

Session 1 Role of sensory neuropeptides in inflammatory mechanisms, Chair Zsuzsanna Helyes

Role of capsaicin-sensitive peptidergic afferents, the TRPV1 receptor and sensory neuropeptides in airway inflammation

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Background: The airways are densely innervated by capsaicin-sensitive sensory neurons expressing Transient Receptor Potential Vanilloid 1 (TRPV1) receptors/ion channels, which play an important regulatory role in inflammation through the release of sensory neuropeptides. Pro-inflammatory peptides such as tachykinins (substance P: SP, neurokinin A: NKA) and calcitonin gene-related peptide (CGRP) induce neurogenic inflammation via NK₁, NK₂ and CGRP1 receptor activation in the innervated area, while somatostatin released into the systemic circulation exerts anti-inflammatory actions presumably by activating subtype 4 receptors (sst₄) receptors. The overall significance of these mediators seems to depend on the pathological mechanisms being involved in certain inflammatory processes.

Aims: We investigated the roles of capsaicin-sensitive nerves, TRPV1 receptors, pro-inflammatory sensory neuropeptides as well as the anti-inflammatory somatostatin and sst₄ receptors in endotoxin-induced airway inflammation and consequent bronchial hyperreactivity. We used high dose resiniferatoxin pretreatment to destroy capsaicin-sensitive nerve terminals, specific receptor antagonists and gene-deficient mice to approach this question with the help of functional (whole body plethysmography), morphological (semiquantitative histological scoring) and biochemical/immunological techniques (myeloperoxidase measurement, ELISA, RIA). *E. coli* lipopolysaccharide was administered intranasally to induce interstitial pneumonitis 24 h later which is a well-defined murine model of airway inflammation.

Results: Our data revealed that capsaicin-sensitive afferents exert a protective role in endotoxin-induced airway inflammation, but contribute to increased bronchoconstriction. Activation of CGRP1 receptors or NK₁+NK₂ receptors participate in granulocyte accumulation, but NK₂ receptors play a predominant role in enhanced airway resistance. These findings were supported by data obtained with preprotachykinin 1 gene- and NK₁ receptor gene-deleted mice. Bronchial hyperreactivity, histopathological changes (perivascular/peribronchial oedema, neutrophil/macrophage infiltration, goblet cell hyperplasia) and myeloperoxidase activity were significantly greater in TRPV1 knockout mice. Inflammation markedly elevated lung and plasma somatostatin concentrations in wildtype, but not in TRPV1 gene-deficient animals. Antagonizing somatostatin receptors by cyclo-somatostatin increased all the inflammatory parameters providing evidence for the functional significance of the released somatostatin. Much more severe inflammation and hyperresponsiveness was observed in sst₄ receptor knockout mice, which revealed that somatostatin-induced inhibitory effects are mediated through the activation of this receptor. Administration of somatostatin-14 as well as the sst₄ receptor agonists, J-2156 and TT-232, diminished inflammation and hyperreactivity.

Conclusions: Although pro-inflammatory sensory neuropeptides participate in the development of endotoxin-induced lung inflammation, the overall role of capsaicin-sensitive fibres as well as the TRPV1 receptor is protective due to the release of somatostatin. NKA increases, but systemically released somatostatin decreases airway hyperreactivity. These studies provide evidence for a novel counter-regulatory mechanism during endotoxin-induced airway inflammation, which is mediated by somatostatin released from sensory nerve terminals in response to activation of TRPV1 receptors of the lung. It reaches the systemic circulation and inhibits inflammation and consequent

bronchial hyperreactivity through sst₄ receptors. These data might open interesting perspectives for the development of a novel group of anti-inflammatory drugs.

Analysis of TNF α -induced inflammatory hyperalgesia.

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We have demonstrated the involvement of TRPV1 in mediating inflammatory hyperalgesia in a murine model of joint inflammation involving wildtype and TRPV1^{-/-} mice. TNF α levels were found to be similar in synovial fluid exudates in WT and TRPV1^{-/-} mice, suggesting an upstream role of TNF α (Keeble *et al.*, 2005). We have now evaluated the influence of TRPV1 in unilateral intra-plantar (i.pl.) TNF α -induced inflammation.

We developed a model involving the intraplantar injection of TNF α . This led to a TRPV1-mediated bilateral thermal hyperalgesia, that was dependent on TRPV1, as it was not observed in TRPV1^{-/-} mice. We found that i.pl. TNF α causes TRPV1-dependent thermal hyperalgesia in the ipsilateral inflamed and contralateral uninflamed hindpaws. However, we could not find evidence for a functional involvement of either substance P or CGRP. In order to learn of the influence of mediators downstream of TNF α , the effects of COX and PKC inhibitors were examined and cytokine and prostaglandin levels were measured. Results showed that COX-2 derived prostaglandins and IL-1 β play roles in mediating the hyperalgesia in the inflamed ipsilateral paw and we provide evidence that IL-1 β plays a crucial role in mediating hyperalgesia in the contralateral uninjured paw. GF109203X, a PKC inhibitor, suppressed TNF α -induced hyperalgesia. This suggests that PKC may be key to TRPV1 sensitisation.

These results indicate that a series of mediator influences are acting in our TNF α model of inflammatory hyperalgesia. We now also realize that reactive oxygen species are involved in mediating thermal hyperalgesia (Keeble *et al* in press), that are known to influence other TRP receptors. We hypothesize that a more informed understanding of mechanisms and inflammatory mediator levels, which can be obtained from models such as this, is important in order to progress the treatment of inflammatory joint diseases.

Keeble J, Russell F, Curtis B, Starr A, Pinter E, Brain SD (2005) *Arthritis Rheum* 52:3248-3256.

Keeble JE, Bodkin JV, Liang L, Wodarski R, Davies M, Fernandes ES, de Faria Coelho C, Russell FA, Graepel R, Muscara MN, Malcangio M, Brain SD. Pain, in press

Supported by the Arthritis Research Campaign and a BBSRC-funded IMB capacity building award, (SB, JK, EF), and a BBSRC/Pfizer Case studentship (FR)

Involvement of Transient receptor potential vanilloid 1 receptors in proteinase-activated receptor 2-induced joint inflammation and nociception

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Background: Proteinase-activated receptor-2 (PAR-2) is a G-protein-coupled receptor activated through proteolytic cleavage by trypsin or tryptase. It is localized on epithelial, endothelial and inflammatory cells as well as capsaicin-sensitive, Transient Receptor Potential Vanilloid 1 (TRPV1) receptor-expressing peptidergic sensory nerves and it plays an important role in inflammatory/nociceptive processes.

Aims: Since there are few data concerning PAR-2 activation in the joints, the aim of the present study was to investigate the effect of intraarticular PAR-2 activation. Secondary hyperalgesia/allodynia in the paw, spontaneous weight distribution and inflammatory cytokine production was measured and the involvement of TRPV1 ion channels was investigated in these pathophysiological changes in rats and mice.

Results: Injection of the PAR-2 receptor agonist SLIGRL-NH₂ (500 µg/kg) into the knee joint decreased touch sensitivity and spontaneous weight bearing of the unilateral limb in both species. The secondary mechanical allodynia/hyperalgesia and spontaneous pain were significantly reduced by i.p. pre-treatment with the TRPV1 receptor antagonist SB366791 in rats, and also in receptor knockout mice. IL-1β concentration increased in the joint homogenates, but this was not dependent on TRPV1 activity, TNF-α levels remained unchanged. For comparison, intraplantar SLIGRL-NH₂ evoked similar primary mechanical hyperalgesia and impaired weight distribution in both WT and TRPV1 gene-deficient mice, but oedema was much smaller in TRPV1 knockouts. The inactive peptide, LRGILS-NH₂, injected in either the knee joint or the paw, did not induce any alterations.

Conclusions: These data provide evidence for a significant role of TRPV1 receptors in secondary mechanical hyperalgesia/allodynia and spontaneous pain induced by activation of PAR-2 receptors in the knee joint. Although intraplantar PAR-2 activation-induced oedema is also TRPV1 receptor-mediated, primary mechanical hyperalgesia, impaired weight distribution and IL-1β production are independent of this ion channel.

Acknowledgements: Hungarian Grants OTKA K73044, OTKA T049027, RET-008/2005. Zs. Helyes is supported by János Bolyai Postdoctoral Research Fellowship. J.J. McDougall is an Alberta Heritage Foundation for Medical Research Senior Scholar and an Arthritis Society Investigator.

