Abstracts

Ethical requirements of The Physiological Society

Experiments on animals or animal tissue

For work conducted in the UK all procedures must conform with current UK legislation. For work conducted elsewhere all procedures must accord with current national guidelines or, in their absence, with current local guidelines.

Experiments on humans or human tissue

All procedures must accord with the ethical standards of the relevant national, institutional or other body responsible for human research and experimentation, and with the principles of the World Medical Association's Declaration of Helsinki.

Abstracts

C01 and PC01

Histamine and its role in the regulation of appetite

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Food intake is essential to all animals. By ingesting food an animal obtains energy to survive. However, food intake must be balanced with energy expenditure otherwise overeating results in obesity. Due to the imbalance between energy intake and output obesity has become is a major worldwide problem. We aim to look at the effects of the central transmitter, histamine, on appetite regulation by measuring feeding behaviour, the induction of the functional marker c-Fos and the electrical activity of histamine responding neurones within the ventromedial hypothalamus (VMN). Pharmacological studies suggest two histamine receptors regulate food intake, H_1R and H_3R. Activation of central H_1R and H_3R leads to a decrease and an increase in food intake, respectively. Our preliminary studies confirmed that 200 nmol histamine given centrally via an intracerebral guide cannula (implanted 1 week prior under 2% isofluorane in O_2 at 1 l/min anaesthesia) is a powerful anorexigen. Examining the effects of H_3R active drugs in rats revealed imetit, a H_3R agonist, caused hypophagia whilst the H_3R inverse agonist thioperamide resulted in hyperphagia. Both thioperamide and imetit can be blocked by proxyfan which, therefore, appears to be acting as a neutral H_3R antagonist. Interestingly murine studies showed both the H_3R agonist and H_3R antagonist caused a decrease in night time feeding which contradicts previously published research. Using c-Fos immunohistochemistry we found the effects of histamine and H_3R based drugs most likely involve the major hypothalamic nuclei involved in homeostatic regulation of body weight; namely the VMN, paraventricular, dorsomedial and arcuate nuclei. Furthermore, extracellular electrophysiological recordings in vitro demonstrate that these effects are likely to be direct on the hypothalamus. For example, histamine activates the majority of VMN neurones (60%). By applying histamine with the H_1R antagonist pyrilamine, we have managed to diminish the excitatory actions of histamine responding neurones within the VMN. We also have evidence for local presynaptic H_3R involvement. Our results show that by applying thioperamide, a H_3R antagonist, we can mimic the actions of histamine causing an increase in the firing rate of neurones within the VMN. Additionally, this increase in neuronal firing caused by thioperamide application can be blocked by applying pyrilamine. These results demonstrate VMN H_3R are presynaptic autoreceptors on histaminergic afferents, rather than heteroreceptors modulating the release of other transmitters. Finally we have found that the neutral H_3R antagonist proxyfan can attenuate the neuronal response of thioperamide suggesting a direct H_3R response. Thus our data supports a role for histaminergic receptors, including postsynaptic H_1R and presynaptic H_3R autoreceptors in the VMN, to modulate feeding.

I would like to thank the BBSRC and Novo Nordisk for funding my work.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C02 and PC02

Enteroendocrine cells in human intestinal inflammation

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Appetite is often reduced in patients with gastrointestinal inflammation, but the precise biological basis for this is extremely unclear. Gastrointestinal satiety signals are produced by enteroendocrine cells (EEC), and signal to the brain to regulate food intake. Polypeptide YY (PYY) and glucagon like peptide-1 (GLP-1) are secreted by L cells and suppress food intake, probably physiologically. Recent animal model research has suggested that immune-regulated upregulation of EEC plays a mechanistic role in the appetite and feeding disturbance observed during gut inflammation (1,2). This has not been explored in humans: Crohn's disease (CD) has been chosen as an exemplar for this, in order to assess EEC and related markers in human intestinal inflammation. Endoscopic terminal ileal biopsies were taken from CD patients with active intestinal inflammation, sampling small and large bowel (SB and LB respectively), and tissue from age/sex matched controls. Symptoms including appetite and satiety were quantified with validated visual analogue scores (VAS). EEC markers and transcription factors were studied by immunohistochemistry and quantitative polymerase chain reaction (qPCR). CD patients with active inflammation displayed a ~6-fold significant reduction in basal appetite as measured by VAS (unpaired t-test; N=18 vs 13; $p<0.0001$). Inflammation was graded independently with endoscopic, clinical, histological and biochemical scoring. GLP-1 cells were significantly increased 2.5-fold in SB CD (unpaired t-test; N=8 vs 11; $p=0.04$), while the general EEC marker chromogranin A showed a 1.5 fold increase in expression. However, PYY cell numbers were not significantly altered, with a trend to decreased numbers. Phox2b (3), a neural transcription factor associated with CD in a recent genome-wide association study, was co-localised to EEC through dual immunofluorescence and showed a 1.5-fold increase in SB CD compared to controls. At mRNA level, significant increases were noted for Chromogranin A (3.3-fold; Mann Whitney (MW) test; N=8; $p=0.009$), GLP-1 (MW test; N=8; 2.7-fold $p=0.05$), Ubiquitination protein 4a (Ube4a) (MW test; N=8; 2.2-fold $p=0.02$). However PYY was not significantly changed. Neurogenin 3, a NOTCH transcription factor central to EEC differentiation showed ~2 fold-upregulation (MW test; N=8; $p=0.04$). These preliminary results show that changes in EEC biology occur in CD, with differential effects on specific cell lineages. This is compatible with a potential role for EEC in appetite dysregulation in intestinal inflammation: enhanced EEC activity may directly suppress appetite in such patients through increased gut-brain signalling. It is now planned to further dissect the signalling pathways implicated.

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C03 and PC03

Hemopressin is a novel peptide ligand at CB_1 cannabinoid receptors that reduces appetite in rodents

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Hemopressin is a short, nine-amino acid peptide, derived from the alpha chain of haemoglobin, which has previously been shown to cause negligible decreases in blood pressure and to have non-opioid antinociceptive effects (Heimann et al. 2007). Hemopressin acts *in vitro* to functionally antagonise CB_1 receptors, and to potentially behave as a CB_1 inverse agonist. We show that injection of hemopressin (peripheral (500 nmol/kg, i.p.) or central (10 nmol/animal, i.c.v.); animals were implanted stereotaxically with guide cannulae into the right lateral ventricle (0.2 mm posterior, 1 mm lateral from bregma for mice, and 0.8 mm posterior, 1.5 mm lateral from bregma for rats) under 2% isoflurane in 1 l/min oxygen) causes a dose-dependent decrease in normal, night-time feeding in outbred mice and rats, occurring without disrupting the normal behavioural satiety sequence, and without causing any obvious, adverse events (Dodd et al. 2010). In fact, hemopressin-induced hypophagia maintains the sequence, but surprisingly shifts it to the left, as has been seen previously with physiological satiety factors. The systemic route of administration was repeated in fasted wildtype and CB_1 receptor knockout mouse littermates. Firstly, hemopressin (500nmol/kg, i.p.) decreased food intake in fasted wildtype mice, showing that it is capable of overcoming a powerful, natural orexigenic drive. Secondly, this response is lost in the CB_1-/- mice. Furthermore, hemopressin (10nmol/animal, i.c.v.) can block CB_1 agonist-induced hyperphagia (CP55940, 0.06 mg/kg, i.p.) in rats, providing further evidence for antagonism of the CB_1 receptor *in vivo* (Dodd et al. 2009). We speculate that hemopressin may act as an endogenous peptide antagonist at CB_1 receptors, capable of modulating appetite pathways in the brain.

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Abstracts

C05 and PC05

A conditional knock-out mouse model for XLαs; a signalling protein involved in the suppression of metabolic rate and energy expenditure

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The regulation of energy homeostasis and an organism's subsequent adiposity and body weight is controlled by a highly complex system of neural, endocrine and metabolic processes which have only recently begun to be identified. With the current epidemic in human obesity, research has focused upon how the dysregulation of these inter-connected physiological processes can cause obesity and on identifying pathways that prevent or reverse obesity development. The development of animal models provides essential tools for investigating the complex systems involved in regulating energy homeostasis; one unique model is the XLαs knock-out mouse. XLαs (eXtra Large αs) is an NH2-extended variant of the 'a-stimulatory' subunit of the trimeric G-protein, Gαs, one of three proteins encoded by the complex imprinted *Gnas* locus. At birth knock-out mice display poor feeding, increased neonatal mortality and very limited adipose development[1], while adult survivors go on to develop a healthy exceptionally lean, insulin-sensitive, hypermetabolic phenotype, weighing ~45% lighter with less than half the body fat of wild-type controls and showing increased sympathetic tone[2]. This knockout provides one of the few lean mouse models and represents a valuable tool that can be used to identify novel pathways involved in obesity prevention or treatment. Given the highly complex phenotypes generated by global gene knockouts a more refined conditional approach is necessary to precisely identify the mechanisms arising from the temporal, tissue and cell-specific effects of *Gnasxl* (XLαs) deletion. In this study we describe the development of a novel, conditionally targeted *Gnasxl* mouse model. In order not to interfere with the complex regulatory mechanisms of the imprinted *Gnas* locus, we designed a conditional gene-trap approach. Tissue-specific Cre expression causes an inversion of the gene-trap cassette, resulting in truncation of XLαs and the formation of a lacZ fusion protein. This conditional *Gnasxl* knock-out provides the tool necessary to dissect the individual tissue-specific mechanisms that contribute to the lean and hypermetabolic phenotype exhibited by global *Gnasxl* knock-out mice. Progress in the analysis of brain-specific XLαs deletions will be presented.

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2. Xie, T., et al., The alternative stimulatory G protein alpha-subunit XLalphas is a critical regulator of energy and glucose metabolism and sympathetic nerve activity in adult mice. J Biol Chem, 2006. 281(28): p. 18989-99.

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C06 and PC06

Mildronate depletes carnitine in fast, intermediate and slow twitch rodent skeletal muscle

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Carnitine has two distinct metabolic roles in skeletal muscle: (i) in translocating long-chain fatty acids across the mitochondrial membranes for oxidation, and (ii) in maintaining a viable mitochondrial free coenzyme A pool (1). Mildronate administration has been shown to reduce heart and liver carnitine concentration by inhibiting carnitine biogenesis and accelerating its renal clearance (2). Given that >95% of the body's carnitine store is located in skeletal muscle, the aim of this present study was to investigate the impact of oral mildronate administration on carnitine moieties in skeletal muscles of differing fibre composition. Sixteen male Wistar rats were randomly assigned to 2 groups that received either drinking water (control, n=8) or drinking water supplemented with mildronate (mildronate, n=8) for 10 days (1,600 mg.kg-1 on days 1-2 and 800 mg.kg-1 thereafter). After 10 days, the extensor digitorum longus (EDL), gastrocnemius (GAS), soleus (SOL) and tibialis anterior (TA) muscles, selected for their different fibre compositions, were excised under terminal anaesthesia (sodium pentobarbital, i.p. 120 mg.kg-1). Mildronate administration resulted in a marked reduction in total carnitine (sum of free and acyls) in all tissues ($P<0.001$), but the reduction in free carnitine was particularly remarkable ($P<0.001$, Table 1). This study has shown for the first time that mildronate depletes free and total carnitine in fast twitch, intermediate and slow twitch rodent skeletal muscle fibres. Furthermore, this present study has demonstrated that oral administration of mildronate provides an ideal model to investigate the significance of carnitine availability on metabolic regulation and physiological function in skeletal muscle in vivo.

