue cultures composed of epithelial cells, macrophages and fibroblasts were exposed to cigarette smoke for 15 minutes, 1 day and 4 days. Hematoxylin-eosin staining was applied to lung sections. Inflammatory cell number in the bronchoalveolar lavage fluid (BALF) was measured with flow cytometry.
TRPA1 expression and upregulation after a 2-month smoke exposure TRPA1 was expressed in the whole mouse lung. Remarkable lymphocyte and macrophage accumulation occured by this time on the histological slides and the inflammation became chronic, as supported by BALF assessment. Epithelial cells and leukocytes expressed TRPA1 mRNA. After 4 days, TRPA1 expression minimally increased in the epithelial cell- and fibroblast-containinig human lung model, but significantly elevated in the macrophage-containing cultures.
Increased TRPA1 gene expression in lung tissue and leukocytes demonstrates its activation by cigarette smoke and a likely function in chronic bronchitis. Similar findings in the human 3D tissue model and in mice indicate translational relevance.

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TREM-1 BLOCKING PEPTIDE LR12 ATTENUATED INFLAMMATORY CELLULAR INJURY IN LPS-INDUCED MACROPHAGES#

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Introduction: Triggering receptor expressed on myeloid cells (TREM)-1 is expressed in neutrophils and monocytes/macrophages, and functions as an amplifying molecule in inflammatory responses. LPS can upregulate the expression of TREM-1. And after activation, TREM-1 promotes the release of abundant pro-inflammatory cytokines, such as TGF-β and IL-1β. It was reported that inhibition of TREM-1 attenuated inflammation and increased the animals model survive of several diseases, such as sepsis and pneumococcal pneumonia. However, the effect of TREM-1 on acute lung injury is unclear. LR12 is a synthetic TREM-like transcript 1-derived peptide composed of 12 peptides (LQEEDTGEYGCV). It inhibits the activation of TREM-1 through competitively blocking the TREM-1 with its natural ligand.
Aim: To investigate the effect of blocking TREM-1 with LR12 on the pro-inflammatory cytokines (IL-1β and TGF-β) in LPS-stimulated macrophages and the LDH activity in cell culture supernatant.
Methods: Murine macrophages RAW264.7 were used in this study. After pretreatment with LR12 (20 μg/ml) or LR scramble (YQVGELCTGEED, 20 μg/ml) for 30 min, murine macrophages were stimulated with 1 μg/ml LPS for 24 h. Then, the LDH activity of culture supernatant was detected with LDH assay kit and the mRNA level of IL-1β and TGF-β was measured by RT-PCR.
Results: After LPS stimulation, the mRNA level of IL-1β and TGF-β increased in RAW264.7 macrophages, and the LDH activation was significantly elevated. LR12 pretreatment decreased the IL-1β and TGF-β mRNA expression, and LDH

activation in the supernatant. However, no effect was detected with LR Scramble pretreatment.
Conclusion: Blocking TREM-1 with LR12 reduced the pro-inflammatory cytokines and LDH leakage of murine macrophage, contributing to recreated inflammatory cellular injury induced by LPS.

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MEMANTINE MITIGATES BLEOMYCIN-INDUCED LUNG INFLAMMATION AND INJURY*

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Background: Pulmonary fibrosis is a life-threatening and progressive dysfunction disease characterized by lung inflammation, excessive proliferation of fibroblasts and increased extracellular matrix. Glutamate and its receptor N-methyl-D-aspartate receptor (NMDAR) have been well known in central nervous system. Several evidences appear NMDAR are present in non-neuronal cells and tissues. It is reported that NMDAR activation may attribute to acute lung injury. However, the roles of NMDAR in lung fibrosis remain largely unknown. We assessed the role of NMDAR in a mouse model of bleomycin (BLM)-induced lung injury.
Methods: C57BL/6 mice were injected with bleomycin intratracheally to induce lung inflammation and injury. Bronchoalveolar fluid (BALF) and lung inflammation cells, cytokines, histology were determined on Day 7 and Day14 after BLM treatment.
Results: Severe lung inflammation and injury were observed in the bleomycin-treated mice on Day 7. Administration of NMDAR antagonist memantine (10mg/kg) significantly reduced the number of total cells in the BALF and the levels of TNF-α, IL-1β, increased IL-10 in the lung homogenates on Day 7. Histological examination revealed that memantine markedly reduced the number of infiltrating cells and improved lung structure on Day 7, and significantly decreased pro-collagen I and collagen III mRNA expression on Day 14.
Conclusions: Our findings demonstrate that intraperitoneal administration of memantine, protected mouse from bleomycin-induced lung injury. These results suggest that further investigations regarding the role of NMDAR in fibrotic lung injury and repair are needed.