Regulatory function of sensory neuropeptides in dermatitis and arthritis

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Neuropeptides released from the activated peripheral terminals of capsaicin-sensitive sensory neurons expressing transient receptor potential vanilloid 1 (TRPV1) receptor exert local efferent function, besides the classical afferent activity. Pro-inflammatory neuropeptides induce neurogenic inflammation in the innervated area. These processes play a significant role in the pathological mechanism of arthritis and dermatitis. The major problem in the treatment of these conditions is the lack of effective therapy for the neurogenic component. On the other hand, several neuropeptides with inhibitory actions are also released from these sensory nerve endings into the systemic circulation and induce potent anti-inflammatory effects. We have provided several lines of evidence for the anti-inflammatory role of neural somatostatin (SOM). The aim of the present study was to examine these modulatory roles of TRPV1 receptor and sensory neuropeptides in complete Freund's adjuvant (CFA)-induced arthritis, oxazolone-evoked allergic contact dermatitis (ACD) and bleomycin-induced scleroderma models in rats and mice.

1. Resiniferatoxin (RTX) pretreatment or the SOM antagonist cyclo-somatostatin (c-SOM) injection significantly increased oedema and mechanical hyperalgesia of both CFA-treated and contralateral paws in the rat. Histological score based on synovial thickening, cell infiltration, cartilage destruction and bone erosion was also significantly higher both in RTX- and c-SOM injected groups. These parameters were dose-dependently decreased by a heptapeptide SOM analogue, TT-232. Plasma SOM-like immunoreactivity elevated 4 folds on the 21st day, which was inhibited by RTX pretreatment, as well as daily administration of TT-232. Our data suggest that SOM released into the circulation from capsaicin-sensitive afferents in response to chronic activation exerts systemic anti-inflammatory and analgesic effects. TT-232 can open new perspectives in the treatment of chronic arthritis.

2. Oxazolone induced a significant ear swelling 24, 48, 72 h after the elicitation of the ACD. It was augmented in TRPV1 knockout mice at all time points and supported by histological analysis and measurement of TNF- α . However, tissue swelling and cytokine generation was significantly reduced in both neurokinin 1 receptor and calcitonin gene-related peptide (CGRP) knockout mice. A protective involvement of the TRPV1 receptor was identified in ACD distinct from mechanisms involving the major pro-inflammatory neuropeptides.

3. Bleomycin treatment induced marked cutaneous thickening and fibrosis compared to the PBS-treated control group. Composite sclerosis score was 18%, dermal thickness 19%, number of α -smooth muscle actin (SMA)-positive cells 47.2%, amount of hydroxyproline 57.5% higher in TRPV1^{-/-} mice than in wild-type counterparts. Similarly, composite sclerosis score was 47%, dermal thickness 29%, number of α -SMA-positive cells 76%, amount of hydroxyproline 30% higher in CGRP^{-/-} mice than in the respective wild-type groups. These results suggest that activation of the TRPV1 receptor by inflammatory mediators induces sensory neuropeptide release, which might exert protective action against fibrosis. We confirmed the protective role of CGRP in the development of cutaneous sclerosis.

In conclusion, the role of capsaicin-sensitive nerves and also the TRPV1 receptor on their terminals in inflammatory processes depends on the significance of the released pro-inflammatory and anti-inflammatory neuropeptides in the pathological mechanisms.

Session 2 Neuropeptides in Health and Disease, Chair Alec Simpson

Cytoprotection by natriuretic peptides: Amelioration of cytotoxic Ca^{2+} responses.

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Natriuretic peptides are well known as vasodilator peptides that elevate cyclic GMP in target tissues. We have shown that in hepatocytes natriuretic peptides attenuate agonist-evoked Ca^{2+} responses and slow oscillations in intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$). This is achieved through a reduction in Ca^{2+} influx and a stimulation of Ca^{2+} efflux. These effects are mediated via protein kinase G. More recently we have demonstrated that natriuretic peptides can provide protection from harmful, cytotoxic elevations of $[\text{Ca}^{2+}]_c$ induced by high concentrations of agonists and also bile salts. We believe that a reduction in harmful elevations of $[\text{Ca}^{2+}]_c$ may be a common mechanism by which natriuretic peptides provide cytoprotection in a number of systems. One such example may be to provide protection from glutamate-induced neurotoxicity. We are therefore examining the ability of natriuretic peptides to reduce $[\text{Ca}^{2+}]_c$ responses and cell damage induced by glutamate in SH-SY5Y neuroblastoma cells. The potential of natriuretic peptides to provide protection in this model will be discussed.

Optogenetic probing of hypothalamus modulation of the sleep-wake cycle.
Antoine Adamantidis

Abstract

While the functions of sleep are still a matter of debate and may include memory consolidation and plasticity, the neural substrates of sleep and wakefulness are the subject of intense study. Successive sleep and wakefulness cycles rely on an appropriate balance between sleep-promoting nuclei of the brain and arousal-promoting nuclei. The posterior hypothalamus contains two distinct neuron populations that have been recently identified as critical modulators of the sleep-wake cycle: the Hypocretins/Orexins (Hcrt./Ox)- and Melanin-Concentrating Hormone (MCH)-expressing neurons. Although the Hcrt system is involved in boundary state control, the precise physiological function and mechanisms of action of the MCH system on the sleep-wake cycle remain unclear. We selectively manipulate the activity of Hcrt and MCH neuronal population using optogenetics to study their modulation of the sleep-wake cycle. We found that activation of the Hcrt facilitates wakefulness. In contrast, activation of the MCH system has the opposite effect and promotes sleep. These results suggested a bimodal switch within the lateral hypothalamus that may modulate behavioral states with different circuit dynamics.

Long Distance Relationships; enhancer-promoter synergy in noxious induction of substance-P expression in sensory neurones.

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Disease is often associated with changes in cell specific gene expression that contribute to the pathology. For example, changes in the expression of substance P (SP) in sensory neurones following noxious stimulation is associated with neuropathic inflammation. In order to define the mechanisms that support expression of SP in sensory neurones we used comparative genomics to identify an enhancer that is active in SP expressing sensory neurones. We provide evidence that this enhancer responds to noxious stimulation by driving gene expression in large diameter DRG neurones via an NK1/p38MAPK pathway. Significantly, these inducible properties are only revealed in the presence of the endogenous TAC1 promoter. The identification of the involvement of enhancer-promoter synergy in the tissue specific regulation of a gene implicated in a human pathology is unique. In addition, the identification of the mechanisms that support this enhancer raises the possibility of the development of novel drug therapies for neuropathic inflammation.

Session 3 PAIN

Pain project management: Considering migraine, neuropathic pain and epilepsy.

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Pharmnovo will create new and superior drugs to treat patients with migraine based on a specific knowledge of drug design, neuropeptide receptors and CGRP receptor interaction mechanisms. In our drug discovery toolbox, we focus on GPCR interactions that can give us 1/ prolonged treatment effect duration, 2/ a well tolerated treatment and 3/ a widened therapeutic window.

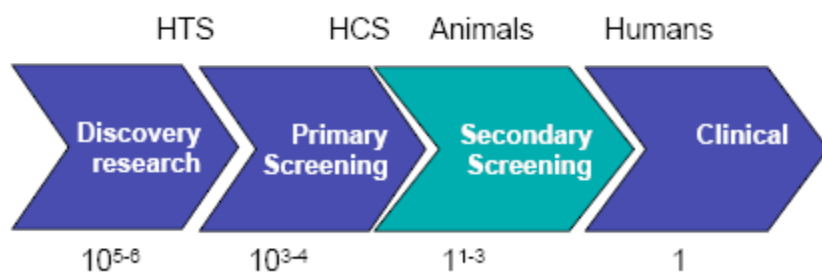
A common approach to migraine and epilepsy can be that both diseases share the same pathophysiology or genetic basis, recent data have shown that as much as 20% of those that have epilepsy also have migraine syndromes. Epilepsy has a prevalence of 0.5 -1% of the population, with almost half of the patients being resistant to current epilepsy treatment. Some epileptic drugs are also used for neuropathic pain. Recent studies have shown that the early phase of migraine pain include neuroimmune interactions with effects on cranial blood vessels and on dura mast cells, both features associated with neuropathic pain.

The present investigation will deal with how Pharmnovo focus on migraine, but discuss also how we like to investigate neuropathic pain and epilepsy from the basis that these disease areas can have common etiology.