Table 1. Muscle carnitine moieties in muscles of differing fibre compositions from Han Wistar rats following 10 days of oral mildronate supplementation.

	Free Carnitine		Long-chain acylcarnitine		Acetylcarnitine		Total carnitine	
	Control	Mildronate	Control	Mildronate	Control	Mildronate	Control	Mildronate
EDL	3.59±0.17	0.49±0.04***	0.63±0.16	0.20±0.05*	0.55±0.10	0.24±0.04*	4.77±0.18	0.92±0.12***
GAS	4.10±0.10	0.50±0.07***	0.52±0.23	0.09±0.02***	0.39±0.07	0.27±0.13	5.01±0.29	0.92±0.12***
SOL	3.03±0.10	0.47±0.03***	0.45±0.15	0.39±0.15	0.49±0.07	0.45±0.07	3.97±0.32	1.13±0.27***
TA	4.31±0.19	0.53±0.04***	0.35±0.17	0.35±0.19	0.49±0.11	0.19±0.12	5.14±0.47	1.07±0.35***

Values expressed as means ± S.E.M. (n=8). Concentrations are expressed as mmol.kg-1 of dry muscle. Mean values were compared using a Student's unpaired t test. *, *** Significantly different from the corresponding control group (P<0.05; P<0.001, respectively).

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C07 and PC07

Expression of 7TM chemo-sensors in isolated enteroendocrine cells

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The secretion of peptide hormones from enteroendocrine cells is controlled by a mixture of different stimuli: neurotransmitters, neuropeptides, nutrients, metabolites and paracrine and luminal messengers - most of which act through 7TM, G protein coupled receptors (1). Enteroendocrine cells have been isolated and characterized based on genetic tagging using promoter elements for GLP-1, GIP and ghrelin (2-4). Here, enteroendocrine cells were isolated from transgenic mice expressing GFP under the control of regulatory elements for CCK and their expression of peptide precursors and 7TM receptors were characterized by QPCR. Immunohistochemistry demonstrated that the GFP expression was restricted to classical enteroendocrine cells which included CCK cells but which was not restricted to these. QPCR analysis of isolated FACS purified cells showed that the GFP labelled cells besides CCK also expressed for example somato-statin. A comprehensive QPCR analysis of 7TM receptor expression demonstrated that this mixed population of enteroendocrine cells – as expected - expressed a number of proposed chemosensors for nutrients and metabolites of which the short chain fatty acid receptor GPR41 was very prominent along with the long chain fatty acid receptors GPR40 and GPR120 as well as the proposed OEA receptor GPR119 were highly enriched as compared to the ordinary enterocytes. Certain, specific subtypes of monoaminergic receptors were clearly, highly enriched as compared to others from the same families – of these the 5HT5b receptor was an unexpected curiosity. Among the neuropeptides receptors the gastrin releasing peptide receptor (BB2) was – as expected - highly enriched and, for example a high level of the galanin R1 receptor expression was also observed. Among the receptors for paracrine substances a number of different somatostatin receptor subtypes were, for example, expressed in the enteroendocrine cells. A number of orphan receptors were also highly enriched in the enteroendocrine cells.

It is concluded that even this rather mixed population of enteroendocrine cells express a surprisingly selective repertoire of 7TM receptors and receptor subtypes. Some of these have previously been shown also to be expressed in more pure populations of enteroendocrine cells (2-4). It remains to be shown which of these 7TM receptors will best serve as targets for novel therapeutics to function as regulators of the release of physiological mixtures of GI tract hormones.

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C08 and PC08

Voltage-gated ion channels in primary murine L-cells

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Background and aims: Glucagon-like peptide-1 (GLP-1) is an enteric hormone secreted by L-cells and is an attractive therapeutic target for the treatment of Type 2 Diabetes. Recently, primary murine L-cells were shown to be electrically excitable, firing action potentials in the presence of glucose, suggesting that voltage-gated ion channels play an important role in stimulus-secretion coupling in this cell type. The purpose of this study was to identify the voltage-gated ion channels expressed in murine L-cells and investigate their role in GLP-1 release. Materials and methods: Transgenic mice expressing the Venus fluorescent protein under the control of the proglucagon promoter were used as a model system. Quantitative real-time PCR (qPCR) was used to quantify the expression of voltage-gated ion channels in Venus-expressing L-cells, purified by flow cytometry. Standard whole-cell patch-clamp experiments and fluorescence calcium imaging were performed on primary cultured colonic L-cells, identified by their expression of Venus. GLP-1 secretion from primary cultures of adult mouse colon was measured by ELISA. Results: Results are expressed as mean ± SEM. Whole-cell voltage-clamp recordings revealed large rapidly-inactivating, tetrodotoxin (TTX)-sensitive sodium currents (-850±123pA cell^{-1} at 0mV, n=9), which exhibited half maximal activation at -17±1mV (n=9), and half-maximal inactivation at -46±1mV (n=10). In agreement with these findings, qPCR analysis showed that L-cells predominantly express *scn3a* (n=3), which is a TTX-sensitive sodium channel isoform. In the presence of TTX (0.3μM), the residual inward current persisted in the absence of Na^+ (n=4) but was eliminated by 5mM Co^{2+}, strongly suggesting that this is a voltage-dependent Ca^{2+} current (p<0.001 by Student's t test, n=10). GLP-1 secretion in the

presence of 75mM KCl was markedly inhibited by the L-type Ca^{2+} channel blocker nifedipine (10μM; p<0.01 by Student's t test, n=5). Furthermore, this selective antagonist attenuated KCl-induced elevation in $[Ca^{2+}]_i$, further confirming that murine L-cells express L-type Ca^{2+} channels. Ω-Conotoxin MVIIC (1μM) also reduced the secretion of GLP-1 (p<0.05 by Student's t test, n=6). Although this toxin is a recognised blocker of Q-type channels, it also non-selectively blocks N- and P-type channels. However, ω-Conotoxin GVIA (1μM) and ω-Agatoxin IVA (200nM), which block N- and P-type channels respectively, had no effect upon hormone release in the presence of KCl, indicating that murine L-cells express Q-type Ca^{2+} channels. Conclusion: L-cells are electrically excitable and changes in membrane potential play an important role in the regulation of GLP-1 release by opening voltage-gated Ca^{2+} channels. Improving our understanding of the stimulus-secretion coupling pathways in L-cells will hopefully facilitate the development of novel therapeutics for the treatment of Type 2 Diabetes.

Wellcome Trust

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C09 and PC09

TGR5 mediated GLP-1 secretion

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Background: The incretin hormone, GLP-1, is secreted from intestinal L-cells in response to food ingestion, however little is known about how L-cells detect luminal nutrients. Bile acids are also released into the gut lumen in response to nutrients and have been associated with glucose homeostasis and GLP-1 secretion [1, 2]. The aim of this study was to investigate the expression of the bile acid sensitive G-protein coupled TGR5 receptor in primary L-cells and its role in GLP-1 secretion. Material and Methods: mRNA expression was analyzed by qRT-PCR in L-cells purified from transgenic mice with fluorescently labeled proglucagon-expressing cells [3]. GLP-1 secretion was assayed in primary colonic cultures and GLUTag cells. FRET based $[cAMP]_i$ and ratiometric $[Ca^{2+}]_i$ imaging experiments were performed on GLUTag cells. Results: TGR5 mRNA expression is highly enriched in L-cells compared to control intestinal epithelial cells. The bile acids deoxycholic acid, lithocholic acid and taurolithocholic acid (TLCA) and a TGR5 agonist increased GLP-1 secretion from primary cultures and enhanced the glucose-triggered response. In GLUTag cells, the GLP-1 secretory responses to TLCA and the TGR5 agonist were attenuated by TGR5 siRNA treatment. Consistent with the idea that TGR5 signals via Gs coupled pathways, the TGR5 agonist and TLCA elevated $[cAMP]_i$ in GLUTag cells. The TGR5 agonist also elevated $[Ca^{2+}]_i$ and enhanced glucose-triggered $[Ca^{2+}]_i$ responses, consistent with previously observed responses of GLUTag cells to elevated cAMP. Conclusion: Primary L-cells express the bile acid sensitive TGR5 receptor, which may contribute to the incretin response via the elevation of $[cAMP]_i$ and $[Ca^{2+}]_i$. The synergistic stimulation of GLP-1 release may be possible by combined activation of GPCRs and glucose-sensing pathways.

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C10 and PC10

GPR119 activation integrates the secretion of gastrointestinal peptides and islet hormones

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GPR119 is mainly expressed in pancreatic islets and in the gastrointestinal (GI) tract. By immunohistochemistry, GPR119 co-localizes with GIP and GLP-1 in K-cells and L-cells, respectively. In islets, GPR119 appears to co-localize with pancreatic polypeptide in rodents, and in both β-cells and α-cells in rodents and humans. We have previously demonstrated that GPR119 agonists potentiate glucose stimulated insulin secretion *in vivo* and *in vitro*, suggesting a role in coordinating secretion of enteroendocrine and islet hormones in response to ingested nutrients. To evaluate the effect of activation of GPR119 on gut peptides, male crl: SD (CD) rats were cannulated (under isoflurane (2% in oxygen) anaesthesia) via the portal vein, recovered for 7 days and tethered in custom cages to facilitate unperturbed sampling. Rats received a single oral dose of a GPR119 agonist (GSK706,n=8) or placebo (Pbo,n=7) one hour prior to the start of the dark cycle and were followed for 24 hours. Measured analytes included plasma glucose (G), insulin, active(a) and total(t) GLP-1, tGIP, tPYY, PP and glucagon.
Prior to the onset of feeding, there were no changes in analytes in the Pbo group. In the agonist treated rats, mean glucose decreased (~0.8mM by 30 minutes post-dose) while (a) and (t)GLP-1, glucagon, tPYY and tGIP were significantly (p<.005 for all, Dunnett's) increased 3, 3, 2.8, 2 and 1.5 fold over baseline respectively with no change in PP. The elevation in glucagon was transient returning to baseline 4 hours post dose. With the onset of feeding, G increased modestly in both groups (~ 1.1mM) and there were no treatment-related changes in plasma insulin. Compared to the other analytes, tGIP increased most robustly in both groups with the onset of feeding (2.3 and 3.8 fold, Pbo and agonist, respectively). During feeding, the changes in all other peptides measured with Pbo were < 1.5 fold and all returned toward baseline values at the end of the dark cycle. In contrast, treatment with the agonist was associated

with sustained significant increases compared to Pbo in (a) and (t) GLP-1, tGIP for 24 hours post dose. tPYY remained elevated compared to Pbo only during feeding.
Perfusions in situ of isolated gut segments with GSK706 in anesthetized (ketamine/xylazine) male crl: SD (CD)rats enabled assessment of the relative contribution of each segment to portal appearance of total GLP-1, GIP, and PYY in the absence of nutrients. Within 5-10 minutes after the start of the perfusion there were rapid and robust increases in tPYY and tGLP-1 during perfusion of the duodenum, lesser change with colonic perfusion, and little with perfusion of jejunum or ileum. There was little change in GIP.
The rapid effects of GPR119 agonism to prime K- and L-cell secretion in concert with islet hormone secretion suggests a role for GPR119 signalling in nutrient sensing and disposal.