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SERUM-FREE CONDITIONED MEDIUM OF LUNG ADENOCARCINOMA A549 CELL PROMOTES A549 AND NIH-3T3 CELLS PROLIFERATION*

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It has been reported that the risk of lung cancer is high in patients suffering with Idiopathic pulmonary fibrosis. Glutamate is one of the major neurotransmitters in the central nervous system. It is essential for a variety of cell proliferation. In this study, we detected concentration of glutamate in serum-free conditioned medium of lung adenocarcinoma A549 cells and observed the proliferation effects of conditioned medium of lung adenocarcinoma A549 cells on A549 cells and NIH 3T3 cells. A549 cells in logarithmic growth phase and grown to 80% confluency were selected , the medium was aspirated, and the A549 cells were rinsed twice with PBS and replenished with serum-free fresh medium in 0.1ml/cm^2 for 24h. Then the supernatant was collected as conditioned medium. The effects of conditioned medium on proliferation of A549 cells and NIH-3T3 cells was detected by CCK8 kits. Concentration of glutamate in conditioned medium was detected by glutamate detection kit. The results showed that glutamate concentration in the conditioned medium of lung adenocarcinoma A549 cells was 497.9±67.4 u mol/L, was 1.25±0.12 times VS serum and 1.81±0.32 times VS NIH-3T3 cells group. The proliferation of A549 cells was increased in a dose-dependent manner ($p<0.01$) with conditioned medium concentration within 10~40% (V/V) .Meanwhile, that of NIH-3T3 cells showed the same way with conditioned medium within 10~30% (V/V) ($p<0.01$). In conclusion, serum-free conditioned medium of lung adenocarcinoma A549 cells promotes the proliferation of A549 cells and NIH-3T3 cells. The conditioned medium contains high concentrate glutamate.

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METABOTROPIC GLUTAMATE RECEPTOR 8 ACTIVATION INHIBITS THE PROLIFERATION OF LUNG CARCINOMA A549 CELLS*

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As a major neurotransmitters in the central nervous system, Glutamate activates both ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) which were subdivided into three groups: Group I (mGluR1 and 5), II (mGluR2 and 3), and III (mGluR4, 6, 7, and 8). In recent years, Researchers have demonstrated that glutamate receptors were also distributed in non-neuronal tissues. Cultured tumor cells, including lung carcinoma A549 cells, showed a decreased proliferation when exposed to MK801, an antagonists of ionotropic glutamate receptors. It remains a mystery what role mGluRs plays on the proliferation of A549 cells. We investigated the 8 subtypes mGluRs mRNA expression levels in A549 cells by the Real - time PCR technique and the effects of mGluRs agonist on the proliferation of A549 cells was detected by CCK8 kits. The results showed there were high level in mGluR8 and mGluR4 mRNA expression , But the expression level of mGluR4 mRNA is less than mGluR8($p<0.01$), low levels in that of mGluR1, mGluR5, mGluR6, mGluR7, no expression in that of mGluR2 and mGluR3 in A549 cells. When exposed to the DCPG, an agonist of mGluR8, within 0.01 ~ 10 u mol/L, the proliferation of A549 cells was decreased ($p<0.01$) While exposed to VU0155041, an agonist of mGluR4, within 0.01 ~ 10 u mol/L, showed no effect on the proliferation of A549 cells. In conclusion, lung carcinoma A549 cells express 6 subtypes mGluRs with the high mGluR8. mGluR8 activation could inhibit proliferation of A549 cells.

