C12

Distinct signalling by leptin and insulin in a neuropeptide Y-expressing neuronal cell line

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Leptin and insulin regulate transcription and alter the excitability of various arcuate nucleus (ARC) neuronal subpopulations. One subtype is the orexigenic neuropeptide Y (NPY) expressing neurons, which are inhibited by leptin and insulin. Obesity is associated with CNS leptin and insulin resistance, which may be due to defective signalling within target neurons. Leptin and insulin stimulate various signalling pathways in ARC neurons, including the phosphoinositide-3 kinase (PI3K) pathway, and affect actin dynamics, in a PI3K-dependent manner, to alter the electrical activity of certain neurons (Mirshamsi et al. 2004). However, these actions have not been investigated within defined ARC neuronal subtypes. The NPY-expressing neuronal cell line (N29/4) was cultured as described (Belsham et al. 2004). For immunoblotting, cells were treated with leptin or insulin for various times in the absence and presence of the PI3K inhibitors LY294002 or wortmannin or the F-actin stabilising agent, jasplakinolide. Levels of the PI3K product phosphatidylinositol 3,4,5-trisphosphate (PIP3) were determined by staining with the pleckstrin homology domain of the general receptor for phosphoinositides-1 protein fused to GFP, and F-actin examined as described by Mirshamsi et al. (2004). Leptin (10 nM) and insulin (1 nM) increased levels of phosphorylated mitogen activated protein kinase (p-MAPK), protein kinase B (p-PKB) and its downstream target glycogen synthase kinase 3 (p-GSK3) (n = 8), and phosphorylation of the latter two proteins was dependent on PI3K activity (n = 4). Stimulation of p-PKB and p-GSK3 levels by insulin was sustained (up to 60 minutes examined; n = 8). In contrast leptin induced a transient increase in p-PBK and p-GSK3, lasting less than 15 minutes (n = 8). Insulin induced a sustained increase in PIP3 (up to 60 minutes; n = 3) whereas the leptin increase in PIP3 was transient (< 15 minutes; n = 3). F-actin staining revealed that insulin (10 nM) did not alter F-actin levels (n = 4) whereas leptin (10 nM) caused rapid (within 15 minutes) F-actin disruption (n = 6). Leptin-driven increased p-PKB and p-GSK3 remained transient after stabilisation of F-actin with jasplakinolide (n = 4). Therefore, the change in F-actin is not responsible for the transient nature of the leptin PI3K response. The differences in leptin and insulin signalling, via the PI3K pathway, observed in this hypothalamic cell line, may be relevant to the mechanism by which leptin and insulin induce differential actions on excitability of ARC neurons (Choudhury et al. 2005).

Belsham DD et al. (2004) Endocrinology 145, 393-400.

Mirshamsi S et al. (2004) BMC Neuroscience 5, 54.

Choudhury AI et al. (2005) J Clin Invest 115, In press.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C13

PTEN inhibition of leptin-induced F-actin disruption in hypothalamic cells is independent of its lipid phosphatase activity

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Leptin hyperpolarizes certain hypothalamic neurons by increasing ATP-sensitive K (K_{ATP}) channel activity, an action that requires F-actin re-organization in a phosphoinositide 3-kinase (PI3K)-dependent manner (Mirshamsi et al. 2004). PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a dual (protein and lipid) phosphatase, with the lipid phosphatase function negatively regulating PI3K-dependent signalling (Leslie & Downes, 2004). The protein phosphatase activity and the C-terminal C2 domain of PTEN are also implicated in its biological activity. As over-expression of PTEN inhibits leptin-induced F-actin re-organization in hypothalamic cells (Ning et al. 2004a), we examined which component of PTEN is responsible.

Hypothalamic cell lines GT1-7 and N29/4 were cultured and plated out as described (Mirshamsi et al. 2004; Belsham et al. 2004). Wild-type and mutant PTEN-GFP constructs were introduced to cells by transfection. PTEN siRNA was designed and used as described by Ning et al. (2004b). Leptin, wortmannin or LY294002 application, F- and G-actin staining, immunoblotting and image acquisition by confocal microscopy were performed as described previously (Mirshamsi et al. 2004). Data are expressed as mean±S.D. of at least 12 cells from 4 experiments, expressed as a ratio of control and analysed by Student's paired t test.