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C11 and PC11

Role of lysophosphatidylcholine in GIP secretion by primary K-cells

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Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) is a hormone secreted by enteroendocrine K-cells found in highest density in duodenal and jejunal epithelium. Apart from being a critical regulator of insulin secretion, GIP modulates pancreatic beta-cell proliferation and survival, and controls dietary fat metabolism. It is secreted in response to the presence of nutrients in the gut lumen and particularly ingested lipids. The aim of this study was to investigate the effects of lipid micelles on GIP secretion by K-cells. Results: Experiments were performed on primary cultures of murine duodenal epithelium and the enteroendocrine model cell line STC-1. To simulate the conditions epithelial cells experience after a lipid rich meal, "post-prandial micelles", comprised of oleic acid (200μM), 2-monooleoyl glycerol (70μM), L-α-lysophosphatidylcholine (LPC) (70μM), cholesterol (17μM) and taurocholic acid (TC) (700μM), were applied. Both primary and STC-1 cells responded to lipid micelles by secreting enhanced amounts of GIP (9.2 fold and 3.1 fold stimulation, respectively compared to baseline, $p<0.001$ for both). The stimulation of GIP secretion by lipid micelles was not attributable to cell lysis, as monitored by lactate dehydrogenase activity released into the supernatant. Fluorescence calcium imaging measurements in STC-1 cells, following loading with Fura-2AM, demonstrated elevations in intracellular calcium in response to lipid micelles (R340/380 increased 1.8 fold compared to baseline $p<0.001$ n=104).
To investigate the relative importance of the different micellar lipids for the secretory response a series of experiments was performed omitting individual components. Exclusion of LPC significantly reduced secretory responses in both primary and STC-1 cells (46% in primary cells n=7 $p<0.05$; 22% in STC-1 n=12, compared to stimulation by micelles containing LPC). Replacement of LPC with phosphatidylcholine (PC) could not compensate (1.14-fold stimulation by micelles containing PC in primary cells; n=4). LPC (in the presence of 700μM TC) promoted the release of GIP in a dose dependent manner over the range of concentrations between 1-100μM. Both in the presence and absence of TC, 70μM LPC stimulated reversible rises in the cytosolic Ca2+ and cAMP concentration monitored in STC-1 cells preloaded with Fura2 or transfected with a Epac2-based FRET-sensor respectively. Conclusion: Lipid micelles stimulate GIP secretion from primary murine cultures and STC-1 cells. One of the components, LPC, enhanced intracellular concentrations of calcium and augmented levels of intracellular cAMP suggesting the involvement of Gs protein- mediated signaling.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C12 and PC12

Ileal administration of zein hydrolysate stimulates glucagon-like peptide-1 secretion and attenuates hyperglycaemia induced by intraperitoneal glucose administration in rats

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Glucagon-like peptide-1 (GLP-1) is one of the incretin hormones that enhance insulin secretion from pancreatic beta cells. GLP-1 is secreted from enteroendocrine L cells mainly located in the distal small intestine, in response to nutrient ingestion. We recently demonstrated that a protein hydrolysate prepared from corn zein (ZeinH) strongly stimulates GLP-1 secretion from rat small intestine under anaesthesia (Hira *et al.* 2009). In this study, we examined whether ZeinH administered into the ileum induces GLP-1 secretion and affects glycaemia in conscious rats during a glucose tolerance test.
Silicone catheters (1 mm diameter) were inserted into the jugular vein and the ileum of male Sprague-Dawley rats (250-300 g) under anaesthesia with sodium pentobarbital (40 mg/kg i.p.). After 2-3 days recovery an intraperitoneal glucose tolerance test was performed in conscious rats. Water (2 ml), ZeinH solution (500 mg in 2 ml) or meat hydrolysate (MHY) solution (500 mg in 2 ml) were administered into the ileum through the catheter at -30 min. Glucose (1 g/2 ml/kg body weight) was injected intraperitoneally 30 min after the ileal administration of test liquids. Blood samples were collected from the jugular catheter before the ileal administration (-30 min) and 0, 15, 30 and 60 min after glucose injection. Glucose, insulin, total GLP-1 and active GLP-1 were measured in plasma.
Peak plasma glucose levels in ZeinH-treated rats (178.7 ± 15.7 at 15 min, n=6, means ± S.E.M.) were significantly lower ($P<0.05$, Fisher's PLSD test) than those in water-treated rats (266.9 ± 33.7 at 15 min, n=7). Glucose levels in MHY-treated rats (n=6) were not significantly lower than those in water-treated rats. Insulin secretion was enhanced by ileal administration of ZeinH, but not by MHY. From these results, it was predicted that GLP-1 secretion would be induced by ileal ZeinH but

not by ileal MHY. However, total GLP-1 levels were increased by both treatments. In addition, GLP-1 increments at 0 min (+0.64 ± 0.13 in ZeinH group and 0.40 ± 0.01 nM in MHY group) and 30 min (+0.62 ± 0.20 in ZeinH group and 0.49 ± 0.07 nM in MHY group) were significantly higher ($P<0.05$, Fisher's PLSD test) than those in water-treated rats (-0.10 ± 0.17 nM at 0 min and 0.03 ±0.24 nM at 30 min). In contrast to total GLP-1, active GLP-1 levels were significantly increased ($P<0.05$, Fisher's PLSD test) only in ZeinH-treated rats (from 6.02 ± 2.62 pM at -30 min to 17.32 ± 3.50 pM at 0 min). These results demonstrate that ileal administration of a protein hydrolysate, ZeinH induces GLP-1 secretion and attenuates hyperglycaemia, and suggest that the activity of secreted GLP-1 is retained following ZeinH but not after MHY administration.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C13 and PC13

Inhibition of GIP and GLP-1 secretion through Gi-coupled receptor activation

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Background and aim: Glucose-dependant insulinotropic polypeptide (GIP) is released in response to nutrient ingestion from specialised enteroendocrine cells (K-cells) scattered throughout the proximal gut epithelium. As an incretin GIP stimulates insulin secretion from the pancreatic β-cell, and together with the other incretin (glucagon-like peptide 1 (GLP-1) secreted from L-cells) is responsible for ~50% of insulin release following oral glucose administration. In addition GIP has been postulated to link over nutrition to the development of obesity, as pharmacological or genetic interference with GIP signalling proved protective in several rodent obesity models. The aim of this study was to investigate if GIP secretion can be inhibited through activation of Gi-coupled receptors. **Results:** Using recently developed transgenic mice with fluorescently labelled K- or L-cells, expression of Gi-coupled receptors was investigated by hybridising mRNA isolated from FACS sorted K-cells, L-cells or non-fluorescent cells from these sorts to Affymetrix 430 2.0 chips. The cannabinoid receptor 1 (Cnr1) mRNA was found to be highly enriched in both small intestinal K- and L-cells relative to the non-fluorescent cells, while it seemed to be absent from colonic L-cells. By contrast, somatostatin receptor 5 (SstR5) seemed to be highly expressed in both small intestinal and colonic L-cells and to a lesser extent in K-cells. To investigate if stimulation of these Gi-coupled receptors affects incretin release, secretion experiments were performed on primary murine cultures of epithelial cells from the duodenum (top ten cm of intestine) and the colon/rectum. GIP and GLP-1 secretion, assayed by ELISA, was stimulated by 10 mM glucose + 100 μM 3-isobutyl-1-methylxanthine (IBMX). Addition of 100 nM somatostatin inhibited both GIP (61±0.2% n=10) and GLP-1 (≥89% n=9) secretion from small intestinal cultures and GLP-1 secretion from colonic cultures (52±0.6% n=9), an effect partially suppressed by coincubation with a SSTR5 selective antagonist. Addition of the CB1R agonist methanandamide (10 μM) attenuated GIP secretion (58±0.3% n=7) and this inhibition was completely suppressed by the concomitant presence of the antagonist AM251 (1 μM; n=7). AM251 alone was without effect on either basal or stimulated GIP secretion. Neither methanandamide nor AM251 did affect GLP-1 secretion from either duodenal or colonic cultures. **Conclusions:** Somatostatin inhibits the secretion of both incretins partially through the activation of SSTR5. GIP secretion can be inhibited by CB1R-activation, which somewhat surprisingly did not affect GLP-1 secretion. The absence of AM251 effects on basal and stimulated secretion argues against a tonic inhibition of K-cells by endocannaboids.

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C14 and PC14

Preproglucagon neurons project heavily to autonomic control regions of the CNS

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Receptors for glucagon-like peptide 1 (GLP-1) are found in many brain regions. Central application of GLP-1 or its analogue exendin-4 inhibits food intake and reduces blood glucose levels. Activation of central GLP-1 receptors also increases blood pressure and heart rate. The only endogenous sources of GLP-1 within the CNS are preproglucagon (PPG) neurons. We suggest that these central GLP-1 neurons modulate sympathetic and vagal outflow. As one step in testing this hypothesis, we analysed the projections of YFP-tagged PPG neurons to key CNS sites involved in autonomic control.

Coronal sections from transgenic mice expressing YFP under the control of the PPG promoter were revealed with an anti-GFP antibody and avidin-biotin-peroxidase. The distribution of immunoreactive cell bodies and fibres was analysed from the anterior commissure to the spinomedullary junction.