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EXPRESSION OF EAATS IN DIFFERENT ORGANS DURING EXPERIMENTAL POLYMICROBIAL SEPSIS*

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Background: Excitatory glutamate transporters (EAATs) mainly includes five subtypes (EAAT1- EAAT5).The major function of EAATs is intake glutamate from the extracellular space to maintain the extracellular glutamate at low concentration level to avoid excessive glutamate produce excitatory toxicity. The present study shows that EAATs also distribution in peripheral organs. We examined the expression and characterization of EAATs mRNA in different organs in sepsis mice.
Methods: Sepsis was induced in BALB/C mice by cecal ligation and puncture (CLP). Organ injury was observed with HIM staining. Reverse-transcription polymerase chain reaction (RT-PCR) analysis the expression of EAATs mRNA in the heart, liver, spleen, lung, kidney, brain of the mice. Glutamate (Glu) content was detected by colorimetry in the above of organs.
Results: Compared with the control group, lung inflammation and injury was the most obviously in sepsis mice.RT-PCR showed that EAATs distributed in the brain, EAAT1 and EAAT3 most widely distributed in peripheral organs. EAAT1 mainly expressed in the spleen and lung; EAAT2 mainly in the liver; EAAT3 mainly expressed in the kidney and lung; EAAT4 and EAAT5 did not observe in heart, liver, spleen, lung, kidney.The expression of EAAT1 mRNA was strongly down-regulated in spleen and lung, and significantly up-regulated in the kidney and brain in CLP group. Consistent with above, the glutamate content also present the same change trend.
Conclusion: The expression of EAATs is changed in several organs in sepsis mice. The results suggest that EAATs may play an important role in the inflammatory process.

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THE EXPRESSION OF AUTOPHAGY BIOMARKERS BECLIN 1 AND LC-3 IN MOUSE MODEL OF PULMONARY FIBROSIS#

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Introduction: Autophagy is a highly conserved cell process in eukaryocyte. It is a catabolic process involving the removal of excess or damaged organelles, degrading misfolded and aggregated proteins as well as recycling these degradation

products to meet metabolic demands. Autophagy plays an important regulatory role in maintaining cellular homeostatic balance. Autophagy is becoming an exciting and focused field in pulmonary diseases. Recent studies have shown that autophagy inducers, such as oxidative stress, endoplasmic reticulum stress and hypoxia, implicated in the pathogenesis of pulmonary fibrosis. Autophagy also regulates expression of fibrogenic genes in embryonic, lung and renal fibroblasts. These associations suggest that autophagy is likely to be related with pulmonary fibrosis.
Aims: To observe the mRNA levels of autophagy biomarkers Beclin 1 and LC-3 in mouse model of pulmonary fibrosis, which may lay a sound basis on the study about autophagy and pulmonary fibrosis.
Methods: 42 male Swiss mice (28-22 g) were bought from Animal Department of Central South University. These mice were randomly assigned to experimental group which receive a single dose bleomycin challenge and control group. The experimental group was treated with 15 mg/kg of intratracheal bleomycin sulfate on day 1. The control group received the same volume saline. These mice were sacrificed and lungs were harvested on Day 7, 14 and 21 respectively. Total RNA was isolated from all the mouse lung tissues using the TRIzol kit. Next, the RNA was reverse-transcribed and amplified.
Results: Lung tissues from bleomycin treated mice demonstrate that the mRNA expression of Beclin1 and LC-3 was significantly decreased, compared with the control group ($P < 0.05$).
Conclusion: The decreased autophagy during pulmonary fibrosis implies that autophagy may play an important role in the development of pulmonary fibrosis.

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SR48692 INHIBITS NON-SMALL CELL LUNG CANCER PROLIFERATION IN AN EGF RECEPTOR-DEPENDENT MECHANISM