From a company perspective we focus early on in vivo studies and on occupancy of receptors in specific brain regions in order to improve our understanding of the therapeutic functionality of compounds.

***In vitro* neurodegenerative models used in a functional setting, *in vivo* linkage and CNS effect. Lars Sundstrom, Capsant Neurotechnologies LTD. Unit 2, Winchester Hill, Romsey U.K. SO51 7UT, U.K.**

One major issue for pharmaceutical research is the linkage between in-vitro screening systems and animal models that are used to gain confidence in potency at specific targets in a biological context, and in determining efficacy and mode of action of new compounds destined for pre-clinical development. Capsant Neurotechnologies has developed several new in-vitro model systems that allow the functional screening of neuroactive molecules in formats that are compatible with secondary drug screening and that allows the profiling of new compounds and the selection of new drug candidates based on measures of function rather than target affinity.



The data presented will explore 3 in-vitro organotypic culture systems maintained at the air liquid interface; models based on cultured organotypic brain slices, reconstituted primary tissues and tissues differentiated in-vitro from stem cells as new model systems to study neurodegenerative and neuroregenerative mechanisms. In addition, data will be presented outlining medium-throughput parallel electrophysiology systems for using functional effects as an end point for candidate selection. Typically these new model systems will be used strategically to link primary high throughput screening systems and in-vivo data prior to clinical candidate selection. These systems also have particular advantages in mode of action studies for drug candidates that may be beneficial in circumstances where chronic or repeated exposures to a molecule is required for studying its effects, as is often the case with neuropeptides.

1. Sundstrom L, Morrison B 3rd, Bradley M, Pringle A. Organotypic cultures as tools for functional screening in the CNS. *Drug Discov Today*. (2005); 10(14):993-1000. Review

2. Sundstrom LE. Thinking inside the box. To cope with an increasing disease burden, drug discovery needs biologically relevant and predictive testing systems. EMBO Rep. (2007); 8:Spec No:S40-3.

Session 4 Pain: Chair Andy Russo

RAMPing up CGRP: A potential animal model for migraine

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The neuropeptide calcitonin gene-related peptide (CGRP) plays a key role in migraine. However, a major challenge for studying migraine is the lack of animal models. We have generated a double transgenic mouse that is sensitized to CGRP as a result of elevated expression of human receptor activity-modifying protein 1 (hRAMP1) in the nervous system. The expression of hRAMP1 is dependent on nestin promoter-driven expression of cre recombinase. RAMP1 is a requisite subunit of the CGRP receptor. It is required for receptor trafficking to the cell surface and subsequent binding of CGRP. Using the nestin/hRAMP1 mice, we measured two associated symptoms of migraine: photophobia and mechanical allodynia. The nestin/hRAMP1 transgenic mice have greater light aversive behavior than littermate controls, which was greatly enhanced by intracerebroventricular administration of CGRP. The CGRP-induced light aversion was prevented by co-administration of the CGRP receptor antagonist BIBN4096BS. The behavior is not simply due to increased anxiety as measured by two anxiolytic assays. Intrathecal injection of CGRP also induced mechanical allodynia, as measured by paw withdrawal to von Frey filaments. These findings suggest that a genetically based sensitivity to CGRP may contribute to migraine susceptibility.

The modulation of neuropeptide signaling by the migraine drug sumatriptan

Andrew H. Ahn

The triptans have anti-migraine properties through their selective activation of a subset of serotonin receptors. In order to identify how one of these receptors contribute to the potentially diverse actions of triptans in the brain, we used a receptor-subtype selective antibody to the serotonin 1D (5-HT_{1D}) receptor, and immunohistochemistry in the rat and mouse brain, and found a dense terminal pattern of immunoreactivity (5HT_{1D}-IR) within the central nucleus of the amygdala (CeA). Double-label immunohistochemistry colocalized these 5HT_{1D}-IR terminals with the peptide neurotransmitter calcitonin-gene related protein (CGRP), but not with the peptide substance P, nor with the serotonin vesicular transporter or tyrosine hydroxylase. Blocking axonal transport of the receptor with intraventricular colchicine revealed a small population of 5HT_{1D}-IR neurons in the subparafascicular, the related parvocellular region of the thalamus, and parabrachial nucleus, which is in agreement the known contribution of CGRP terminals to CeA. We next tested whether sumatriptan administration could alter amygdala function, and indeed found that it shortens the latency to explore the exposed portions of the elevated zero/circle maze. Together with recent physiologic and behavioral evidence that indicates a pain-modulatory role for CGRP in the central amygdala, we hypothesize that sumatriptan modulates amygdala function through the inhibition of CGRP release.

Role of peptides of the galanin family in inflammation

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The galanin peptide system consists of four members: galanin and galanin message-associated peptide (GMAP), which are encoded by the galanin precursor (ppGAL); galanin-like peptide (GALP) and alarin, a splice variant of GALP. Our objectives were to investigate different aspects of the function of the galanin family in innate immunity and inflammation.

I: The substantial expression of ppGAL in keratinocytes of the skin is a feature also specific for antimicrobial peptides, which participate in innate immune response by providing a rapid first line defense against infection. We were able to demonstrate that GMAP possesses growth-inhibiting activity against *Candida albicans*. GMAP inhibits the yeast- to- hyphal transition, which is one of the most important virulence factors of *C. albicans*.

II: In the dermis galanin and alarin like immunoreactivity was detected around blood vessels. Therefore, the vaso-modulatory activity of peptides of the galanin family was studied in a model of substance P and calcitonine-gene related peptide induced plasma- extravasation. Galanin, GALP and alarin exhibited potent and dose-dependent vasoconstrictor and anti-oedema activity in the cutaneous microvasculature via a postsynaptic mechanism. An antagonist specific for GALR3 abolished the anti-oedema activity of galanin, which indicates GALR3 as the receptor subtype mediating the anti-inflammatory effect of this neuropeptide. In GAL-KO mice, development of inflammatory oedema of the skin was impaired.

III: Co- culture of keratinocytes with *C. albicans* resulted in a 2-fold upregulation of ppGAL expression. The 3-fold upregulation of ppGAL expression upon exposure to lipopolysaccharides in keratinocytes but also an intestinal epithelial cell line (T84) indicates that the expression of galanin is modulated during an ongoing microbial infection in different organ systems.

IV: Since sepsis is a systemic inflammatory condition, we investigated the expression of the galanin peptide system in organs of septic animals. ppGAL was massively upregulated (40-fold) in lungs of septic mice but also elevated in other organs. Future studies will reveal if galanin is a counter-regulator of overshooting plasma extravasation in an ongoing sepsis.

There is strong evidence from our data but also the literature that the galanin system is involved at different levels during host defense and inflammation. This starts with the antimicrobial activity of GMAP on fungal growth, continues with anti-oedema effects in the dermis, extends to the immune system where the galanin system can affect different immune cells and also target inflammation of other organ systems.

Supported by a FEBS Short- term- Fellowship and the FWF (P14306).

Diverse signaling and trafficking pathways in neurons, lessons learned from the neurokinin 3 receptor.

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Intracellular trafficking of proteins has shown to have an immense effect on neuronal function. Membrane receptor endocytosis and trafficking can greatly affect signaling via ion channels, second messenger cascades, and gene expression. It is well established that steroid receptors are trafficked into the nuclei of neurons in an activity dependent manner. G-protein coupled receptors (GPCR) can also follow this nuclear transport pathway in an activity dependent manner. Some GPCRs have been shown to be incorporated into the nuclear membrane to affect ion movement, cellular responses, and gene expression. Our understanding of how receptors move from the plasma membrane to cytoplasm to nucleus is limited at best. There is information about the possible proteins involved in the trafficking pathway there is little information about which of these transport molecules are present in neurons. Importins (Karyopherins) have been shown to be involved in the nuclear transport of various proteins, like steroid receptors and transcription factors, which contain a nuclear localization sequence (NLS). Although the importin pathway is an established protein trafficking pathway there is little evidence demonstrating its function *in vivo*. Previously we demonstrated using confocal microscopy, immuno-electron microscopy, and Western analysis that the Neurokinin 3 receptor (NK3R) is trafficked to the nucleus of hypothalamic neurons in an activity dependent manner. Here we questioned if importins are involved in the intranuclear trafficking of the NK3R. Co-immunoprecipitation results identified that NK3R associates with importin beta 1 in hypothalamic tissue. These are the first data to establish that NK3R is transported across the nuclear membrane by the importin pathway and raises the possibility that other GPCRs containing a NLS may be similarly transported to the cell nucleus to exert genomic actions. (Supported by P20 RR15640 and NS 57823 to FWF.)