YFP-immunoreactivity was intense and axons were clearly visible. YFP-immunoreactive cell bodies were located in the caudal brainstem, primarily within the caudal nucleus tractus solitarius (NTS). Additional somata were observed in the intermediate reticular nucleus, at the ventral border of the hypoglossal nucleus and in the raphe obscurus. The caudal NTS contained a dense network of dendrites, some of which extended into the area postrema (AP). Immunoreactive axons were widespread throughout the NTS, the dorsal vagal nucleus and the reticular nucleus (except for the parvicellular section) but more limited within the hypoglossal nucleus and the pyramids. The AP, rostral ventrolateral medulla, pontine central

grey, parabrachial nucleus and locus coeruleus were moderately innervated and some axons extended into the amygdala. In contrast, dorsomedial and paraventricular hypothalamus, periaqueductal grey and the paraventricular nucleus of the thalamus exhibited heavy innervation with YFP-immunoreactive axons.
These results demonstrate that PPG neurons innervate primarily brain regions involved in autonomic control. Our data also show that YFP-PPG neurons in the mouse project more widely than GLP-1 immunoreactive neurons in the rat. This finding highlights the greatly increased sensitivity provided by immunohistochemical detection of neurochemically distinct populations of neurons that have been genetically modified to express GFP or a GFP analogue. Hence, central PPG neurons are in a prime position to modulate sympathetic and parasympathetic outflow through input at a variety of central locations.

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C15 and PC15

L-Glutamine stimulates the release of GLP-1 from primary murine L-cells

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Glutamine has been shown to elevate plasma levels of the incretin hormone, GLP-1, in human subjects (1). The mechanisms behind glutamine-evoked GLP-1 release have previously been investigated using the model cell line, GLUTag (2), but remain poorly defined. This project has sought to clarify the validity of this cell model whilst further elucidating the pathways behind glutamine-induced GLP-1 secretion. Calcium imaging and static secretion experiments were performed on colonic cultures from adult transgenic mice expressing the fluorescent protein, Venus, driven by the proglucagon promoter. GLP-1 release was measured from 24hr old cultures and fura2 loaded cells were monitored in real time to track intracellular Ca^{2+} levels in individual identified L-cells. Real time intracellular cAMP levels were investigated in GLUTag cells transfected with a cAMP FRET sensor. A range of L-amino acids and meAIB, a specific substrate of system A transporters (SNAT), elevated Ca^{2+}_i to comparable levels in primary L-cells. The glutamine-evoked Ca^{2+} response was shown to be dependent on Na^+_o (n=8, p<0.01). However, only glutamine (Gln), asparagine (Asn) and phenylalanine (Phe) stimulated a significant release of GLP-1 from the cultures (n=9, p<0.05), with Gln elevating GLP-1 levels significantly higher than Asn and Phe (n=9, p<0.01). Gln triggered the release of GLP-1 in a dose-dependent fashion with an EC_{50} of 0.1 mM (n=6), comparable to that seen in GLUTag cells (2). The response was attenuated ~20% by the inhibition of either L-type voltage gated Ca^{2+} channels or TTX-sensitive Na^+ channels (n=9, p<0.01). In addition, Gln stimulated secretion via a mechanism that was downstream or independent of membrane depolarisation-evoked Ca^{2+} entry (n=6, p<0.01). Antagonising components of the Gq pathway were without effect as was inhibition of a range of Gln-dependent metabolic pathways. In the GLUTag cell line, both Gln and Asn triggered a transient rise in $cAMP_i$ (n=21-66, p<0.001), which was independent of Na^+_o (n=15-36). Together, these data suggest a role for SNAT in the release of GLP-1. The coupled Na^+ entry accompanying Gln influx, depolarises the membrane, triggering action potentials and Ca^{2+} entry via L-type Ca^{2+} channels. However, this pathway alone is not sufficient to stimulate secretion of GLP-1, given the lack of effect of meAIB. In addition, Gln elevates intracellular cAMP levels, a known and potent signalling pathway in enteroendocrine cells, thus potentiating the secretory response. The findings validate the use of GLUTag cells for the study of GLP-1 secretion in relation to the sensing of L-Gln. The ability of amino acids to modulate $cAMP_i$ suggests the involvement of an amino acid-sensitive plasma membrane receptor, the molecular identity of which is under current investigation.

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C16 and PC16

CCK-stimulation of GLP-1 neurons involves α_2 adrenergic receptor activation

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Cholecystokinin-octapeptide (CCK-8) is released from the gut postprandially and inhibits food intake. Peripheral application of CCK-8 activates c-FOS in the vagal complex. Immunohistochemical characterisation of these c-FOS-positive cells revealed that these include catecholaminergic and glucagon-like-peptide 1 (GLP-1) producing pre-proglucagon (PPG) neurons. Hindbrain catecholaminergic neurons have been implicated in glucoprivic feeding and GLP-1 injected into the brain causes satiety. In order to analyze functional effects of CCK-8 and catecholamines on PPG neurons we used transgenic mice expressing YFP under PPG promoter control, thus allowing patch-clamp recordings from PPG cells identified by fluorescence.
Coronal slices (200μm) were obtained from adult transgenic mice after halothane anaesthesia. Perforated patch-clamp (current clamp) and conventional whole-cell (voltage clamp) recordings were performed on PPG neurons under visual control at 32°C.
In current-clamp, 10 μM adrenaline (AD) and 100 μM noradrenaline (NA) increased action potential firing rate (by 58±14 % and 85±37 %, respectively), but had no effect on membrane potential and conductance. In voltage-clamp, AD and NA increased the frequency of spontaneous excitatory synaptic currents (sEPSCs). Kynurenic acid (1mM) reduced the frequency of sEPSCs from 2.4±0.5 Hz to 0.2±0.04 Hz and blocked the AD-induced increase in sEPSC frequency (n=5). The AD effect was

also prevented by the α_2 receptor antagonist yohimbine (n=5). TTX reduced sEPSC frequency from 3.0±0.6 Hz to 0.7±0.1 Hz in 15 out of 24 PPG cells and blocked the effect of AD. In the remaining 9 PPG neurons TTX did not affect sEPSC frequency and the AD effect remained in 5 of these cells. 100 nM CCK-8 increased the frequency of sEPSCs by 79±1 % in 3 out of 8 cells tested. Yohimbine (10 μM) blocked this response in all 3 cells. PPG neurons do not receive a direct adrenergic input, but their glutamatergic input is modulated by α_2-adrenergic receptor activation. This modulation takes place at either of two sites: at the glutamatergic synapse (TTX-resistant) or distant from the synapse (TTX-sensitive). CCK-8 stimulation of PPG neurons is indirect and involves activation of α_2-adrenergic receptors, thus suggesting that catecholaminergic neurons are essential for the effect of CCK on PPG neurons.

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PC18

Central cannabinoid signalling in the rat: a pharmacological-challenge MRI and functional histology study

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Endocannabinoids have a variety of effects by acting on CB1 receptors located throughout the brain. The presynaptic location of CB1 receptors and differences in the strength of negative coupling means that expression studies alone do not provide the basis for interpreting site of action. Likewise, to date, most functional studies have used high drug doses which can bias results toward non-relevant adverse effects, and which mask more behaviourally-relevant actions. To better define sites of action of cannabinoids we have combined blood-oxygen-level-dependent (BOLD) pharmocolgical-challenge magnetic resonance imaging (phMRI) with whole brain c-Fos functional activity mapping to characterise structures responsive to behaviorally- relevant orexigenic or anorectic doses, respectively, of a CB1 agonist (CP-55940, 0.06mg/kg; i.p.) and an inverse agonist (Rimonabant, 0.1mg/kg, i.p.). We also demonstrate the use of a new fMRI analysis tool, which determines regions where the drugs functionally antagonise each other.
For phMRI, rats were imaged using a T2*-weighted gradient echo in a 7T magnet for 70 mins under α–chloralose anaesthesia (30 mg/kg/h i.v.), while those for immunohistochemistry were unanaesthetised and freely behaving. These complementary methods demonstrated functional activity in the cortico-striatal-hypothalamic pathway which is key to the motivational drive to eat. Furthermore, regions of this pathway affected by CP-55940 were functionally antagonised by co-administration of Rimonabant.
These results provide strong evidence for target sites of cannabinoid signaling with whole brain coverage, and identify common neuronal substrates underlying cannabinoid central effects, elucidating potential targets for the development of viable, cannabinoid-based therapies.

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PC19

Long day length potentiates leptin secretion in female Wistar rats

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Leptin is predominantly secreted by adipocytes and plays a central role in the regulation of energy homeostasis and food intake. Additionally, leptin is implicated in other physiological processes, including mainly reproductive functions by providing a link between metabolic status and related hypothalamic areas. It is proposed that leptin provides information to the brain that there are enough energy stores for reproductive functions, and may be a major determinant or only a permissive factor of the timing of puberty. Our previous studies showed that serum leptin levels were significantly elevated in the pinealectomised rats, and were suppressed by exogenous administration of melatonin. Day length (DL) can affect leptin secretion by modulating melatonin secretion and therefore leptin-regulated physiological functions. The aim of this study was to investigate the effect of a long DL cycle on serum leptin levels and puberty onset in female rats fed high-fat. For this aim, the groups were fed diets that contained 24%, 4.3% or 2.4% fat by weight. The groups were reared under conditions of 12-h light/dark or 18-h light/6-h dark cycles from day 21 when the

rats were weaned. Body weight and food intake were daily determined, and vaginal opening was daily monitored starting from day 26. The animals were decapitated when the first estrus, which was determined by vaginal smears, was observed. Puberty onset was advanced only in the rats fed a high-fat (24%) diet. Serum leptin levels were found to be higher (P<0.05) in the groups fed the diets with 24% and 4.3% fat compared to the rats fed the diet with 2.4% fat. Under long DL, serum leptin levels increased significantly (P<0.05), and puberty onset was delayed in the three groups. The results of the present experiment show that photoperiod affects leptin secretion and the onset of puberty independent of metabolic status in the female rats. (The experimental protocol was approved by the Firat University Ethical Committee).

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PC20

Remodelling of the left ventricle in the ageing type 2 diabetic Goto-Kakizaki rat

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Type 2 diabetes mellitus (T2DM) is associated with alterations in cardiac function, partly due to structural remodelling of the left ventricle (LV)[1]. This study investigates features of LV remodelling in 18 month old spontaneously T2 diabetic Goto Kakizaki (GK) (n=8) rats compared to age-matched Wistar controls (n=8). Blood was collected from the tail vein and blood glucose measured at time zero and 30, 60, 120 and 180 min following intraperitoneal glucose injection (2 g (kg body weight)$^{-1}$) using previously established methods[2]. Myocyte size, collagen area fraction, capillary supply and apoptosis in the LV were estimated by histo-morphometric techniques. Quantitative PCR was used to measure the relative expression of mRNA for natriuretic peptides ANP and BNP, collagen type(s) 1& 3α, elastin, fibronectin, matrix-metalloproteinase 2 (MMP2), tissue inhibitor of metalloproteinase(s) 1&4 (TIMP-1, TIMP-4), vimentin, transforming growth factor β1 (TGFβ1), connective tissue growth factor (C-TGF) and calcium handling proteins namely sarcoendoplasmic reticulum Ca^{2+}ATPase, (SERCa2a), sarcolemmal Na+/Ca2+ exchanger (NCX), ryanodine receptor (RyR) and L-type calcium channels (Cav1.2 & Cav1.3).