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Neurotensin (NTS) is an autocrine growth factor for some cancer cells e.g. small cell lung cancer (SCLC). NTS is synthesized in and secreted from SCLC cells, binds with high affinity to SCLC cells and causes increased cellular proliferation. The actions of NTS on SCLC are blocked by SR48692, a NTSR1 non-peptide antagonist. Here the mechanism by which SR48692 inhibits the proliferation of non-small cell lung cancer (NSCLC) cells was investigated. By Western blot, a 55 KDal band of NTSR1 immunoreactivity was detected in A549 and NCI-H1299 cells. Treatment of A549 or NCI-H1299 cells with siRNA for NTSR1, reduced significantly NTSR1 protein and the ability of SR48692 to inhibit the proliferation of A549 or NCI-H1299 cells. Treatment of A549 and NCI-H1299 cells with siRNA for NTSR1 reduced the ability of NTS to cause epidermal growth factor receptor (EGFR) transactivation. SR48692 or gefitinib (EGFR tyrosine kinase inhibitor) inhibited the ability of NTS to cause EGFR and ERK tyrosine phosphorylation. NTS transactivation of the EGFR was inhibited by PP2 (Src inhibitor), GM6001 (matrix metalloprotease inhibitor), Tiron (superoxide scavenger), or U73122 (phospholipase C inhibitor) but not H89 (PKA inhibitor). NTS stimulates whereas SR48692 and/ or gefitinib inhibits the clonal growth of NSCLC cells. The results indicate that SR48692 inhibits NSCLC growth in an EGFR-dependent manner.

SYNTHESIS AND IN VITRO AND IN VIVO EVALUATION OF A ^{99M}TC-LABELED LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) PEPTIDE AS A BREAST CANCER IMAGING AGENT

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Objectives: The overexpression of LHRH receptors on various commonly occurring human cancers including breast, prostate and ovarian cancer and the low expression in most normal tissues making LHRH a potential molecular target for tumor imaging and therapy with radiolabeled LHRH peptide analogs. Here, we prepared and evaluated a novel LHRH analog for its ability to target breast cancer in vivo.
Methods: Acetyl-Gly-Gly-Cys-Asp-Glu-His-Trp-Ser-Tyr-Trp-Leu-Arg-Pro-Gly-$CONH_2$ was prepared by solid-phase synthesis according to Fmoc strategy and labeled with ^{99m}Tc by stannous-tartrate exchange method. In vitro cell-binding was performed on various breast cancer cell lines (MDA-MB-231, MCF7, T47D) and in vivo biodistribution was determined in healthy and nude mice with MDA-MB-231 tumors.
Results: The structure and purity of the synthetic peptide was confirmed by mass spectrometry and HPLC. The peptide labeled efficiently with ^{99m}Tc (>90%) as revealed by HPLC. ^{99m}Tc-LHRH showed high affinity binding to all three breast cancer cell lines with the Kd values ranged between 3-15 nM. In healthy mice, the radiopeptide displayed efficient clearance from the blood and excreted largely through the renal pathway. The elimination via the hepatobiliary system was low. The accumulation in the lungs, liver, stomach and pancreas was low (<3% ID/g) both at 1 and 4 h p.i. The kidneys showed the highest uptake (up to 7% ID/g). In nude mice with MDA-MB-231 xenografts, ^{99m}Tc-LHRH exhibited a somewhat low tumor uptake (0.95±0.31%

ID/g, 1 h). The uptake in the tumor was always higher than the uptake in the blood and muscle resulting in good tumor/blood and tumor/muscle ratios.
Conclusions: This initial study towards the development of a peptide-based agent for breast cancer detection suggest that it is worth to perform further studies in order to determine the full potential of this peptide for targeting breast or other LHRH receptor-positive tumors.

TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 (TRPA1) AND VANILLOID 1 (TRPV1) ION CHANNELS ARE EXPRESSED AND UPREGULATED IN RESPONSE TO ESTROGEN IN THE RAT ENDOMETRIUM

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Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) cation channels localized predominantly on capsaicin-sensitive peptidergic sensory nerves play essential roles in pain, hyperalgesia and neurogenic inflammation. They are activated by a variety of noxious stimuli, chemical irritants and cold or heat, respectively. Besides sensory nerves, both receptors have been described on epithelial and immune cells. Estrogen-induced TRPV1 up-regulation in the human uterus suggests its potential involvement in pain during the reproductive cycle. Since there are no data regarding TRPA1 expression in the endometrium and little is known about TRPV1 regulation, we investigated estrogen- and progesterone-dependent alterations of these channels in the rat endometrium.
Different groups of 4-week-old and 4-month-old female rats were treated with synthetic estrogen analog diethylstilbestrol (DES), progesterone and their combination for 8 or 12 days, respectively. Ovariectomy was performed in separate groups of 4-month-old animals. TRPA1 and TRPV1 mRNA levels were measured with quantitative PCR, the localization of the receptor proteins was determined with immunohistochemistry on paraffin-embedded sections.
Both TRPA1 and TRPV1 were detected in the rat endometrium at mRNA and protein levels, showing their remarkable local, non-neuronal expression. DES treatment resulted in a 5-fold and 7-fold significant up-regulation of TRPV1 mRNA in young and adult rats, respectively, which were absent if progesterone was added simultaneously. DES also induced significant elevation of TRPA1 mRNA in both groups. In young rats, weak TRPV1 and A1 staining were observed in the epithelium, while in adult animals it was detected in the stroma and the glands with weak expression in the epithelium. Further investigations are in process to elucidate the functions of TRPA1 and TRPV1 in conditions related to pain and inflammation.