Session 5 **Neuropeptidergic Systems in Psychiatric Disease, Chair Robert Ring**

Novel Roles for VGF in Psychiatric Disorders

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Our recent studies have demonstrated a potential role for the neuropeptide VGF (non-acronymic) in the pathogenesis of depression. We have previously shown that VGF expression is induced by factors that have antidepressant activity including brain-derived neurotrophic factor (BDNF), serotonin (5-HT) and imipramine. Conversely, VGF was downregulated in the hippocampus in the learned helplessness and forced swim test (FST) models of depression. Along with others, we have demonstrated that VGF infusion in the hippocampus of mice subjected to FST reduced the time spent immobile for up to 6 days, thus demonstrating a novel role for VGF as an antidepressant-like agent. VGF infusion also reduced the latency to consume in the novelty induced hypophagia paradigm suggesting that VGF is effective in models of depression which only respond to chronic antidepressant treatment and have a time course consistent with that of neurogenesis. Our studies have also shown that VGF enhances neurogenesis as well as synaptic activity in the hippocampus, which may mediate VGF's antidepressant-like effects. We have begun to examine the expression of VGF in affective disorder patients. Postmortem tissue sections from the mid-hippocampus as well as the prefrontal cortex were obtained from the Neuropathology Consortium of the Stanley Brain Collection consisting of coded samples from control, depression, bipolar and schizophrenia patients and subjected to in situ hybridization. A trend toward decreased expression of VGF mRNA in the CA regions of the hippocampus was detected in all diagnoses with significantly reduced expression in bipolar disorder. When the data was analyzed by sub-region within the hippocampus, CA2 showed significantly reduced expression for bipolar compared to controls. Cellular analysis revealed a reduction in the number of silver grains per cell but not in the number of cells expressing VGF mRNA. No change in expression in BA9 of the prefrontal cortex was detected but analysis of BA46 is currently underway. The effect of VGF in a model of mania indicates that VGF infusion into the hippocampus can reduce amphetamine-induced hyperlocomotion. Taken together, our findings suggest a role for the neuropeptide VGF in the pathogenesis of affective disorders and its potential usefulness as a mood stabilizer.

Neuropeptide S receptor expression and regulation of neurotransmitter release indicates a potential role in learning and memory

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Central administration of Neuropeptide S (NPS) has been reported to promote arousal and anxiolysis, effects hypothesized to be mediated by the NPS receptor. The distribution of the NPS receptor protein has not yet been described but could ultimately provide valuable information in further elucidating the precise function of the NPS system. We determined the distribution of the NPS receptor with a polyclonal antibody directed against the second extracellular loop of the NPS receptor protein. Consistent with the reported mRNA distribution, NPS receptor was localized to the rat subiculum, ventral hippocampus, piriform cortex, medial amygdala, dorsal raphe, and several hypothalamic and thalamic nuclei. Based on this distribution, we investigated NPS receptor regulation of neurotransmitter release in the rat dorsal raphe nuclei and hippocampal formation. NPS (100 nM, 1 μ M) was infused directly through a microdialysis probe while extracellular concentrations of dopamine, serotonin, norepinephrine (DRN) or acetylcholine (subiculum) were measured. NPS preferentially increased dopamine levels in the DRN by approximately 2-fold, an effect consistent with the reported arousal-promoting properties of this peptide. In the subiculum and ventral hippocampal area, NPS dose-dependently increased extracellular levels of acetylcholine without affecting concentrations of amino acids. Overall, these data confirm the presence of NPS receptors in brain regions involved in regulating the stress response as well as learning and memory. Furthermore, these preclinical results provide the first neurochemical investigation of the NPS system and may ultimately be useful in providing valuable insight into the regulation of classical neurotransmitter systems by the NPS receptor.

Delineating the important pathways for neuropeptide expression associated with psychiatric disorders

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Susceptibility to psychiatric disorders can occur by polymorphism within transcriptional regulatory domains of many neurotransmitter genes. We are complementing bioinformatics and association studies with biochemical and functional analysis to determine regions that are both involved in tissue specific and stimulus regulated neurotransmitter gene expression with important correlates of clinical importance, drug response and predisposition to disease. We are currently applying such technologies to genome wide association data on depression. Plans are to extend to addiction, epilepsy and motor neurone disease

We are characterising two classes of domain 1) the most evolutionary conserved regions (ECRs) which are not in exons and 2) subclasses of microsatellite DNA (variable number tandem repeats – VNTRs). The VNTR is obviously polymorphic, however many ECRs also contain variants, such as SNPs, that could change the function of that domain. By concentrating on genetic variation in functional regions of the genome it will greatly accelerate the association of SNPs and VNTRs within these regions with susceptibility to various conditions and the development of more effective and personalised drug therapies. We are currently addressing ECRs in a number of neuropeptide genes including VIP and TAC1.

Many of the ECRs and VNTRs are responsive to drug exposure such as lithium, cocaine and amphetamine, thus they are also a target for pharmacogenetic studies. Interesting our data indicates differential epigenetic profiles over promoters depending on the genotype in response to drug exposure which could modulate medium to long term gene expression profiles.

We will discuss our data to demonstrate how a more global analysis of VNTR and ECR variation correlated with disease predisposition would be a step to understanding the integrated cellular response to a specific challenge or drug.

Placental Corticotropin Releasing hormone concentrations during gestation and structural brain differences in 6-9 year-old children

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A significant proportion of variation in health outcomes and disease risk is attributable to developmental processes during fetal life in response to various environmental, social, psychological, physiological and genetic influences. Programming refers to the action of a factor during a sensitive period affecting the development and organization of specific organs. Elevations in stress sensitive hormones, such as placental corticotropin-releasing hormone (pCRH), are believed to have the potential to “program” fetal development.

In the current prospective longitudinal study pCRH concentrations in plasma have been assessed repeatedly during pregnancy in 58 women, whose children’s neurodevelopmental stage was assessed by a structural MRI scan that was performed between six to nine years of age. With the application of voxel based morphometry analyses, specific brain areas could be identified where gray matter density was significantly associated with pCRH concentrations during pregnancy after controlling for children’s age, sex and gestational age at birth. Interestingly, variation in gray matter density in the same brain areas were found to be related to cognitive function suggesting functional implications of those structural changes.

This is the first human study to show that stress hormones during gestation are related to structural brain differences in the developing individual and therefore support the programming hypothesis.

Session 6 Adult Neurogenesis and Stem Cells , Chair William Gray

Differential modulation of hippocampal neurogenesis by NPY and VIP

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We have shown that neuropeptide Y (NPY), a peptide neurotransmitter released by hippocampal interneurons, is proliferative via the Y1 receptor, for neural stem / progenitor cells (NSPCs) in the normal dentate gyrus and in the subventricular and subcallosal stem cell niches around the hippocampus. We have recently found that another neuropeptide VIP has both trophic and fate determining effects for dentate NSPCs. In this presentation I will review the roles of NPY and VIP in the neural stem cell niche and will describe novel and important interactions between these neuropeptides.

NPY is a proneurogenic peptide in the subventricular zone - A single cell calcium imaging approach

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Neural stem cells residing in neurogenic niches of the adult brain represent a potential source of new neural cells to treat brain diseases. Particularly, the robust neurogenic activity of the subventricular zone, and the peculiarities of neuroblast migration in the rodent brain, contribute to elect this niche as a key brain structure in the study of adult neurogenesis.

However, the scientific community dedicated to the study of neurogenesis, and factors involved in its regulation, face a major problem related with the lack of good methods to evaluate the functional and pharmacological properties of differentiating cells. We took advantage of single cell calcium imaging technology to develop a novel and unique procedure that allows us to identify living and functional properties of cells differentiating from neural stem cell cultures. Using this principle, we are able to identify a variety of functional cells based on particularities of functional responses to a protocol of stimulation. In 15 minutes we are able to identify, simultaneously, immature cells, progenitors, astrocytes, neurons and oligodendrocytes. Because cells are identified on the basis of function, we are also able to characterize the pharmacological properties of differentiating cells, and to screen and identify new proneurogenic and pro-oligodendrogenic factors.