Over-expression of wild-type PTEN decreased (to 0.35±0.11), and depression of endogenous PTEN by siRNA (n = 4) or dominant negative PTEN C124S (lipid and protein phosphatase inactive; n = 4), increased levels of the PI3K product phosphatidylinositol 3,4,5-trisphosphate (PIP3) by 2.8±1.0 and 3.3±1.5, respectively ($P<0.05$). The increased PIP3 was associated with reduced F-actin levels, to 0.27±0.12 and 0.36±0.11, respectively ($P<0.05$), and prevented by PI3K inhibition (n = 4). The C2 domain per se had no effect on PIP3 or F-actin and did not block leptin action. In contrast, over-expression of the PTEN G129E mutant (lipid phosphatase inactive, protein phosphatase active), increased PIP3 (by 3.3±1.4), did not change F-actin levels but inhibited leptin-induced reduction of F-actin (leptin: 0.32±0.09, G129E and leptin: 0.98±0.14; $P<0.05$). This latter effect was prevented by deletion of the PDZ (PSD-95/Dig/ZO-1) interacting motif of PTEN G129E, but not by PDZ motif deletion of C2 or wild-type PTEN (n = 4).

Thus, PTEN protein phosphatase activity inhibits leptin-induced rearrangement of the actin cytoskeleton in hypothalamic cells by virtue of PDZ motif membrane targeting.

Belsham DD et al. (2004). Endocrinology 145, 393-400.

Leslie NR & Downes CP (2004). Biochem J 382, 1-11.

Mirshamsi S et al. (2004). BMC Neurosci 5, 54.

Ning K et al. (2004a). J Physiol 557P.

Ning K et al. (2004b). J Neurosci 24, 4052-60.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C14

Reversal and reoccurrence of insulin resistance in streptozotocin-treated, hyperglycaemic rats

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The streptozotocin-induced diabetes mellitus (STZ-DM) rat model is commonly used to study diabetes. However, it differs from human diabetes in its insulin resistance. This study was designed to elucidate if insufficient replacement with long-acting insulin improves the insulin sensitivity in the STZ-DM rat. Male Sprague-Dawley rats were injected with streptozotocin (55mg (kg body wt)$^{-1}$), resulting in a sustained hyperglycaemia (22.5±1.0 mM) measured using a glucose oxidase method on blood obtained from the cut tips of the tails (10-15 µl). After treating the rats with vehicle for 14 days, their blood glucose (BG) had reached 26.1±1.1, and the response to an injection of fast-acting insulin (5 IE (kg body wt)$^{-1}$) was investigated. Thereafter, the rats were treated with long-acting insulin for 7 days (5 IE (kg body wt)$^{-1}$ day^{-1}), whereby BG decreased to 19.4±2.7. The response to fast-acting insulin was again recorded. After treating the rats with vehicle for a last set of 7 days, BG had increased to 26.9±1.7. The effect of fast-acting insulin was investigated to evaluate if resistance had reoccurred. Animals were humanely killed at the end of the experiments.

The reduction in BG was more pronounced when pre-treated with long-acting insulin for a week (Day 22, 61%) as compared to the untreated groups (Day 15 and 28, 23% and 34%, respectively). When pre-treated with long-acting insulin the rats had a longer duration of effect in response to fast-acting insulin (150 min, as opposed to 90 min in the vehicle-treated rats). There was no difference between the vehicle-treated rats (Day 15 and Day 28) in their response to fast-acting insulin.

In summary, insulin resistance developed in the STZ-DM rats, leading to a both brief and modest response to fast-acting insulin. The insulin resistance was reversed by treatment with long-acting insulin, but rapidly redeveloped after ceasing treatment. This should be taken into consideration when using this animal model.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C15

Prolactin-releasing peptide (PrRP) mediates CCK-induced satiety in mice

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We have previously shown that central administration of prolactin-releasing peptide (PrRP) inhibits food intake and body weight gain in rats (Lawrence et al. 2000), and have suggested that PrRP, acting through its putative receptor GPR10, may play a role in mediating the central anorexigenic effects of cholecystokinin (CCK) (Lawrence et al. 2002). Here we examine the effects of CCK and PrRP on feeding in mice, including animals in which the PrRP-receptor gene has been disrupted (GPR10-/-).