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THE CYTOKINES OF SKELETAL MUSCLE CELLINHIBIT THE PROLIFERATION OF A549

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Introduction:As a major organ of the body, skeletal muscle is rich in lymphatic vessels and blood supply, but nearly without cancer metastasis. The study was designed to investigate this physiological phenomenon by exploring the relationship between skeletal muscle and the proliferation of cancer cells.
Methods: A549, the human lung adenocarcinoma cells, was studied in this study. We used transwell plates, which only cytokines was allowed to exchange through these two layers.First, we cocultured the supernatant ofskeletal muscle cells and cancer cells. Then, we cocultured skeletal muscle tissue with cancer cells. At last, we cocultured skeletal muscle cells and cancer cells. DMEM culture group was used as the control. Cell proliferation was detected by MTT andcell morphology was examined under optical microscope.
Results: MTT results showed that the proliferationof cocultured cancer cells in experimental groups was significantly inhibited compared with the control groups, the rates of skeletal muscle tissue coculture group is the lowest (OD:experimental group 0.17±0.07,control group 0.35±0.27, n=8,$P<0.05$). The group of skeletal muscle cell's supernatant coculture group has a minimal impact(OD:experimental group 0.30±0.26,controlgroup 0.33±0.81, n=8,$P<0.05$).The morphology of cancer cells changes from the original elliptic into fusiform or thin strip.
Discussion: Cytokines in the supernatant of skeletal muscle cells caninhibit the proliferationof A549 andaffect the growth morphologyof A549.

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OVER-EXPRESSION OF BOMBESIN RECEPTOR-ACTIVATED PROTEIN INHIBITS APOPTOSIS OF HEK293T CELL UNDER OXIDATIVE STRESS

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Bombesin receptor-activated protein(BRAP) was identified as a protein interacted with bombesin receptor subtype 3 by bacteria two hybrid screening. Our previous study found that it is highly expressed in human lung tissue and may participate in oxidative stress responses of bronchial epithelial cells. In this study, we explored the roles of BRAP in cellular apoptosis under oxidative stress. A recombinant BRAP protein was over-expressed in HEK293T cells by introducingan expressionplasmid into cells. Then the cells were stressed with ozone exposure and the levels of reactive oxygen species (ROS) were detected using fluorescent DCFH-DA. BRAP over-expression loweredcellular ROS level and decreased the activity of antioxidant response element (ARE) by luciferase reporter assay after ozone exposure. Cell apoptosis was analyzed by annexin V-FITC staining followed by flow cytometry detection and BRAP over-expression led to a decreased number of annexin V-FITC stained cells under ozone stress. Since the aspartic acid specific protease caspase-9 has been linked to the mitochondrial pathway of apoptosis, caspase-9 activity was observed bya luminescent assayand it was decreased in BRAP over-expressed cells.The mitochondrial membrane potential of the cell was also measured by detecting a fluorescent JC-1probe. BRAP over-expression maintained the mitochondrial membrane potential under ozone stress. Our data suggests that BRAP might inhibit ROS production and regulate apoptosis under oxidative stress via mitochondrial pathway.

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Both contributes equally.