By using a complementary approach combining immunocytochemistry and functional responses of neural stem cells cultures, we were able to identify new factors able to increase the differentiation of new neurons from these cultures. Among these factors, neuropeptide Y (NPY) is particularly attractive since this peptide was shown to be particularly up-regulated following epileptic seizures and is a potent neuroprotective peptide. Moreover, we were able to perform a detailed pharmacological characterization of NPY as a proneurogenic factor, involving activation of NPY Y1 receptors. Furthermore, we also show that the proneurogenic effect of NPY involves activation of JNK pathway and axonogenesis.

The identification of new proneurogenic peptides, and the dissection of the intracellular signalling mechanisms involved in neurogenic fate, will foster the scientific community to provide new strategies for brain repair. For instance, it is possible to stimulate the neural stem cultures to differentiate into neuronal phenotype before grafting in brain disease models or, alternatively, to identify new drugs, and their molecular targets, as key elements to take in consideration in developing new pharmaceutical strategies for self-brain repair.

Acknowledgments: We are grateful to the Fundação para a Ciência e a Tecnologia by supporting the research project PTDC/SAU-NEU/68465/2006.

Neuropeptide Y stimulates proliferation, migration and differentiation of neural precursors from the subventricular zone in adult mice: Potential therapeutic application for Huntington's disease.

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Abstract

The neuropeptide Y (NPY) is widely expressed in the central nervous system and has been shown to stimulate neurogenesis in the hippocampus and the olfactory epithelium. Here, we demonstrate that intracerebroventricular injection of NPY stimulates proliferation of neural stem cells in the mice subventricular zone (SVZ), one the most neurogenic areas of the brain. Newly generated neuroblasts migrate through the rostral migratory stream to the olfactory bulb and also directly to the striatum, as evidenced by BrdU labeling and cell phenotyping. Using knock-out mice, specific NPY receptor agonists and antagonists, we report that this neuroproliferative effect is mediated by the Y1 receptor subtype that we found to be highly expressed in the SVZ both at the mRNA and protein levels. Our data suggest that stimulating endogenous SVZ neural stem cells by NPY may be of a potential interest in cell replacement based therapies of neurodegenerative diseases affecting the striatum such as Huntington's disease.

The NPY system and neurogenesis in the olfactory epithelium: Insights from knockout models

Kharen Doyle, Yvonne Hort & Herbert Herzog, Neuroscience Program, Garvan Institute of Medical Research, Sydney, NSW 2010, AUSTRALIA

While the regenerative capacity of the olfactory neuroepithelium has been well studied less is known about the molecular events controlling precursor cell activity. Neuropeptide Y (NPY) is expressed at high levels in the olfactory system, and NPY has been shown to play a role in neuroregeneration of the brain. In our studies, we show that the numbers of olfactory neurospheres derived from NPY, NPY/peptide YY, and Y1 receptor knockout mice are decreased compared with wild type (WT) controls. Furthermore, flow cytometric analysis of isolated horizontal basal cells, globose basal cells, and glandular cells showed that only glandular cells derived from WT mice, but not from NPY and Y1 receptor knockout mice, formed secondary neurospheres suggesting a critical role for NPY signaling in this process. Interestingly, olfactory function tests revealed that olfaction in Y1 knockout mice is impaired compared with those of WT mice, probably because of the reduced number of olfactory neurons formed. Together these results indicate that NPY and the Y1 receptor are required for the normal proliferation of adult olfactory precursors and olfactory function.

Epilepsy and Epileptogenesis

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Epilepsy is the commonest serious neurological disorder. It frequently develops following a specific brain insult (such as prolonged seizure, head injury stroke). The process that leads to the development of epilepsy (the propensity to have recurrent, spontaneous seizures) is termed epileptogenesis and this is distinct from the process of ictogenesis (the generation of seizures). During epileptogenesis, brain excitability increases due to molecular, cellular and network alterations. These changes are thought to be initiated by one or more brain “insults” which may be naturally occurring events such as traumatic brain injury, but can also be modelled in animals, using insults such as chemically or electrically induced status epilepticus.

Here I will describe work in which we have shown alterations to neuronal excitability, GABAergic inhibition and glutamatergic transmission following brain insults (status epilepticus and traumatic brain injury) which may all promote network hyperexcitability. Alterations in peptide expression have also been described. Our previous work has demonstrated changes in the expression of REST and REST4 following a prolonged seizure (status epilepticus) which may promote not only changes in peptide expression, but also some of the other cellular alterations associated with epileptogenesis. We now show that REST and REST4 expression can be modified by treatment with sodium valproate (an inhibitor of histone deacetylation), but interestingly valproate does not prevent epileptogenesis. This work suggests that the epileptogenic process consists of complex temporal alterations to neurons, neurotransmission and network connectivity and that the expression of epilepsy is not secondary to one of these but may result from the accumulation of changes that lead to the hyperexcitable state.

Roles of the PAC1 receptor in mouse neurogenesis.

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The pituitary adenylate cyclase activating polypeptide (PACAP) and its high affinity receptor PAC1 are highly expressed in the central nervous system during embryonic development and in adulthood. We have investigated the role of PAC1 receptor in neurogenesis, through the gain of function approach with a 130 kb transgene encoding the human PAC1 receptor. Transgenic mice are shown to develop transgene dose-dependent neuroanatomical abnormalities including enlarged ventricles, reduced cerebral cortex and corpus callosum which are shared by the neuropathology of hydrocephalus and schizophrenia. Mechanistically, neuronal proliferation is significantly reduced and neuronal apoptosis is massively increased in addition to elevated PKA/PKC levels. Furthermore, the retinal development and visual acuity are compromised in transgenic mice. These data demonstrate that appropriate levels of PAC1 expression are crucial for embryonic neurogenesis and retinogenesis.

Session 7 Stem Cells, Chair Pranela Rameshwar

The hematopoietic stem cell activity of Sar⁹, MetO₂¹¹-Substance P contributes to effective treatments for diverse areas including: radiation exposure, infectious disease, adjuvant activity and wound healing

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The tachykinin analogue Sar⁹, MetO₂¹¹-Substance P specifically targets and activates the neurokinin-1 receptor. In early studies under ImmuneRegen, the peptide (termed Homspera®) increased survival rates of lethally irradiated C57/B6 mice when treated once daily with Homspera after radiation exposure. We believe this increase in survival may be due to the effects of Homspera on the hematopoietic system, which subsequently lead to enhanced immune system function. Studies using human bone marrow aspirate have confirmed the ability of Homspera to target and stimulate hematopoietic stem cells. Homspera increases colony formation of precursor cells to all three lineages in the blood as demonstrated by *in vitro* colony forming assays. Specifically, Homspera targets the multi-lineage progenitor CFU-GEMM as well as more mature progenitors including CFU-GM, CFU-Mk and CFU-E and stimulates cell formation 2-3 fold above controls. Additionally, the stimulatory activity of Homspera is greater than that of Substance P and this may be a result of Homspera's increased resistance to proteolytic degradation. We are currently investigating the mechanism of action for Homspera in an effort to better understand its potential role as a therapeutic for strengthening the immune system. We believe the ability of Homspera to stimulate hematopoietic stem cells contributes to the efficacy observed in other areas such as the anti-inflammatory activity of Homspera in the lungs following influenza infection and the ability of Homspera to accelerate wound healing, amongst others.

Neuropeptide-mediated effects on the prototypical hematopoietic stem cells: Suddenly in the background

Pranela Rameshwar

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The etiology of hematological disorders and the role of neuropeptides have been studied at the cellular and molecular levels. These studies have provided insights on the pathophysiology of myeloproliferative disorders. Cells of interest are resident bone marrow mesenchymal stem cells (MSC). These stem cells surround the abluminal areas of blood vessels and trabeculae of humans. MSCs form contact with innervating fibers of bone marrow where the released neurotransmitters can interact with specific receptors on MSCs. Myelofibrosis could occur secondarily to myeloproliferative disorder. Since the neuropeptide/neurotransmitter substance P (SP) has been linked to the pathophysiology of myelofibrosis, we hypothesize that SP functions in conjunction with the resident MSCs in the pathophysiology of myeloproliferative disorders and then predispose the patients for secondary bone marrow fibrosis. MSCs from healthy subjects express MHC-II, making them antigen presenting cells (APC) in bone marrow. In contrast, MSCs from patients with myeloproliferative disorders show decreased MHC-II expressions with of their ability to elicit allo-responses and to exert immune suppressor functions, as third party cells. Retrospective analyses of bone marrow biopsies showed increases in MSCs in patients with fibrosis and SP. While it is unclear of the source of SP, these results indicate dysfunctions of MSCs from patients with hematological disorders and increases in the neuropeptide, SP. These findings open avenues to address a lingering question on the etiology of hematological disorders. The findings have clinical implications for future therapies on bone marrow- related diseases to combine stem cell therapy and targeting of neuropeptide receptors.