GK rats were moderately hyperglycaemic relative to controls, as verified by fasting blood glucose levels and glucose tolerance testing. Hypertrophy was evident by an increased heart weight to body weight ratio, myocyte diameter and expression of ANP and BNP mRNA (P<0.05, Student's *t* test). Diabetes produced alterations in sarcomeric structure and reductions in capillary density. Although cardiac pathology frequently manifested as focal scarring, myofibrillar loss, vacuolisation and large clusters of cells showing end-stage apoptosis, caspase-3 activity was unchanged in diabetic vs. control rats (P<0.05, Student's *t* test). Increased extracellular matrix (ECM) proliferation in the LV of GK rats was concomitant with an augmented expression of mRNA for collagen type(s) 1 & 3α, MMP2, elastin, TIMP-1, TGF-1 and C-TGF(P<0.05, Student's *t* test). mRNA expression for SERCA2a and the L-type calcium channels were also found to be elevated relative to controls (P<0.05, Student's *t* test) which may contribute to the altered calcium transient kinetics previously observed in this model[2]. Results indicate that chronic mild-to-moderate increases in glucose levels appear to accentuate the effects of aging in the LV and elicit a gene expression profile that promotes remodelling of the ECM.

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PC21

The effect of specific phospholipids that may have possible role in propagating or inhibiting glioma

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Cancer is one of the major causes of death in spite of a substantial increase in understanding of the molecular mechanism behind its occurrence 1. Glioma is the type of brain cancer which arises in the glial cells of the brain. Glioma is categorized into three areas namely: astrocytoma, oligodendroglioma and astro-oligodendroglioma (mixture of both) 1, 2. In our day-to-day diet, the public consume phospholipids from various sources of food products including egg, milk, soybean, offal and some vegetables. Environmental factors such as food habits could also be contributing factors of glioma. This study was designed to investigate the possible effects of dietary phospholipids in either proliferating or inhibiting the growth of glioma 3. In this study, the soy phospholipids, Lipoid S-100, Phospholipon ® 90H and L-α-phosphatidylcholine were tested individually on three different glioma cell lines namely 1321N1, GOS-3 and U87-MG.

Tissue culture techniques were employed to measure the activity of each phospholipid by *in vitro* studies. The ATP release by 1321N1, GOS-3 or U87-MG cell line treated with each soy derived phospholipid was measured after 48 hrs of incubation. On measurement using the ATP assay, the results obtained from 1321N1 and GOS-3 cell lines showed significant ($P < 0.05$) increases in growth on the treatment with either Lipoid S-100, Phospholipon ® 90H or L-α-phosphatidylcholine when compared with untreated cells and treated cells with 0.002% isopropyl alcohol (IPA). In contrast, Phospholipon ® 90H was found to enhance the growth of 1321N1 and GOS-3 cell lines and this

effect was significantly ($P < 0.05$) larger when compared to the effects of Lipoid S-100 and L-α-phosphatidylcholine. Treatment of cells with either Lipoid S-100 or Phospholipon ® 90H showed a significant ($P < 0.05$) decrease in the growth of U87-MG cell line when compared with untreated cells and cells treated with 0.002% IPA. Following treatment with L-α-phosphatidylcholine, no effect on the growth of U87-MG cell line was observed when compared with either untreated cells or 0.002% IPA treated cells. There was also a significant inhibition when compared with untreated cells. These results have indicated that soy derived phospholipids can enhance the growth of the low grade astrocytoma 1321N1 and GOS-3 cell lines and they do not support the growth of high grade glioblastoma U87-MG. Further experiments are required to determine the mechanism of action of soy derived phospholipids in either proliferating or inhibiting cancer cells.

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PC22

Effects of Momordica charantia fruit extract with the combination of temazolamide, cisplatin in the treatment of glioma cancer

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Prior to the availability of chemotherapeutic agents, dietary measures, including traditional medicines derived from plants, were the major forms of cancer treatment1. M.charantia (Family: Cucurbitaceae) also known as bitter gourd. M. charantia has many different chemical components that help medicinally. Mainly, it is the fruit part of the bitter gourd that is useful medicinally. It is made up of many different proteins and steroids that are chemically active. One of its chemicals displays cytotoxic activity and can inhibit guanylate cyclase, which is thought to be cause of psoriasis and leukemia. Another protein has been clinically shown to serve as anti-cancerous in animals. Alpha and Beta momorcharin has been tested as anti-HIV and shows to stop virulence while the host cells were unaffected2,3. This study characterizes the active ingredient (s) of M. charantia and the crude extract and investigates its potential chemotherapeutic effect with the combination of temazolamide, cisplatin in the cancer therapy. Different concentration (100 μM - 400 μM) of the crude fruit extract with (40 μM - 160 μM) of temazolamide, (50 μM - 200 μM) of cisplatin were treated (24 hrs incubation) separately with four different glioma cell lines (1321N1, GOS-3, U87-MG, and WERI-Rb1) and normal L6 muscle muscle cell line in different sets of experiments. In another series of experiment the active protein content α and β Momocharin was subsequently tested in all five cell lines including normal L6 skeletal muscle cells employing different concentrations (200 μM - 800 μM) and (40 μM - 160 μM) of temazolamide, (50 μM - 200 μM) of cisplatin in different sets of experiments using 2500 cells in each 200 μl 96 well plates. The cell viability was measured by using MTT assay kit for every 8 hrs, 16 hrs, 24 hrs. Initial results have shown that the crude extract of M.charantia and α and β Momocharin evoked a significant ($P<0.05$; Student's-t-test) decrease in cell viability for each cell line compared to untreated cells of each cancer cell line. In another sets of results there is no combined drug effect of temazolamide and cisplatin along with crude extract and α and β Momocharin had no much significant effect in cancer cell lines. In contrast, either the crude extract or α and β Momocharin had no significant effect on control L6 skeletal muscle cell line. These effects of crude extract and α and β Momocharin were dose dependent. In conclusion, the results have shown that both the crude extract and α and β Momocharin can elicit marked anti-cancer effects in different glioma cell lines, when compared to the commercially available chemotherapeutic agents. The next stage of the study is to determine the mechanisms whereby M. charantia extract can induce cell death measuring cytosolic calcium, P53, caspase- 3 activities and cytochrome c4.

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PC23

Preclinical studies on the effect of ethanolic extract of ginger on the renal antioxidant enzyme system and hormonal regulation in STZ-induced diabetic male albino rats

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Diabetes is a major socio-economical burden with serious health consequences(1). The reactive oxygen species generated in this pathology alters the internal milieu of the cellular systems paving way for metabolic disorders(2,3). The aim of the present study was to evaluate the effect of the ethanolic extract of ginger rhizome on oxidative stress in relation to hormonal regulation in kidney of streptozotocin (STZ) induced diabetic rats. The parameters like superoxide dismutase (SOD) activity, ascorbic acid (vitamin C), alpha tocopherol (vitamin E) levels, calcitriol, renin were decreased and uric acid level, glutathione-s transferase (GST), xanthine oxidase (XOD) activity were

increased in diabetic control rats. Ginger treatment (30 days) remarkably improved the tissue antioxidant defense system and all the above parameters attained near normality after treatment with ginger in STZ-diabetic rats. On the basis of the results obtained, it is concluded that the treatment of ginger may effectively normalize the impaired oxidative stress in response to hormonal changes in streptozotocin-induced diabetes than the glibenclamide-treated groups. Moreover, degenerative changes of renal cells in the diabetic group were minimized by the administration of ginger as shown by histopathological examination. Based on these findings, it is suggested that consumption of ginger is beneficial in terms of defensive action against oxidative stress.

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PC24

Does ethanol promote endoplasmic reticulum stress-induced cell death during pregnancy: a study on involvement of oxidative stress?

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When pregnant woman drink, alcohol reaches the baby through the placenta. However, the baby cannot process it as fast as the mother can, so it is exposed to greater amounts of alcohol for longer than the mother, which can seriously affect the baby's development and it may also lead to miscarriage (1,2). Oxidative stress is central to alcohol-induced cell death during pregnancy (3,4). The underlying cellular/molecular mechanisms remain unclear. The role of the endoplasmic reticulum (ER) in this process is uncertain. In ER signalling, PERK-Nrf2 and Ire-CHOP are two pathways that determine cell fate under stress (5). Using human choriocarcinoma JEG-3 cells, we explored ER stress response induced experimentally by treatment with different doses of ethanol (100 mg to 800 mg/ml) and tunicamycin (0.125 to 1 μg/ml). We demonstrated that exposure to ethanol alone had little effect on the expression of markers for ER stress; however, ethanol drastically enhanced the expression of GRP78, CHOP, ATF4, ATF6 and phosphorylated PERK and eIF2–alpha when induced by tunicamycin. We speculate that sustained ER stress may contribute to the placental dysfunction seen in human pregnancy complications. However, further studies are required to elucidate whether ethanol promotes ER stress-induced cell death in the placenta during pregnancy.

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PC27

Hypothalamic expression pattern and molecular and physiological markers indicate elevated sympathetic stimulation of metabolism in XLαs-deficient mice

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XLαs constitutes an NH_2-terminal splice variant of the stimulatory G-protein α-subunit Gsα, which is encoded by the imprinted *Gnas* locus. We previously generated a knock-out specific for the *Gnasxl* transcript (XLαs) [1], which exhibits a lean, hypermetabolic, glucose tolerant and insulin sensitive phenotype [2]. Increased catecholamines in urine and increased cAMP in brown adipose tissue (BAT) suggest elevated sympathetic nervous system (SNS) activity in XLαs deficient mice. To further define the causes of this phenotype, particularly in relation to the SNS, we are investigating the brain expression pattern, as well as additional molecular and physiological parameters. Immunohistochemistry in adult brain shows XLαs to be expressed in areas associated with SNS activity, including the paraventricular nucleus of the hypothalamus, the raphe magnus and the raphe pallidus. Other areas involved in feeding regulation and energy metabolism are also positive for XLαs, including the arcuate nucleus, the lateral and dorsomedial hypothalami, the zona incerta, the amygdala and the locus coeruleus. Co-localisation studies show that XLαs is expressed in a subgroup of Orexin positive neurons in the lateral and dorsomedial hypothalamus and in tyrosine hydroxylase positive neurones in the arcuate nucleus, but this does not account for all XLαs neurons in these regions. Q-RTPCR results of adult knock-out hypothalami show decreased expression of the ghrelin receptor, MCH receptor 1 and malonyl CoA decarboxylase, which is in line with their increased energy expenditure. We also assessed additional peripheral markers of SNS outflow. Western blotting of BAT shows increased hormone-sensitive lipase (HSL) phosphorylation in XLαs knock-outs, indicating increased sympathetic stimulation. Uncoupling protein 1 (UCP1) expression, as determined by Q-RTPCR, was also found to be increased in adult BAT [2]. However, in neonatal BAT no correlation of HSL phosphorylation (increased) and UCP1 expression (decreased) was found. This might indicate an additional cell-autonomous role of XLαs, which is transiently expressed in the neonatally developing BAT. Furthermore, using tail plethysmography under anaesthesia (1.3-2.15 g/kg urethane i.p.), adult knock-outs were found to have increased blood pressure. These results support the suggestion of increased SNS outflow in XLαs-deficient mice and provide further hints as to which central nervous system mechanisms may be involved.