BOMBESIN RECEPTOR-ACTIVATED PROTEIN DOWN-REGULATES NF-κBACTIVITY IN HUMAN BRONCHIAL EPITHELIAL CELLS

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Our previous studies found that bombesin receptor-activated protein (BRAP) is widely expressed in the airway epithelium of human lungs and may play a role during the stress response of lung epithelium. However, the actual functions of BRAP in lung epithelium remain largely unknown. A jmjC-domain was predicted in the C terminus of BRAP and may have enzyme activity. In this study we tested the regulatory effects of BRAP on NF-κB activity in human bronchial epithelial cells and explored its underlying structural mechanism. BRAP was over-expressed by introducing a recombinant BRAP expression plasmid into 16HBE14o-, an immortalized human bronchial epithelial cell line. The down-regulation of BRAP expression was achieved by RNA interfering techniques. The activity of NF-kB was monitored by performing NF- κB luciferase reporter assays. LPS, Poly (I:C) or ozone exposure were used to induce activation of NF-κBvia TLR4, TLR3 or oxidative stress dependent manners.. Over-expression of BRAP in 16HBE14o- cells decreases the basal level of NF-κB without any stimulus and abolished the increase of NF-κB activity induced by the above stimuli as well. Inhibition of BRAP expression can enhance the activity of NF-κB. A plasmid that expresses a mutated BRAP protein without jmjC-domain was constructed and used to transfect 16HBE14o- cells. Over-expression of this mutated BRAP protein has no regulatory effect on NF-κB activation, which indicates that BRAP could regulate NF-κB activation via its putative jmjC-domain in human bronchial epithelial cells.

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Both contributes equally.

DOUBLE STIMULATION-INDUCED NLRP3 AND DOWNSTREAM MOLECULES EXPRESSION WERE INHIBITED BY CGRP IN A549 CELLS AND HUMAN BRONCHIAL EPITHELIAL CELLS

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Introduction: Inflammasomes are intracellular PRRs, such as nod like receptors (NLR), among which NLRP3 inflammasome is closely related to inflammation in pulmonary diseases. It is recognized that NLRP3 activates caspase-1, leading to maturation of interleukin-1β (IL-1β) and enhancement of inflammatory response. It suggests that reducing NLRP3 could attenuate inflammation. Recent evidence points to that endogenous mediator have the advantage of regulatory effect on inflammation. Calcitonin gene related peptide (CGRP), a neuropetide widely located in pulmonary neuroendocrine cells and lung nerve fibers, is one of these mediators. CGRP has the ability to regulate inflammation

cytokines and protect lung. However, the effect of CGRP on NLRP3 and its downstream molecules expression has not yet been identified.

Aims: To observe the effect of CGRP on NLRP3 and its downstream molecules in A549 cells and human bronchial epithelial cells (HBECs) after stimulation of lipopolysaccharides (LPS) plus ATP.

Methods: A549 cells and HBECs were divided into three groups respectively. Control, LPS (500 ng/mL) +ATP (5 mM), CGRP (10^{-6} M) + LPS (500 ng/mL) +ATP (5 mM). Double-stimulation protocol as follows, cells were pretreated with CGRP for thirty minutes, and next stimulated with LPS for three hours and simultaneously with the last forty-five minutes in the presence of ATP. NLRP3, caspase-1 and IL-1β mRNA in A549 cells and HBECs were analyzed by RT-PCR.

Results: In LPS plus ATP group, NLRP3, caspase-1 and IL-1β mRNA in A549 cells and HBECs were significantly increased ($P<0.05$) compared with control. Pretreatment with CGRP, LPS plus ATP induced NLRP3, caspase-1 and IL-1βmRNA expression were obviously reduced ($P<0.05$).

Discussion: These results suggested that inhibition of the NLRP3, caspase-1, IL-1β mRNA on A549 and HBEC s by CGRP during acute inflammation could be part of the negative feedback mechanism controlling the extension of acute inflammatory responses.

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RECEPTORIAL MECHANISMS OF THE INHIBITORY ACTION OF PACAP ON THE VASCULAR CHANGES IN NEUROGENIC INFLAMMATION

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The related neuropeptides PACAP and VIP are known to have potent actions in the development of the immune system and in inflammatory reactions. PACAP-38 diminishes neurogenic inflammation, but no data have been reported on the contribution of its three receptors, PAC1R and VPAC1/2R, to the inhibitory effect.