NFκB: A Dictator of Cell Differentiation in Stem Cell Therapy

Cecile King

Human adult mesenchymal stem cells (hMSCs) are pluripotent and are crucial in the mediation of the human inflammatory response. MSCs are primarily found in the adult bone marrow (BM), are of mesodermal origin and can differentiate into lineage specific cells of the connective tissue, fat, stroma, cartilage, and bone. hMSCs are capable of preserving, producing and restoring terminally differentiated cells of their lineage as a result of physiologic cell turnover or tissue damage due to injury. This characteristic enhanced the current appreciation of the vast therapeutic potential of hMSCs. Thus far, MSCs have demonstrated therapeutic use in diseases such as: coronary artery disease, spinal cord injury, Parkinson's disease and liver regeneration. Scientists must however, fully understand the underlying mechanism by which these cells sustain their pluripotency.

Although few genes, such as *Oct4*, *Nanog* and *Sox2* have been linked to pluripotency, it is imperative to identify the master regulatory gene of pluripotency in these cells. More specifically, researchers must fully understand how to manipulate the microenvironment surrounding the site of injury to maintain the curative potential of these cells *in vivo*. NFκB is a family of transcription factors found abundantly in sites of injury and known to be important regulators of inflammatory responses. Various stimuli such as cytokines, stress and pathogens have been shown to elicit NFκB regulated immune responses or more specifically, the activation of various genes involved in the inflammatory response. The NFκB family shares Rel homology and is inhibited by inhibitory kappa B (IκB) proteins. Inflammatory mediators such as NFκB and others at regions of tissue injury affect the development of human MSCs. It is hypothesized that NFκB maintains pluripotency in MSCs by regulating the expression of *Oct4*, *Nanog* and *Sox-2* and may ultimately be the master regulator of pluripotency in these cells.

Session 8 Hypothalamic Peptides, Chair Luis de Lecea

The role of the vasopressin V1b receptor in the HPA axis response to acute stress: molecular and pharmacological studies

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The neurohypophyseal hormone arginine vasopressin (Avp) is the hormonal regulator of water resorption in the kidneys and moderates vascular smooth muscle tone. Avp also has many central effects on behaviour and acts as an adrenocorticotrophic hormone (ACTH) secretagogue in the hypothalamic-pituitary adrenal (HPA) axis. These actions are mediated through a family of G-protein coupled receptors: the V1a receptor controls vasoconstriction in peripheral vasculature and has many putative roles in the brain; the V2 receptor regulates renal collecting duct permeability via aquaporin 2 membrane insertion; and the V1b receptor (V1bR), predominantly located in anterior pituitary corticotropes, stimulates the release of ACTH into the circulation.

In response to diverse homeostatic challenges, corticotropin-releasing factor (Crf) and Avp from parvocellular paraventricular origin synergistically act to promote the release of ACTH from the anterior pituitary leading to corticosteroid release from the adrenal cortex. Although Crf is seen as the dominant ACTH secretagogue, Avp is preferentially released in response to some stressors (e.g. insulin induced hypoglycaemia) and may have an important role in chronic stress situations.

To further investigate the role of the V1bR in the HPA axis response to acute stress, we measured the HPA axis response to acute restraint and shaker stress in adult mice lacking a functional V1bR and wildtype controls after pre-treatment with a recently described antagonist specific for the V1bR. Using this knockout mouse model we have shown that a functional V1bR is necessary for a normal ACTH but not corticosterone (CORT) response to acute restraint (30 minutes) and shaker (10 minutes) stress when compared to wildtype groups. Administration of antagonist 2 hours prior to the stress reduced plasma ACTH levels after both restraint and shaker stress in wildtype animals to levels comparable with KO groups showing effective blockade of the V1bR. This, together with our previous studies, demonstrates that loss or blockade of the V1bR is not compensated for by other ACTH secretagogues confirming AVP's vital role in the modulation of the HPA axis response to stress.

Our studies reveal a dissociation between plasma ACTH and CORT following acute restraint and shaker stress. It is clear that the nature and/or severity of the stress is a critical consideration when interpreting the HPA axis response to stress as factors that regulate the CORT response to stress may be dynamically influenced by different types of stressor.

These results suggest that the V1bR plays an important role in the HPA axis response to acute restraint and shaker stress and the development of V1bR antagonists will prove useful in further studies of this receptor.

GPR30 a novel G protein-coupled oestrogen receptor is present in the hypothalamic paraventricular and supraoptic nuclei, and multiple peripheral tissues.

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Oxytocin (OT) and vasopressin (VP) are produced predominantly by the magnocellular neurosecretory cells of the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) and are released into systemic circulation from neurones terminating in the posterior pituitary. The primary functions of OT and VP are to contract reproductive tract smooth muscle and increase renal water reabsorption, respectively. Oestrogen (17 β -oestradiol or E2) has been reported to up-regulate OT and VP mRNA expression and modulate OT and VP release *in vivo*. E2 effects are typically mediated through two structurally related oestrogen receptors alpha and beta (ER α and ER β) that function as ligand-activated transcription factors. On activation ERs can either bind directly to their target DNA sequences in the nucleus or interact with other nuclear proteins to alter gene activation, and this genomic action occurs slowly (hours-days). However it is well documented that E2 also has fast non-genomic effects and rapidly stimulates intrahypothalamic and peripheral OT and VP release. These could be mediated by extranuclear ERs or by non-classical oestrogen receptors.

Recently the G protein-coupled receptor (GPCR) GPR30 was shown to specifically bind E2 with low nanomolar affinity and evoke rapid effects; GPR30 stimulates Ca²⁺ mobilisation from intracellular stores directly or via epidermal growth factor receptor (EGFR) transactivation, induces *c-fos* expression, stimulates adenylyl cyclase and cAMP mediated signalling and activates ERK-1/2 in a variety of cell types. The development of GPR30 knockout mice has indicated that GPR30 mediates E2 stimulated insulin release in females, has a role in cardiovascular homeostasis and bone development, but is not essential for reproduction.

To date a full characterisation of GPR30's anatomical distribution has not been determined. To further understand the possible functions of GPR30 we explored its central and peripheral distribution in rodent tissues using *in situ* hybridisation histochemistry with GPR30-specific riboprobes and immunohistochemistry with a GPR30 antibody. In the CNS GPR30 is abundantly expressed in the hypothalamus, particularly in the PVN and SON where GPR30 is present in OT and VP neurones. In the periphery highest GPR30 expression is observed in all three lobes of the pituitary, adrenal medulla, renal pelvis and ovary.

The localisation of GPR30 within OT and VP neurones and its expression in the pituitary provides a likely means by which E2 can modulate fast non-genomic effects on these specific neuropeptide systems in the rodent. GPR30's peripheral distribution also suggests other roles for this novel E2 receptor.

GnRH receptors outside the hypothalamo-pituitary-reproductive axis

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GnRH is a hypothalamic decapeptide with an established role as a primary regulator of gonadal function. It exerts this regulation by controlling the release of the pituitary gland gonadotropins. While this role for GnRH is undisputed, it is increasingly apparent that GnRH may have a variety of other vital roles in normal physiology. This has become evident from work implicating actions of GnRH in an array of non-pituitary tissues. Initial studies have identified potential roles for GnRH within the brain by using immunohistochemistry to label the specific areas expressing GnRH receptors.