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PC28

Pharmacological effect of DGAT1 inhibition on food intake and post-prandial lipaemia - determination of the mechanism of action

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Diacylglycerol acyltransferase 1 (DGAT1) functions in triglyceride synthesis and triglyceride intestinal absorption. DGAT1 catalyses the formation of triglycerides from diacylglycerol and acyl-coA and is the final committed step in the mammalian triglyceride synthesis pathway. Dietary triglycerides are emulsified and hydrolysed to fatty acids and 2-monoacylglycerol in the intestinal lumen, are then transported to the enterocyte where triglyceride is re-synthesised and incorporated into chylomicrons for secretion into lymph. DGAT1 enzyme is distributed across the metabolic tissues with high levels of mRNA expressed in human and rodent small intestine. DGAT1 global knock out mice have decreased adiposity when fed a diet high in fat and accumulate neutral-lipid droplets in the cytoplasm of the enterocyte. DGAT1 knock out mice also show a reduced rate of triacylglycerol absorption after an acute lipid challenge.
We have conducted studies to evaluate the effects of a small molecule DGAT1 inhibitor. DGAT1 inhibition produces a dose-dependent decrease in post-prandial lipaemia in Han Wistar rats administered a corn oil challenge orally (n=15, EC50 = 0.02μM). Moreover it produced a decrease in the acute intake of high fat,

but not low fat food (>50% decrease at 1 hour post-refeed, n=12, p=<0.001). Studies in DGAT1 knockout mice have shown that DGAT1 deficiency produces an increase in PYY and GLP-1 levels in response to a lipid load and these gut peptides represent one potential anorectic mechanism. DGAT 1 inhibition has, however, additional potential effects in the gastrointestinal tract. Indeed DGAT1 inhibitors divert fatty acids away from triglyceride synthesis into fatty acid oxidation in CaCo2 (human colonic cell line) (EC50 = 0.03μM, n=1) and HuTu80 (human duodenal cell line) (EC50 = 0.008μM, n=3) cells as assessed by uptake of 14C-oleate or 14C-palmitate and release of 14C-CO2 and 14C-acid-soluble products. These data suggest multiple potential effects of DGAT1 inhibition in the gastointestinal tract that may have therapeutic benefit in metabolic disease.

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PC29

The association of body mass index with gastric emptying and effect of an exercise intervention on gastric emptying and appetite in adolescent girls

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Gastric emptying (GE) plays a role in appetite regulation and has been implicated in the pathogenesis of obesity. Regular exercise may be one factor which moderates GE. We investigated (i) the relationship between GE and body mass index (BMI) and (ii) the effects of an exercise intervention on GE, appetite and food intake in adolescent girls. Nineteen healthy schoolgirls (BMI 22.1±2.1 kg m^{-2}; age 16.3±0.3 years) attended the laboratory, after giving written informed consent, on 2 occasions, at week 0 and week 8. GE was assessed by ^{13}C octanoic acid breath test, subjective appetite sensations by visual analogue scales and food intake at a buffet meal. During weeks 1 to 7 subjects attended three classes per week of either a moderate intensity exercise class (exercise group; (EXE): n = 9) or a non-exercise class (control group (CTL); n = 10). Statistical analysis was conducted using Pearson correlations and Mixed ANOVA (group x time). Increasing BMI was significantly associated with increases in GE lag (r = 0.68, p = 0.001), latency (r = 0.62, p = 0.005) and half times (r = 0.62, p = 0.005) all indicating slower gastric emptying. Exercise intervention did not significantly alter GE or food intake compared to the CTL group. GE half time significantly increased in both EXE and CTL over time (main effect, p = 0.043). Hunger ratings at 30min after the standardised test meal were reduced in the EXE group over time (-24.9 ± 29.7%) compared to a mean increase (43.3 ± 100.1%) in the CTL group (p = 0.032). Interestingly, others have reported increases in glucagon-like peptide 1 at this time point following exercise intervention. Findings indicate increasing BMI is associated with slower GE in adolescent girls. The 7 week exercise intervention reduced 30 min postprandial hunger ratings but did not significantly influence GE compared to a control group.

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PC30

The effect of a 10 week exercise intervention on gastric emptying, appetite, food intake and cardiac autonomic function in multiple sclerosis

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Multiple sclerosis is a progressive autoimmune disease in which autonomic nervous system dysfunction, delayed gastric emptying (GE) and sedentary lifestyles have been reported. Regular exercise is associated with accelerated GE in healthy adults. We investigated the effect of an exercise intervention on GE, heart rate variability (HRV; as an indicator of autonomic balance), appetite sensations and food intake in MS patients. Twelve MS patients (BMI 27.5±4.5 kg m^{-2}; age 51.3±9.5 years) attended the laboratory at baseline and post test, after giving written informed consent. Six subjects participated in a ten week exercise intervention (1 supervised strengthening exercise class in addition to independent aerobic exercise increasing from 2 to 3 sessions per week at week 5; exercise group (EXE); n = 6). Six others were instructed to maintain their habitual lifestyle over the same time period (control group (CTL); n = 6). GE was assessed by ^{13}C octanoic acid breath test, HRV in the frequency domain from resting electrocardiogram, subjective appetite sensations by visual analogue scales and food intake by food diary. Statistical analysis was conducted using Pearson correlations and Mixed ANOVA (group x time). A large inter and intra individual variability was evident in all parameters. There were no significant correlations between any variables. The exercise intervention did not significantly change GE or HRV. Mean daily energy intake increased in the EXE group by 10.4±6.4% compared to a 26.9±24.6% decrease in the CTL group over time (p = .007). Area under the curve for 4hr postprandial hunger sensations was significantly reduced in the EXE group following intervention compared to the CTL group (p=.047). Findings suggest that GE rate is not significantly associated with cardiac autonomic function in MS. The exercise intervention did not improve autonomic function as reflected by GE and HRV parameters but increased daily energy intake and paradoxically decreased postprandial hunger sensations. Further studies with larger sample sizes are desirable.

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PC31

A Prospective Study of Cardiovascular Risk Markers in Type 2 Diabetic patients

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Type 2 Diabetes mellitus (T2DM) is a complex metabolic disorder with major epidemiological impacts affecting over 200 million people worldwide. This number is expected to reach indefinite proportions by the year 2030 (Zimmet et al., 2001). Individuals with T2DM have an increased risk of cardiovascular diseases (CVD). British Medical Association has reported that CVD accounts for up to 80% mortality associated with T2DM in the United Kingdom. Type 2 diabetics (T2DPs) carry an array of risk factors including dyslipidemia, hyperglycaemia, insulin resistance and elevated concentrations of various biomarkers in their circulation, which lead to an accelerated probability of CVD (Erdmann, 2005). Consequently, rates of cardiovascular mortality and morbidity are very high in T2DPs. Since the accelerated atherosclerosis and cardiovascular diseases in diabetes are likely to be multifactorial, there is an urgent need for consideration of different therapeutic approaches. The main aim of this study is to identify a number of risk factors and biochemical parameters, which serve as predisposition factors and govern susceptibility to CVD in T2DM and to evaluate their possible roles as cardiovascular risk markers among T2DPs patients comparing them with healthy age-matched controls. Various biochemical parameters including plasma glucose, insulin, lipid profile, urea, C - reactive protein (CRP), HbA1C were analysed. Initial observations indicate a highly significant ($P<0.01$) increase in the levels of glucose (Control 4.726±0.1897 (n=19), Diabetic 11.2357±1.337 (n=14)), Hba1c (Control 5.55±0.098 (n=18), Diabetic 8.277±0.407 (n=18)), urea (Control 4.204±0.275 (n=21), Diabetic 8.616±0.881 (n=18), C- reactive protein (Control 1.470±0.2313 (n=17), Diabetic 8.441±0.591 (n=17)) in T2DPs when compared with healthy age-matched controls. Data are presented as mean ± standard error of mean (SEM). Data were compared using student's t-test and statistical significance was defined as $P<0.01$. These results clearly indicate that different biochemical markers may be used for the assessment of CVD risk in T2DM patients. Further studies following these preliminary findings are currently in development and are focussed on age-related changes that would probably predispose T2DPs to CVD in future. This study will assist in the development of potential clinical strategies for the impediment and management of CVD in a high risk population.

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Abstracts

SA01

Therapeutic relevance of the entero-endocrine system

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Obesity is now classified as an epidemic by the World Health Organisation and represents one of the greatest threats to the health of the developed world. It is estimated that there are over one billion overweight adults worldwide and an estimated 22 million children under 5 are obese, with annual figures rising sharply. Obesity increases the risk of heart disease, type 2 diabetes, stroke, some forms of cancer, arthritis, respiratory disease and translates into healthcare costs of over half a billion pounds every year in the UK alone. The obesity crisis in the developed world is largely attributed to the easy availability of cheap, calorie dense, palatable food accompanied by an increasingly sedentary lifestyle. The current crisis has prompted public health initiatives to improve diet and promote exercise, however these have proved largely ineffective, highlighting the urgent need for improved therapies for the treatment of obesity.

Weight regulation in individuals depends on energy intake (in the form of food) and energy expenditure. Hunger leads to initiation of eating and when a meal is ingested, satiety hormones contribute to digestion and a feeling of fullness. Central circuits in the brain integrate satiety signals and signals of long term energy status to produce a coordinated response to the change in nutritional status. The nuclei of the hypothalamus and brainstem are important regions for regulation of energy homeostasis. The actuate nucleus (ARC) can access signals from the periphery. The signals act on two distinct neuronal populations. One population co-expresses the orexigenic Agouti-related Peptide (AgRP) and Neuropeptide Y (NPY); the other population releases cocaine and amphetamine regulated transcript (CART) and proopiomelanocortin (POMC), which inhibit feeding. Both of these populations project to the paraventricular nucleus (PVN) and other nuclei involved in energy regulation.

Gut hormones are released in response to a meal acting on both central neural circuits and peripheral tissues. They affect diverse physiological functions including appetite, gastrointestinal motility and acid secretion, nutrient absorption and cell proliferation. Receptors for gut hormones can be found on neuronal populations within the actuate nucleus of the hypothalamus. Recent work has identified the gut hormones PYY, oxyntomodulin and pancreatic polypeptide (PP) that inhibit appetite, and ghrelin, which stimulates appetite. These hormones occur naturally, are active within the plasma range observed in humans and may therefore represent a novel approach to the treatment of obesity. Our continuing research has identified oxyntomodulin and PYY as strong candidates for therapeutic agents.