We used mustard oil-induced neurogenic inflammation, induced in the mouse ear. Mustard oil is an activator of TRPA1 receptors on the capsaicin-sensitive nerve terminals. The accompanying neurogenic edema was quantified by measuring ear thickness with engineer's micrometer and vasodilation was assessed by laser Doppler scanning. Increased plasma protein leakage, another crucial process in neurogenic inflammation, was determined by measuring the albumin-bound Evans blue extravasation with intravital videomicroscopy. Myeloperoxidase activity, an indicator of neutrophil infiltration, was also evaluated. CGRP, SP and somatostatin concentrations were assessed by radioimmunoassay.

In our study we found that maxadilan, a specific PAC1R agonist, significantly diminished the mustard oil-induced neurogenic edema and attenuated the vascular permeability indicated by the decreased Evans blue extravasation. Inhibitory action of the VPAC1/2 receptor agonist VIP on neurogenic edema was milder, without exerting any effect on the plasma leakage. Both peptides exhibited inhibitory actions on mustard oil-induced vasodilation, while activation of neither PAC1R, nor VPAC1/2 receptors influenced the cellular components of the inflammation. VIP significantly increased the CGRP, SP and SST concentrations in the mouse ear. These results show that the specific PACAP receptor, PAC1R, is primarily responsible for the inhibitory properties of PACAP on both the venous and arterial components of neurogenic inflammation, while activation of VPAC1/2R is involved in the attenuated vasodilation only.

BIOMARKERS IN LATE STAGE NEUROENDOCRINE TUMOURS OF THE SMALL BOWEL

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Introduction: Small bowel neuroendocrine tumours (MGC) comprise 20-25% of all neuroendocrine tumours (NETs). They present early with obstruction or later with diarrhoea, flushing and in some right sided heart lesions (CHD). Biomarkers for MGC include serotonin or urinary 5 hydroxy indole acetic acid (U5HIAA), the general NET marker chromogranin A (CGA) and a specific marker, neurokinin A (NKA). We have shown that NKA >50ng/l indicates poor prognosis. Many treatment options for MGC exist.

Methods: We reviewed clinical notes from 20 patients who died from MGC, to identify the most useful markers at late stage disease. We noted biomarkers during the 2 years to date of death, at 24, 18, 12 & 6m.

Results: All 20 patients were symptomatic, 20 with diarrhoea, 19 with flushing and 10 with CHD. All received somatostatin analogue therapy. Thirteen had surgery (5/20 right hemi-colectomy, 4/20 tumour resection). Eight had received radio nucleotide therapy, 3 hepatic embolization and 7 interferon alpha therapy.

From 24-6m median U5HIAA (with ranges), rose through 285 (54.3-1,230), 274 (82.4-704), 397 (62-1,985) to 701 (78.1-1,995)umol/l, CGA through 375 (140->1,200), 1,200 (40->1,200), >1,200 (220->1,200) to >1,200 175->1,200)U/l and NKA through 57 (20-580), 109 (22-917), 112 (23-1,350) to 134 (30-2,425)ng/l. (Ref Ranges: U5HIAA <47umol/l, CGA <30U/l, NKA<20ng/l).
Across the 20 patients and 4 sample times, 4 specimens were missed for NKA, 6 for CGA and 15 for 5HIAA.
In 2 patients U5HIAA remained <150umol/l. Six patients had no sample at 6m. In 4 patients CGA remained <500U/l throughout, 47% of CGAs were >1,200U/l. In 2 patients NKA remained <50ng/l throughout, in all others NKA was >50ng/l from 6m.
Conclusions: The DACO Assay used for CGA does not dilute parallel to the standard above 1,200U/l making CGA difficult to measure in advanced disease. Missed sampling for U5HIAA is a problem in late disease. NKA is a reliable test in 90% of patients heralding terminal disease.