Intracerebralventricular (i.c.v.) administration of PrRP (1-4 nmol, in 1µl) to wild type C57B6/J mice, was found to inhibit feeding in a dose-dependent manner under both fasted and ad libitum-fed conditions. Over the first hour of feeding, PrRP (2nmol) reduced feeding in pre-fasted mice by approximately 40% (vehicle: 0.53 ± 0.05g, PrRP: 0.32 ± 0.05g; $P < 0.05$) and over 50% in ad libitum-fed mice (vehicle: 0.26 ± 0.03g, PrRP: 0.12 ± 0.01g; $P < 0.05$). Food intake was inhibited to a similar degree following intraperitoneal (i.p.) administration of CCK (8µg/kg). Furthermore, as shown previously in the rat, immunohistochemical detection of c-fos protein synthesis, a common marker of neuronal activation, revealed some overlap in the brain regions activated by icv administration of PrRP and i.p. administration of CCK.

GPR10-/- mice demonstrate adult-onset obesity, exhibiting a significantly higher mean body weight at 18 weeks of age (GPR10-/-: 41.3 ± 1.6g; GPR10+/+: 34.6 ± 1.0g; $P < 0.01$), and significantly higher epididymal fat pad mass (GPR10-/-: 1.3 ± 0.2g; GPR10+/+: 0.6 ± 0.1g; $P < 0.01$). In contrast to the observations made in wild type mice, neither PrRP (2nmol, i.c.v.) nor CCK (15µg/kg, i.p.) inhibited food intake in GPR10-/- mice. These findings suggest that PrRP is involved in regulating the balance between food intake and energy expenditure, and may be a key intermediary in the central satiating actions of CCK.

All animals were humanely killed at the termination of chronic experiments.

Lawrence et al. (2000). Nat Neurosci 3, 645-647.

Lawrence et al. (2002). Endocrin 143, 360-367.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA14

Central pathways involved in regulation of body weight

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The development of obesity, a condition facilitated by the availability and increasing consumption of high caloric food and beverages as well as a general decrease in physical activity, has become a rapidly increasing health problem. Obesity and overweight increases the risk for developing diseases that shorten life, including type 2 diabetes, coronary heart disease, hypertension and cancer. Progress in understanding the precise and powerful biological mechanisms that maintains body weight within a relatively narrow range is crucial for future therapeutic strategies that may help to prevent and treat obesity. Over the past two decades, major advances have been made to clarify the neuronal pathways, chemical mediators and signal transduction mechanisms, which contribute to the process of energy homeostasis. A primary central nervous site for control of energy balance is the hypothalamus. Several peripheral tissues produce peptides/hormones that act via the brain, the arcuate nucleus in particular, to regulate short- and long-term food intake and energy expenditure. At least 25 transmitters have been suggested to play key roles in feeding behavior. During the last two decades attention has been focused on the role of different neuropeptides in hypothalamic control of feeding behavior. Some of the transmitter molecules that influence feeding behavior and which are produced in hypothalamic cell bodies will be reviewed, with special emphasis on GABA and glutamate, two relatively neglected regulators of energy balance. Hypothalamic transmitter molecules that participate in the control of ingestive behavior are primarily produced in neuronal cell bodies of the arcuate nucleus and/or the lateral hypothalamic area. These neurons send projections to a large number of regions of the central nervous system. Apart from producing orexigenic or anorexigenic compounds of peptidergic nature, the hypothalamic neurons also produce excitatory and inhibitory amino acid neurotransmitters. The role of GABA and glutamate in regulating energy balance have received less attention in comparison to neuropeptides. The arcuate nucleus-median eminence area, a region with a weak blood-brain barrier, contains at least two neuronal cell populations that extert opposing actions on energy balance. The majority of the neurons located in the ventromedial aspect of the arcuate nucleus, which produce the orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AGRP), contain in addition the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) and the vesicular GABA transporter (VGAT), thereby supporting their GABAergic nature. Some neurons producing pro-opiomelanocortin (POMC)- and cocaine- and amphetamine-regulated transcript (CART), located in the ventrolateral division of the arcuate nucleus have recently been reported to contain the vesicular glutamate transporter 2 (VGLUT2), a marker for glutamatergic neurons. In the lateral hypothalamic area, hypocretin/orexin neurons express VGLUT1 or VGLUT2, but not GAD, whereas some melanin-concentrating hormone (MCH) cells contain GAD. These observations support the view that GABA and glutamate, two relatively neglected feeding transmitters candidates, are present in key neurons that regulate body weight and consequently represent important orexigenic/anorexigenic mediators that convey information to other neurons within the hypothalamus as well as from the hypothalamus to other brain regions that participate in regulation of energy balance.