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SA02

Incretins and bariatric surgery

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Bariatric surgery operations were designed to promote weight loss. However, it was soon realized that these operations were also associated with impressive and sustained improvements in type 2 diabetes mellitus (T2DM). In fact, it has recently been recognized by the American Diabetes Association that bariatric surgery has the potential to offer complete remission of a T2DM. Certainly, weight loss plays an important role in the amelioration of T2DM following bariatric surgery. Nonetheless, mounting evidence shows that certain intestinal diversionary operations improve T2DM through mechanisms beyond weight loss and reduced caloric intake. Although the precise weight-independent mechanisms mediating T2DM remission are not yet clear, it has been proposed that changes in the secretion of gastrointestinal hormones secondary to the rearrangement of the gastrointestinal tract anatomy could be involved. In that respect, two different theories have been put forward. According to the hindgut hypothesis, the bypass of the proximal gut results in an expedited delivery of ingested nutrients to the lower bowel accentuating the secretion of GLP-1. Consequently, glucose tolerance would be enhanced shortly after surgery by virtue of the stimulatory effects of GLP-1 on insulin secretion, and its inhibitory effects on glucagon secretion. In the long run, GLP-1 would also enhance glucose tolerance by increasing the pancreatic beta cell mass. In contrast, the foregut hypothesis proposes that it is the exclusion of the duodenum from contact with ingested nutrients what exerts direct antidiabetes effects. The absence of nutrient contact with the duodenum and proximal jejunum would preclude the secretion of an unidentified pro-diabetogenic factor (anti-incretin). Several studies have now demonstrated that gastric bypass surgery is associated with a marked increase in the GLP-1 secretory response to peroral nutrient stimulation in non-diabetic and diabetic subjects. It has been shown that the exaggerated GLP-1 response is independent of weight loss, and is associated with remission of T2DM. Interestingly, we have shown that GLP-1 could also be involved in the resolution of T2DM following sleeve gastrectomy. Therefore, there is compelling data to suggest that GLP-1 could be a contributing factor accounting for the early resolution of T2DM independent of the bypass of the proximal intestine. The long-term effects of the increased GLP-1 secretion following bariatric surgery in human subjects are yet to be clarified. Elucidating the mechanisms underlying the metabolic benefits of bariatric surgery could be critical to identify novel anti-diabetic therapeutic targets and/or to propose safer modalities of metabolic surgery.

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SA03

Gut microbiome, lipid metabolism and inflammation

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Over the last decades the nutritional status has changed where a fat-enriched diet progressively replaced dietary fibers. This is now considered a leading cause for the growing occurrence of metabolic diseases. An increased inflammatory tone is defined as a cornerstone of all metabolic diseases and is characterized by an augmented circulating and tissue cytokine concentration associated with an increased infiltration of cells from the innate immune system. However, the progressive onset of the disease in patients feeding on a fat-enriched diet leading to inflammation had no molecular origin. In the quest of a factor triggering inflammation we demonstrated that changes of intestinal metagenomic (i.e. bacterial genome) profiles occurred after a short period of high-fat feeding and were associated with an increased plasma concentration of lipopolysaccharides (LPS). Using genetic, antibiotic and pharmacological approaches in mice we causally demonstrated the role of intestinal microflora and LPS as triggering factors of adipose tissue, liver and muscle inflammation, leading to the onset of insulin resistance, overt hepatic glucose production, and fat pad enlargement. We next showed that LPS could directly target the adipose fat pads in mice with wild type or with CD14 inactivated adipose depots i.e. the LPS receptor. We also demonstrated that the origin of plasma LPS was linked to an increased leptin-regulated intestinal bacterial translocation process. Hence, a change in intestinal microflora induced by a fat-enriched diet was causally linked to plasma LPS concentration and metabolic inflammation at the onset of the development of metabolic diseases.

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SA04

Gut-expressed sweet taste receptor and regulation of intestinal glucose transport

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Many of the receptors and downstream signalling elements involved in taste transduction are also expressed in enteroendocrine cells where they underlie the chemosensory functions of the gut. We showed, for the first time, that taste receptor 1, T1R, family members, T1R1, T1R2 and T1R3 are expressed in the intestinal epithelium, and proposed that the sweet taste receptor, T1R2+T1R3, functions as the luminal sugar sensor. Recent work in our laboratory has determined that T1R2+ T1R3, and the partner G-protein, gustducin, are co-expressed in glucagon like peptide 1 (GLP-1), glucose-dependent insulinotropic peptide (GIP) and serotonin containing endocrine cells. We have been interested in the role played by the intestinal sweet taste receptor in regulation of the intestinal glucose transporter, Na+/glucose cotransporter 1, SGLT1. SGLT1 is the major route for the transport of dietary sugars from the lumen of the intestine into enterocytes. Regulation of this protein is essential for the provision of glucose to the body and, thus, is important for maintenance of glucose homeostasis. We demonstrated that dietary sugars and artificial sweeteners increase SGLT1 mRNA and protein expression and glucose-absorptive capacity in wild type mice, but not in T1R3, or gustducin, knockout mice, indicating that T1R3 and gustducin are required for enhanced expression of SGLT1 in response to lumenal sugars and sweeteners.

The findings that gustducin and T1Rs reside in enteroendocrine cells, whereas SGLT1 is expressed in neighbouring absorptive enterocytes implies a signalling event taking place between the chemosensory enteroendocrine cells and absorptive enterocytes. I shall discuss progress made in understanding the mechanisms underlying this cell to cell communication.

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SA05

Electrophysiology of the L-cell

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Glucagon like peptide 1 (GLP-1) is released from enteroendocrine L-cells in the gut epithelium and plays an important role in post-prandial glucose homeostasis and appetite control. Following the successful introduction of GLP-1 mimetics and DPP4 inhibitors to treat type 2 diabetes, attention is now turning towards the L-cell, to address the potential benefit of stimulating the endogenous release of L-cell peptides in vivo. These cells release not only GLP-1 but also the anorexigenic peptides, PYY and oxyntomodulin, as well as GLP-2 which stimulates epithelial regeneration and repair. Understanding the mechanisms underlying secretion from L-cells is key to discovering ways to target the cells therapeutically. In vitro studies on L-cell physiology, previously largely restricted to the use of cell lines, can now be performed on primary intestinal cultures from transgenic mouse lines in which GLP-1 secreting cells are identifiable by their expression of a fluorescent protein.

Primary L-cells are electrically active and nutrient responsive. The pattern of action potential firing is determined by tetrodotoxin-sensitive voltage-gated sodium currents. Secretion, by contrast, is dependent on voltage-gated calcium currents which are largely carried by L-type and Q-type calcium channels.

Agents that stimulate GLP-1 secretion employ a number of transporter and receptor pathways, including electrogenic nutrient uptake, metabolism and G-protein coupled receptor activation. Targeting these pathways provides potentially novel strategies to increase L-cell secretion in vivo. GPR119 and TGR5 are two Gs coupled receptors that are highly expressed in L-cells. Agonists of both receptor types result in elevated cAMP

in L-cells, with downstream effects on membrane potential and hormone secretion.
Although the new protocols for studying single primary enteroendocrine cells provide novel avenues for the discovery of L-cell specific drug targets, definitive trials are now required to demonstrate that L-cell stimulation will translate into a clinically useful therapeutic strategy.

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SA06

Lipid sensing in the L cell

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Stimulus-secretion coupling in the enteroendocrine L cell leads to release of the proglucagon-derived peptides, glucagon-like peptide-1 (GLP-1), GLP-2 and oxyntomodulin, as well as of peptide YY. These biologically-active peptides play diverse roles in the physiological response to nutrient ingestion, including regulation of glycemia, satiety and intestinal function. Although stimulated by a diversity of ingested nutrients, the L cell is notably sensitive to fatty acids, with a marked specificity for long-chain monounsaturated fats such as oleic acid. Consistent with these findings, diets that are rich in oleic acid, such as the Mediterranean diet, increase circulating levels of GLP-1 in association with improved glycemic control, in both rodents and humans. Recent studies have now begun to elucidate the mechanism of action of fatty acids on the intestinal L cell, with a number of different signaling pathways acids now being implicated, including the Galpha q-linked G protein-coupled receptors, GPR40 and GPR120, the Galpha s-coupled receptor, GPR119, and the intracellular enzyme, protein kinase Czeta. Expression of a variety of different fatty acid transport proteins, as well as of the bile acid receptor, TGR5, further illustrates the complexity of the intestinal L cell response to ingestion of fat. Collectively, these findings have piqued interest in potential therapeutic applications for L cell secretagogues. Notwithstanding, the large number of biologically–active peptides produced by the L cell must be taken into account in any such considerations.

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SA07

PYY and the regulation of food intake

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The last decade has witnessed a marked increase in our understanding of the importance of gut hormones in the regulation of energy homeostasis. In particular, the discovery that the gut hormone peptide YY 3-36 (PYY3-36) reduced feeding in obese rodents and humans fuelled interest in the role of PYY3-36 in body weight regulation. Pharmacological and genetic approaches have revealed that the Y2-receptor mediates the anorectic effects of PYY3-36 whilst mechanistic studies in rodents identified the hypothalamus, vagus and brainstem regions as potential sites of action. More recently, using functional brain imaging techniques in humans, PYY3-36 was found to modulate neuronal activity within hypothalamic and brainstem, and brain regions involved in reward processing. Several lines of evidence suggest that low circulating PYY concentrations predispose towards the development and or maintenance of obesity. Subjects with reduced postprandial PYY release exhibit lower satiety and circulating PYY levels that correlate negatively with markers of adiposity. In addition, mice lacking PYY are hyperphagic and become obese. Conversely, chronic PYY3-36 administration to obese rodents reduces adiposity, and transgenic mice with increased circulating PYY are resistant to diet-induced obesity. Moreover, there is emerging evidence that PYY3-36 may partly mediate the reduced appetite and weight loss benefits observed post-gastric bypass surgery. Taken together these findings, coupled with the retained responsiveness of obese subjects to the effects of PYY3-36, suggest that targeting the PYY system may offer a therapeutic strategy to help treat obesity.