RADIOIMMUNOASSAY EXAMINATION OF PACAP IN HUMAN TISSUE AND BLOOD SAMPLES OF DIFFERENT PATHOLOGICAL CONDITIONS

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional neuropeptide with well-known neuroprotective and general cytoprotective effects. Earlier we found significantly lower level of PACAP-like immunoreactivity (PACAP-LI) in both lung and colon tumor samples compared to normal tissues, most probably due to degeneration of PACAP containing nerve fibers in the tumor. We also showed that in cardiac samples PACAP-LI is significantly higher in ischemic heart diseas compared to valvular abnormalities.
In the present study we investigated the PACAP-LI with radioimmunoassay examination from human blood and tissue samples of patients. 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, endocrinological disorders, sleep apnea syndrome and ischemic cardiac diseases.
We found significantly higher PACAP-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 PACAP-LI compared with healthy tissue samples. We did not find significant alterations in PACAP-LI between healthy and tumorous urinary bladder and prostate samples. Our results in the human blood samples also showed significant correlations with PACAP-LI and severity of diabetes, sleep apnea syndrome and ischemic cardiac disorder, but further investigations are necessary to describe the exact function of PACAP in different pathological conditions.

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EXAMINATION OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE AND MACROPHAGE MIGRATION INHIBITORY FACTOR IN HUMAN BREAST MILK SAMPLES

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Breast milk contains several bioactive compounds that play important roles in development of the nervous system and gaining immuncompetence. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide with important functions in reproductive and developmental processes. Recently, we have shown that high level of PACAP is present in breast milk and we have described changes of PACAP levels during lactation. Earlier we have also examined the presence of macrophag migration inhibitory factor (MIF), a proinflammatory cytokine, in human milk samples, but previous studies have only focused on the water phase of milk. In the first experiment we aimed to examine the changes of MIF level both in the water and lipid phases of milk samples during the first 6 months of lactation. It is well-known that the constitution of breast milk is influenced by numerous external agents, e. g. the gender of the newborn. Therefore, in the second part of our research we analyzed the difference in PACAP concentrations of the milk samples of male and female newborns.
We separated the milk samples to lipid phase and water phase by centrifugation. We used ultrasonication to factor the lipid phase to additional lipid and water fractions. We measured the

MIF concentration with ELISA and the PACAP level of the milk by radioimmunoassay examination.
We detected, for the first time, the presence of MIF in the lipid phase of human milk. We measured higher MIF concentrations in the water phase than in the lipid phase, and we showed an increasing tendency of MIF concentration in the lipid fraction during the first 6 months. Our preliminary examinations did not find significant differences between the PACAP level of milk samples from male and female newborns. Our future aim is to determine the exact influence of MIF and PACAP in the process of lactation with additional clinical and molecular biological experiments.

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A SELECTED INHIBITOR OF SOLUBLE EPOXIDE HYDROLASE TPPU INHIBITS THE EXPRESSION AND ACTIVATION OF INFLAMMASOME#

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Introduction: Acute lung injury (ALI) was the diseases induced by many extreme conditions including severe sepsis, trauma and burn. It is believed that the pathophysiological mechanism of ALI is associated with the uncontrolled inflammatory response in lungs. Inflammasomes are a group of protein complexes that control the production of important pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18. Inhibition of inflammasome maybe a powerful strategy to attenuate ALI. cis-epoxyeicosatrienoic acids (EETs) is cytochrome P450 epoxygenase metabolites of arachidonic acid and is hydrolased mainly by soluble epoxide hydrolase (sEH). EETs have anti-inflammatory effects. However, the effect of EETs on inflammasome is unclear.
Aim: To observed the effect of a selected sEH inhibitor TPPU on the inflammasome expression and activation.
Methods: Murine ALI model was intraperitoneal injected with LPS (10 μg/kg). TPPU, administered by gavage dosing, was used to increase the concentration of EETs. RT-PCR was used to detect the mRNA levels of main inflammasome (NLRP3 and NLRC4), and its downstream cytokine IL-1β. ELISA was employed to determent the IL-1β concentration in the mice BALF. LDH activation was tested to inflect the intrapulomanory cellular injury.
Results: The NLRP3 and NLRC4 mRNA were significantly increased in ALI mice. TPPU treatment decreased both inflammasomes gene expression. It indicated EETs inhibited the inflammasome expression. Furthermore, we found TPPU treatment decreased the IL-1β mRNA level in lung tissue and IL-1β concentration in BLAF, indicating EETs inhibited the inflammasome activation. Then we found TPPU also reduced the LDH activation in BALF.
Conclusion: A selected inhibitor of sEH TPPU inhibited the inflammasome expression and activation, resulting to attenuate of LPS-induced intrapulomanory cellular injury.

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