van den Pol AN. Weighing the role of hypothalamic feeding neurotransmitters. Neuron 2003; 40:1059-1061.

Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev 1999; 20:68-100.

Collin M, Backberg M, Ovesjo ML, Fisone G, Edwards RH, Fujiyama F, Meister B. Plasma membrane and vesicular glutamate transporter mRNAs/proteins in hypothalamic neurons that regulate body weight. Eur J Neurosci 2003; 18:1265-1278.

Hentges ST, Nishiyama M, Overstreet LS, Stenzel-Poore M, Williams JT, Low MJ. GABA release from proopiomelanocortin neurons. J Neurosci 2004; 24:1578-1583.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA15

Gut Regulators

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Obesity is the main cause of premature death in the UK and worldwide its prevalence is accelerating rapidly. Our understanding of the physiological systems that regulate food intake and body weight has increased immensely over the past decade. Weight regulation in individuals depends on energy intake (in the form of food) and energy expenditure. It has previously been established that the hypothalamic arcuate nucleus receives appetite input signals from both the brain stem and the peripheral circulation to regulate energy homeostasis. Two types of neurone control food intake: an inhibitory neurone secreting alpha melanocyte-stimulating hormone (MSH) and cocaine and amphetamine-regulated transcript (CART) and a stimulatory neurone secreting neuropeptide Y (NPY) and agouti-regulated protein (AGRP). It was established that leptin activated the inhibitory neurone and inhibited the appetite stimulating neurone. The intestinal hormone, peptide YY (PYY), released after food ingestion acted in a similar way. In contrast the hormone of hunger ghrelin, released from the stomach in the fasting state, stimulated the appetite stimulating neurones and inhibits the appetite inhibitory neurones i.e. acts in an opposite direction. We have now identified two further gut hormones that influence appetite, oxyntomodulin and pancreatic polypeptide. Oxyntomodulin is released after food intake in a similar way to PYY and has a similar action on appetite. It belongs to the same family of peptides as glucagons-like peptide-1 (GLP-1), which was previously shown to inhibit appetite. GLP-1, however, also releases insulin (thereby causing hypoglycaemia) and has an affect on both gastric emptying rate and glucagon release. Pancreatic polypeptide (PP) belongs to the same family as NPY and PYY and is released from the PP cells in the islets of Langerhans, again

after food ingestion and in parallel with insulin. Together these gut hormones form an integrated appetite regulatory system that tells the brain when the gut is empty and when it is full and regulates meal size. Of considerable interest is the finding that in obesity, the release of these hormones is altered and at least some of these changes tend to perpetuate the obesity and may thus provide clinically useful targets for pharmaceutical correction.

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SA16

The hypothalamus: studies with knockout mice in obesity

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Energy homeostasis is tightly controlled via hormonal signals communicating the energy state of peripheral tissues to the central nervous system. It has been demonstrated that insulin provides such a signal rising both short-term in response to increased blood glucose concentrations and long-term in correlation with body fat stores. Insulin receptors are widely expressed across the central nervous system with the highest density in hypothalamic areas responsible for the regulation of food intake and energy expenditure. Moreover, peripherally administered insulin is capable of crossing the blood/brain barrier to act on these receptors. Insulins ability to inhibit food intake and to increase energy expenditure appears to be regulated via a neuronal network in the arcuate nucleus of the hypothalamus involving both the regulation of the melanocortin pathway as well as neurons expressing neuropeptide Y in the agouti-related peptide. The presentation will focus on the genetic determination of the anatomical sites in the central nervous system and signal transduction pathways targeted by insulin.

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