The GnRH receptor was expressed in a variety of regions including the cerebral cortex, cerebellum, hippocampus, amygdala, olfactory system, preoptic region, lateral septum and hypothalamus. GnRH has been shown to affect GnRH neurons and we confirmed the presence of GnRH receptors in these neurons. Specific GnRH receptor expression on cerebellar Purkinje neurons raises the possibility that persons with Gordon Holmes' syndrome (cerebellar ataxia and hypogonadism) may suffer defects in the GnRH receptor signal transduction pathway. GnRH receptor expression in the mesencephalic central gray was consistent with research showing that GnRH can affect sexual behavior in rodents. However, several studies on ungulates could not confirm this potential role for GnRH. Through intracerebroventricular GnRH administration, sexual behavior was induced in ewes. In addition, we demonstrated that very high dose of intravenous GnRH were required to elevate CSF GnRH concentrations. GnRH receptor expression in neural regions vital to thermoregulation, led us to determine if GnRH could affect skin temperature change. After designing a system in sheep through which peripheral skin temperature changes at the ears and face could be induced by lipopolysaccharides, intravenous bolus injections of GnRH were administered. No change in peripheral skin temperature was evoked.

In addition to brain expression, we detected GnRH receptor expression in several peripheral tissues, including the liver, kidney and heart. Indeed, these data are in keeping with earlier studies investigating the binding of GnRH agonist/antagonist binding but it is noteworthy the little or no discussion of these findings can be found in the literature. Our preliminary investigations have shown that even at very low concentrations GnRH can potently affect cardiomyocyte contractility. Moreover, we have found that the heart is capable of synthesizing GnRH, leading to the novel hypothesis that this non-reproductive organ may be a self-contained GnRH producing and responsive system.

Clearly GnRH has effects that are not involved in reproductive physiology and it may be that our naming of this decapeptide to its first discovered role may have promoted some scientific myopia. Indeed, is it time to rethink the name?

Neuropeptides signaling in hypothalamic neurons.Mark A Smith^{a,b}, Dominic J Withers^a, Michael LJ Ashford^b^aCentre for Diabetes and Endocrinology, Rayne Institute, University College London, London, UK.^bBiomedical Research Institute, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK.

Obesity leads to a number of associated disorders that can severely reduce the quality of life and lower life expectancy. There are powerful signals in the body that maintain body weight in which the peripheral hormones insulin and leptin play a pivotal role. However, insulin and leptin levels are elevated in obese individuals which may suggest that these hormones are dysfunctional. Two major types of neuronal population that are targeted by insulin and leptin are located in the arcuate nucleus of the mediobasal hypothalamus. One population makes and releases weight reducing neuropeptides (pro-opiomelanocortin, POMC) whereas the other population expresses and releases neuropeptides that promote weight gain (agouti related peptide and neuropeptide Y, AgRP and NPY respectively). Together these two populations form the melanocortin pathway and dysfunction of this system profoundly disrupts metabolism and satiety in rodents and humans alike¹. Insulin and leptin modulate the activity of POMC- or AgRP/NPY- containing neurons by modulating the amount of neuropeptide expressed and released by these cells. The type of response (i.e. inhibition or stimulation) that leptin and insulin induces differs depending on the population type. In studies from our laboratories and others, we have shown that insulin inhibits or hyperpolarizes and leptin excites or depolarizes POMC neurons. In contrast, under our recording conditions, we have shown that insulin depolarizes AgRP neurons and leptin is generally without effect^{2,3}.

This presentation will describe electrophysiological data from AgRP and POMC neurons in which the catalytic subunit for AMP-dependent kinase (AMPK) has been specifically deleted and phosphatidylinositol 3-kinase (PI3-kinase) has been pharmacologically inhibited. These data suggest that AMPK is not essential to the actions of insulin and leptin in these neurons but allow these neurons to acutely sense changes in extracellular glucose. However, inhibition of PI3-kinase prevents both the depolarizing and hyperpolarizing actions of insulin and leptin. These data thus suggest that there are alternative divergent signalling pathways downstream of PI3-kinase activity that underscores the opposing excitable properties of insulin and leptin on these neurons.

1. Cone RD (2005) *Nat. Neurosci.* 8:571-578
2. Choudhury AI et al (2005) *J. Clin. Invest.* 115:940-950
3. Claret M et al (2007) *J. Clin. Invest.* 117:2325-2336

The role of neuropeptides in the control of energy intake and energy expenditure

Margriet Veldhorst

The increasing prevalence of overweight and obesity is a major public health concern. Overweight and obesity are the result of energy intake exceeding energy expenditure. In the context of research on prevention and treatment of overweight and obesity, relatively high protein diets have come into focus as having the potential to act on the different metabolic targets regulating body weight, including increased postprandial and postabsorptive satiety and increased energy expenditure. Several neuropeptides, *e.g.* Glucagon-like peptide-1 (GLP-1), cholecystokinin, peptide YY, insulin, ghrelin, and leptin, have been shown to play a role in energy intake and/or in energy expenditure.

In a series of experiments, effects on changes in concentrations of peptides and changes in satiety were measured after consumption of different types of protein in either a normal or a high concentration (10 and 25% of energy from protein, respectively). Concentrations of GLP-1 and insulin were increased more and concentrations of ghrelin were decreased more ($p < 0.05$) after a breakfast with 25 En% whey-protein than after a breakfast with 10 En% whey-protein, whereas changes in satiety were increased more after 10 En% whey-protein than after 25 En% whey-protein ($p < 0.05$) (1). Satiety was more increased after a breakfast with 25 En% casein-protein than after 10 En% casein-protein, whereas insulin concentration was more increased after the 10 En% casein-breakfast and there were no differences in concentrations of GLP-1 or ghrelin (2). In the comparison of breakfasts with 25 En% from casein-, soy-, or whey-protein, whey-protein triggered strongest responses in concentrations of GLP-1 and insulin compared with casein and soy ($p < 0.05$), however, there were no differences in satiety ratings (3).

Neuropeptides also have been shown to be able to influence energy expenditure. Effects of a high (25 En%) versus a normal (10 En%) gelatin-protein diet on 24h energy expenditure and the role of several neuropeptides have been studied in 24 healthy subjects. Total energy expenditure was higher when subjects were on the 25 En% gelatin diet compared with the 10 En% gelatin diet ($p < 0.05$), however, there were no differences in concentrations of GLP-1, PYY, and ghrelin (4).

In conclusion, with regard a specific type of protein in a meal or diet, a direct relationship between changes in peptides like GLP-1, PYY, insulin or ghrelin and changes in satiety or energy expenditure was not present. Neuropeptides may support satiety or energy expenditure, but are not mathematically related to energy intake or energy expenditure.

1. Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, et al. Effects of complete whey-protein breakfasts versus whey without GMP-breakfasts on energy intake and satiety. in press, *Appetite*.
2. Veldhorst MA, Nieuwenhuizen AG, Hochstenbach-Waelen A, et al. Comparison of the effects of a high- and normal-casein breakfast on satiety, 'satiety' hormones, plasma amino acids and subsequent energy intake. *Br J Nutr* 2009;101:295-303.
3. Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A, et al. Dose-dependent satiating effect of whey relative to casein or soy. in press, *Physiology&Behavior*.
4. Hochstenbach-Waelen A, Veldhorst MAB, Nieuwenhuizen AG, Westerterp-Plantenga MS, Westerterp KR. Effects of gelatin in a 25 vs 10 energy% single protein diet on energy expenditure, substrate balances and appetite. submitted 2008.

PYY and obesity

Shu Lin, Lei Zhang, Amanda Sainsbury & Herbert Herzog, Neuroscience Program, Garvan Institute of Medical Research, Sydney, NSW 2010, AUSTRALIA

The gut-derived hormone peptide YY (PYY) is most commonly known for its effect on satiety, decreasing food intake and body weight in animals and humans. However, PYY is also involved in a wide range of digestive functions including regulating insulin secretion and glucose homeostasis. Obese people exhibit reduced circulating PYY levels, but it is unclear whether this is a consequence or cause of obesity. PYY knock-out significantly increases bodyweight and increased fat mass by 50% in aged females on a normal diet. Male chow-fed PYY^{-/-} mice are resistant to obesity but become significantly fatter and glucose-intolerant compared with wild-types when fed a high-fat diet. PYY^{-/-} animals exhibit significantly elevated fasting or glucose-stimulated serum insulin concentrations vs wild-types, with no increase in basal or fasting-induced food intake. Male but not female knock-outs exhibited significantly increased growth hormone-releasing hormone expression in the ventromedial hypothalamus and significantly elevated serum IGF-I and testosterone levels. On the other hand in PYY transgenic mice increased PYY levels protect against diet-induced obesity in association with increased thermogenesis and sustained expression of thyrotropin-releasing hormone. Moreover, PYY over-expression in genetically obese (*ob/ob*) mice decreases weight gain and adiposity, reduces hyperlipidemia and improves glucose tolerance. PYY over-expression also results in low circulating IGF-1. Together, these findings suggest that long-term administration of PYY, PYY-like compounds or agents that stimulate PYY synthesis *in vivo* may be able to reduce the excess adiposity associated with obesity but also highlight a role of PYY in the negative regulation of circulating IGF-1 levels.