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SA08

Gastric inhibitory polypeptide and its role in insulin regulation and metabolic disturbances in obesity

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Recognition of the importance of gut hormones in the insulin response to feeding was quickly followed by isolation of the first incretin, gastric inhibitory polypeptide (GIP). This 42 amino acid peptide, together with it's sister incretin hormone glucagon-like peptide 1 (GLP-1), is released into blood following feeding and exerts various actions on nutrient homeostasis despite rapid degradation by the enzyme dipeptidyl peptidase-4 (DPP-4). GIP acts in several distinct ways to lower blood glucose, the most notable being stimulation of insulin secretion. Insulin release occurs in a glucose-dependent manner, serving to minimise the risk of hypoglycaemia. GIP also enhances the growth, differentiation, proliferation and survival of pancreatic beta cells. These actions are complemented by effects in peripheral tissues including inhibition of hepatic glucose production, depression of insulin clearance and promotion of glucose uptake. Recently, other important and initially unsuspected actions have been uncovered for GIP, including central neuroprotection and promotion of bone formation. However, particularly interesting is the finding of GIP receptors on adipocytes and appreciation that GIP secreted strongly in

response to fat ingestion may be involved in translation of excessive amounts of dietary fat into adipocyte tissue stores. Established effects of GIP on adipocytes include increase of lipoprotein lipase, stimulation of lipogenesis, enhancement of fatty acid and glucose uptake, augmentation of insulin-induced fatty acid incorporation and inhibition of both glucagon- and adrenergic receptor mediated lipolysis. These actions open up an unexpected therapeutic channel of exploiting GIP receptor antagonism for treatment of obesity and associated insulin resistance. This scenario is borne out by studies in high fat fed mice or ob/ob mice with either genetic knock-out of GIP receptor or diminished GIP action. Further studies are needed to evaluate the applicability to human obesity-diabetes. However, rapid cure of diabetes in grossly obese subjects undergoing Roux-en Y bypass surgery may be partly mediated by surgical bypass of GIP secreting K-cells in upper small intestine. This operation results in altered patterns of gherlin and GLP-1, but also low levels of circulating GIP with restoration of normoglycaemia due to substantial improvement of insulin resistance and beta cell glucose responsiveness.

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SA09

CART is a regulator of islet function and a possible incretin hormone

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CART (cocaine- and amphetamine-regulated transcript) is an anorexigenic peptide widely expressed in the central and peripheral nervous systems as well as in islet endocrine cells and nerve fibers. CART knock out mice display impaired glucose-stimulated insulin secretion *in vivo* and *in vitro*, together with impaired glucose elimination and reduced expression of GLUT-2 and PDX-1. In addition, a mutation in the human CART gene cosegregates with obesity and type 2 diabetes. CART regulates islet hormone secretion from isolated rat islets and is upregulated in the beta cells of type 2 diabetic rodents.
More recently, we have studied the effect of CART on insulin secretion *in vivo* in mice. In addition, we have examined CART expression in human pancreas and GI-tract. Furthermore, we have studied regulation of beta cell CART expression *in vivo* in rats as well as in clonal beta cells. Peripherally administered CART lowers plasma glucose and enhances glucose stimulated insulin secretion after an intra-venous glucose tolerance test (IVGTT) in mice. CART increases glucose stimulated insulin secretion from isolated mouse islets stimulated with an array of secretagogues. CART is markedly upregulated in the beta cells of rats made type-2 diabetic with daily injections of dexamethasone; this is prevented by daily insulin treatment. The gene expression and protein levels of CART in INS-1 (832/13) cells seem to be regulated by both glucose and glucocorticoids. Interestingly, CART mRNA and protein are expressed also in human islet cells and in nerve fibers innervating the islets. Furthermore, CART is a constituent of the gastrin producing G-cells in the antrum of the stomach as well as of endocrine cells in the upper small intestine, paving the way for CART as an incretin hormone.
In conclusion, CART may play important roles in glucose homeostasis and in the pathophysiology of type 2 diabetes.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA10

The endocrinology of ghrelin

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Ghrelin, a peptide hormone first discovered as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R), is predominantly produced and released into the circulation by ghrelin-cells (X/A-like) of the stomach fundus cells. Ghrelin has multiple actions in multiple tissues. In particular, it is the most potent known endogenous orexigenic peptide, and plays a significant role in glucose homeostasis: deletion of the genes encoding ghrelin and/or its receptor prevents high-fat diet from inducing obesity, increases insulin levels, enhances glucose-stimulated insulin secretion and improves peripheral insulin sensitivity. In addition, stimulation of pituitary hormones secretion, regulation of gastric and pancreatic activity, modulation of cardiovascular and hemodynamic activities, cartilage and bone homeostasis, sleep and behavioral influences, and modulation of the immune system, as well as effects on cell proliferation, are other relevant actions of ghrelin.
The orexigenic action of ghrelin depends on hypothalamic GHS-R 1a which is expressed in several neuronal populations well known for their involvement in the control of food-intake. The work carried out by different groups over the last few years have uncovered the molecular mechanisms involved in ghrelin orexigenic effect. We have recently reported that central (ICV) administration of ghrelin decreases hypothalamic de novo fatty acid synthesis and increases fatty acid oxidation through selective activation of AMPK and CPT1, respectively. Importantly, the orexigenic effect of ghrelin was mediated by this hypothalamic AMPK/malonyl-CoA/CPT1 axis; in fact, pharmacologic

or genetic inhibition of AMPK or CPT1 blunted ghrelin feeding-promoting effects. The molecular details of this interaction will need further investigation, but recent data have demonstrated that they were linked to 1) calmodulin-dependent protein kinase kinase 2 (a upstream kinase of AMPK), and 2) uncoupling protein 2 (UCP2) and hypothalamic free radicals. This, AMPK-driven changes in hypothalamic lipid metabolism will subsequently influence neuropeptide gene-expression possibly through the transcription factor Bsx. Finally, recent evidences indicates that ghrelin acting at central level influence peripheral lipid metabolism in liver and white adipose tissue.

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SA11

Gut peptides and responses to infection and inflammation

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The role of gut peptides such as CCK in the physiological regulation of energy homeostasis has been the main driver of recent enteroendocrine (EEC) research. However, apart from their contribution to maintaining mucosal integrity, little is known about the role of EEC in the response to intestinal inflammation. Gut inflammation is associated with reduced food intake and nutritional compromise. We have established that increased activity in the EEC system is a central component of this. In humans with upper gut infection plasma levels of the peptide CCK are significantly elevated, returning to normal following antimicrobial therapy. Modelling this in nematode models of murine enteritis has confirmed a role for CCK in the hypophagic response operating via the CCK1 receptor, presumably vagal. CCK cell hyperplasia is observed during the inflammatory response. This is directly effected by the immune response: immunoneutralisation of CD4+ T-lymphocytes abolishes both hypophagia and EEC hyperplasia, in an IL-4/13 dependent manner. This does not occur in immunodeficient SCID or nude mice, but the response can be adoptively transferred from immunocompetent animals and is independent of TNF-α. In addition, the EEC cell line STC-1 shows enhanced CCK secretion in response to nutrients when exposed to pro-inflammatory mediators. Preliminary data also indicate amplification in EEC function in human inflammatory bowel disease. These findings indicate that EEC may present therapeutic targets in the aetiology and management of reduced food intake observed in GI disease.

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SA12

Gut peptides and signalling to the CNS

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The endocrine cells of the gut constitute a luminal surveillance system selectively responding to either the presence or absence of food in the gut lumen. Collectively, their secretory products regulate the course of digestion and determine nutrient delivery to the gut by controlling food intake. In both cases the CNS is a major target. Although some gut regulatory peptides may act directly on CNS neurons, there is an established body of evidence to indicate activation of CNS pathways as a consequence of stimulation of vagal afferent neurons. Important consequences of stimulation of these neurons after a meal include inhibition of food intake and activation of autonomic reflexes regulating gastric and pancreatic secretion, gut motility, intestinal immune responses and gastric cytoprotection. Vagal afferent neurons express receptors for intestinal satiety peptides such as cholecystokinin (CCK), GLP-1 and PYY3-36 ie CCK1R, GLP1R and Y2R, respectively. Cholecystokinin stimulates vagal afferent neuron discharge; this effect is potentiated by leptin and by gastric distension, and inhibited by the gastric orexigenic peptide ghrelin. In addition, CCK controls the expression of both G-protein coupled receptors and peptide neurotransmitters by vagal afferent neurons. When plasma CCK concentrations are low, for example after fasting, the expression by these neurons of cannabinoid (CB)1 and melanin concentrating hormone (MCH)1 receptors is increased, as is that of the neuropeptide transmitter MCH; all three are associated with stimulation of food intake. Secretion of CCK leads to a rapid down-regulation of expression of CB1, MCH1R and MCH and to increased expression of Y2R and of the neuropeptide transmitter, cocaine and amphetamine regulated transcript (CARTp), both of which are associated with inhibition of food intake (1-3). Ghrelin blocks these actions of CCK at least in part by excluding phosphoCREB from the nucleus. Thus, vagal afferent neurons (a) are capable of integrating different peripheral signals known to control food intake and (b) exhibit different neurochemical states depending on previous nutrient ingestion. In the fasted state there is enhanced capacity for orexigenic signalling and in the fed state there is enhanced capacity for anorexic signalling. Cholecystokinin acts as a gatekeeper switching vagal afferent neurons between these states and so determining their capacity to respond to other signalling molecules produced by the gastrointestinal tract.

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SA13

GLP-1 agonism: from physiology to pharmacology. Incretin physiology and pathophysiology

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In healthy subjects, the incretin effect ensures that plasma glucose concentrations after oral carbohydrates remain low regardless of the amount of glucose taken in. It is due to proportional increases in GLP-1 and GIP secretion and corresponding insulin responses. We studied this phenomenon in obese subjects with T2DM and matched controls and found that incretin release was unimpaired and proportional to the glucose load, but in T2DM the effect on insulin was dramatically reduced regardless of load. We also identified gastric emptying rates of glucose to contribute to both the release of incretin hormones and to regulation of postprandial glycaemia. The incretin effect was also reduced in diabetes secondary to chronic pancreatitis suggesting that the loss is secondary to diabetes. In further studies, we were able to identify that obesity and glucose intolerance are associated with the loss, and a similar loss could be brought about in perfectly healthy subjects during glucocorticoid induced insulin resistance and glucose intolerance. In patients with T2DM, postprandial GLP-1 secretion is generally impaired, and several factors including BMI, insulin resistance and poor metabolic control are associated with this. The insulinotropic actions of physiological amounts of GLP-1 and GIP are lost, but pharmacological amounts of GLP-1 can still normalize glucose induced insulin secretion and glucagon suppression. As a result, pharmacological doses of GLP-1 may greatly improve diabetic metabolism. It can be demonstrated that insulinotropic and glucagonostatic effects of GLP-1 contribute equally to its glucose lowering action. The suppression of glucagon may be one of the most important actions of GLP-1 as indicated from studies of the effects of the antagonist exendin 9-39. Recent studies of GLP-1 secretion in patients with RYGB suggest that hypersecretion of GLP-1 plays a prominent role in the antidiabetic actions of this operation. Convincing clinical results with GLP-1 agonists support this view.

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