Neuropeptides and the gut in obesity

Mark Berner Hansen

The signaling systems underlying appetite, eating behavior control and obesity are complex. This talk will focus on gastrointestinal (GI) signaling systems as physiological key functions for metabolic and appetite control and gut-related interventions, that are or might be effective in the treatment of obesity. Many of the peptides and hormones, that are involved in the regulation of food intake in the brain are also found in the enteric nervous system and enteroendocrine cells of the mucosa of the GI tract. The only identified hunger-driving signal from the GI tract is ghrelin, which is mainly found in the mucosa of the stomach. Satiety signals from the GI tract act through the brain stem, where neuronal networks directly linked to hypothalamic centers for food intake and eating behavior are activated. Pros and cons for targeting the gut (e.g. fat sensation and absorption, stomach functions, etc) for the treatment of obesity will be discussed.

POSTERS

Atypical antipsychotic-induced adiposity may be modulated by peptide hormones

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Weight gain is not only a common problem for most Americans, but with the use of many antipsychotic drugs it is a prominent side-effect. Olanzapine is an atypical antipsychotic drug that has been reported to increase appetite, with subsequent changes in body weight, in female rats compared to male rats. For rats, the traditional routes of administration for most studies utilizing antipsychotic drugs employ daily gavage, intraperitoneal injection, or subcutaneous injection. A less invasive technique is oral administration of a drug by adding it into the normal diet or into another desirable food that the rat will intake voluntarily. In the present study, olanzapine was placed in a “treat” (condensed milk) to ensure complete consumption of the daily dose of the drug at a specified time, which simulates a human taking the drug on a typical once-a-day schedule. Twelve male and female Long Evans rats were assigned to either a control or experimental group. One week after arrival at our laboratory, animals were implanted with biotelemetry transmitters to record gross motor activity. They were then subjected to a three week habituation period, in which they all received a condensed milk “treat”. Following habituation, six experimental rats, in their respective genders, received olanzapine dissolved in ethanol in the condensed milk, while the control rats received only ethanol in the milk, in amounts based on body weight. The drug period lasted for three weeks. Daily body weight, food intake, and water intake, as well as continuous monitoring of activity levels were recorded. Even though male rats weighed more than female rats, there were no differences between the control and experimental groups in either gender. Male animals consumed more food than female animals, but the only difference between experimental and control animals occurred during the first week of drug administration, where experimental female animals consumed more food than control animals. There was a similar difference found in water intake of these two groups. Of particular significance was the observed difference in adiposity between genders at the conclusion of the experiment, with male rats having less fat than female rats and female rats receiving olanzapine developed more adiposity than female rats not exposed to the drug. Comparing body activity, the female rats had higher levels of activity than the male rats overall, but the drug did not disrupt circadian rhythms of activity in either gender.

Underlying mechanisms in the observed adiposity results suggest a possible modulatory role for some peptides such as ghrelin, leptin, and/or neuropeptide Y.

Characterization of species-related differences in the pharmacology of tachykinin NK receptors 1, 2 and 3.

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Tachykinin NK receptors (NKR) differ to a large degree among species with respect to their affinities for small molecule antagonists. The aims of the present study were to clone NKRs from gerbil (NK₂R and NK₃R) and dog (NK₁R, NK₂R, NK₃R) in which the sequence was previously unknown and to investigate the potency of several NKR antagonists at all known human, dog, gerbil and rat NKRs.

The NKR protein coding sequences were cloned and expressed in CHO cells. The inhibitory concentrations of selective and non-selective NKR antagonists were determined by inhibition of agonist-induced mobilization of intracellular Ca²⁺. Receptor homology models were constructed based on the rhodopsin crystal structure to investigate and identify the antagonist binding sites and interaction points in the trans membrane (TM) regions of the NKRs.

Data collected using the cloned dog NK₁R confirmed that the dog NK₁R displays similar pharmacology as the human and the gerbil NK₁R, but differs greatly from the mouse and the rat NK₁R. Despite species-related AA differences located close to the antagonist binding pocket of the NK₂R, they did not affect the potency of the antagonists ZD6021 and saredutant. Two AA differences located close to the antagonist binding site of NK₃R likely influence the NK₃R antagonist potency, explaining the 3-10-fold decrease in potency observed for the rat NK₃R. For the first time, detailed pharmacological experiments in vitro with cloned NKRs demonstrate that not only human, but also dog and gerbil NKR displays similar antagonist pharmacology while rat diverges significantly with respect to NK₁R and NK₃R.

Impaired nocifensive behaviours and mechanical hyperalgesia, but enhanced thermal hyperalgesia in pituitary adenylate-cyclase activating polypeptide deficient mice

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Background: Pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) and its receptors (PAC1 and VPAC) have been shown in the spinal dorsal horn, cell bodies in the dorsal root ganglia, as well as both central and peripheral terminals of capsaicin-sensitive sensory neurons. Data concerning the role of PACAP in central pain transmission are controversial and we have recently published its divergent peripheral effects on nociceptive processes.

Aims, experimental models: The aim of the present study was to investigate acute somatic (2.5% i.pl. formalin-evoked paw lickings) and visceral (3% i.p. acetic acid-induced writhing movements) nocifensive behaviors, partial sciatic nerve ligation-evoked neuropathic as well as the TRPV1 receptor agonist resiniferatoxin (0.6 ng i.pl.)-induced inflammatory mechanical and thermal hyperalgesia in PACAP deficient (PACAP^{-/-}) mice to elucidate its overall function in pain transmission.

Results: The number of paw lickings in the early (0-5 min) and late (20-45 min) phases of the formalin test which reflect acute somatic chemonocifensive behavior and acute inflammatory nociception, respectively, was markedly diminished in PACAP^{-/-} mice. Similarly, i.p. injected acetic acid-evoked abdominal contractions referring to acute visceral chemonociception was also significantly attenuated in the PACAP knockout group. Neuropathic mechanical hyperalgesia was absent in PACAP-deficient animals. Intraplantarly injected resiniferatoxin-evoked mechanical hyperalgesia of the paw which involves both peripheral and central mechanisms was decreased, but thermal hyperalgesia mediated by only peripheral mechanisms was increased in PACAP^{-/-} mice.

Conclusions: These data clearly demonstrate that the overall role of PACAP in pain transmission originating from both exteroceptive and interoceptive areas is excitatory, it is involved in central sensitization presumably at the levels of the spinal cord, spinothalamic tract, thalamus and sensory cortex. These findings can be explained by the signal transduction mechanisms of its identified receptors, both PAC1 and VPAC activation leads to neuronal excitation. In contrast, it is an inhibitory mediator at the level of the peripheral sensory nerve endings and decreases sensitization of the capsaicin-sensitive terminals to heat. This result which is in agreement with our previous data showing that PACAP inhibits sensory neuropeptide release from sensory nerve endings, suggests the existence of a potential, presently unknown, inhibitory mechanism in the peripheral nerve terminals.

Acknowledgements: Hungarian Grants K72592, K73044, RET-008/2005, ETT-06-348/200 ETT-06-284/2006 and Janos Bolyai Postdoctoral Research Fellowship. PACAP^{+/-} mice were kindly provided by Dr. Nobuhisa Iwata, RIKEN Brain Science Institute, Saitama, Japan.

WNPC Lecture

Neuropeptides organize behavior: Or what I learned organizing neuropeptide conferences

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In a research program that has spanned over 40+ years, we have found that a family of neuropeptides play a central role in organizing the brain and behavior. Fetal and infant exposures to physiological levels of peptides result in permanent changes in behavior and the structure of the nervous system. Paralleling our research program has been the organization and development of the Winter Neuropeptide Conference (WNPC). When our research began, the term “neuropeptide” had not been uttered. As research in this area blossomed around the world, and the number of neuropeptides swelled from a literal handful to an unimaginable quantity, the need developed for focus in our research program and for a forum to discuss this rapidly expanding area. Thus, our research program focused on the HPA axis and the WNPC, now in its thirtieth year, was initiated to provide an opportunity to present new findings with newly discovered peptides. The historical (perhaps autobiographical) roots, both of our research program and the WNPC will be discussed.