Peripheral glucose metabolism is controlled by hydrogen peroxide-mediated hypothalamic glucose-sensing

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There is increasing evidence that sensing of changes in blood glucose in the brain, particularly in the hypothalamus, plays a key role in controlling peripheral glucose homeostasis. Hypothalamic levels of reactive oxygen species (ROS) have been suggested to act as an indirect signal reflecting peripheral glucose levels, perhaps being generated by a glucose-dependent increase in flux through the mitochondrial electron transport chain. ROS consist of a number of different chemical compounds, including hydrogen peroxide which has also been implicated as a physiological neurotransmitter. We hypothesised, therefore, that lowering hypothalamic HP would be detected by nutrient sensors as a fall in circulating glucose and lead to a compensatory increase in endogenous glucose production/decrease in insulin sensitivity.

To test this hypothesis, we performed 180 minute hyperinsulinaemic euglycaemic pancreatic clamps (infusion of insulin 2 mU/kg/min and somatostatin 3 μg/kg/min) on adult Sprague Dawley rats with vascular and intracerebroventricular [ICV] catheters inserted 1 week previously under isoflurane anaesthesia. On study days, starting 90 min prior to clamps and then continuing until the end of studies, conscious free moving animals received an ICV infusion into the third ventricle of either the antioxidant catalase (to reduce hypothalamic hydrogen-peroxide levels) (CAT, n=6) or vehicle in controls (VEH, n=5).

Using this insulin clamp technique, plasma glucose levels were adjusted to and maintained at equal levels of euglycaemia in both groups of rats (6.0 ± 0.3 vs 6.1 ± 0.3 mM vehicle and catalase infused rats respectively during the last 30 mins of clamps, p = NS).

As shown in figure below, despite being maintained at equivalent plasma glucose, the requirements for exogenous dextrose infusion in the 2 groups were different. In keeping with our hypothesis, whole body insulin-sensitivity, as gauged by the dextrose infusion rates required to maintain euglycaemia during last 30 minutes of clamp studies, was significantly reduced in the ICV catalase group compared with vehicle controls (p<0.05).

These data support our hypothesis that changing levels of ROS in the hypothalamus, specifically levels of hydrogen peroxide, are a key physiological factor controlling plasma glucose levels.

The central effects of leptin are mediated partially by pituitary adenylate cyclase activating polypeptide in the ventromedial hypothalamic nucleus

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Energy homeostasis involves extensive interactions between hypothalamic neuronal populations, and their communication with both central and peripheral targets. The adipocyte-derived hormone leptin, acts in the hypothalamus to control food intake and energy expenditure. It is widely assumed that the major site of leptin’s effects within the hypothalamus is the arcuate nucleus. However, recently Dhillon et al. (2006) demonstrated clearly the functional importance of another hypothalamic leptin target, the ventromedial nucleus (VMN). The VMN has important functions in controlling feeding, energy expenditure and blood glucose levels. Even so, there is still little known about the VMN cell types or transmitters involved. We have evidence that pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide that is strongly expressed in the VMN, may have a significant role in leptin’s central actions. Both leptin and PACAP dose-dependently decrease fast-induced re-feeding in outbred CD1 mice when administered to freely behaving animals via an ICV cannula inserted under isoflurane 1 week earlier. PACAP also has robust effects on metabolism, increasing oxygen consumption and core-body temperature, and induces c-Fos protein expression in discrete brain regions associated with thermogenesis and appetite suppression. The effects of PACAP appear to be mediated by PAC1 receptors rather than VPAC receptors (that also bind vasoactive intestinal peptide).
peptide), since they are blocked by the partially-selective PAC₁ receptor antagonist, PACAP₆-₃₈. Moreover, our data indicate that leptin-induced effects on feeding and body temperature are significantly attenuated by pre-treatment with the PAC₁ antagonist.

Semi-quantitative in situ hybridisation histochemistry reveals that PACAP mRNA expression in the VMN, but not elsewhere in the forebrain, is regulated according to metabolic state. Either fasting or genetic leptin deficiency, both states of hunger and hypometabolism, are accompanied by a significant reduction in the relative expression of VMN PACAP compared with controls. Conversely, both high-energy diet and exogenous leptin treatment increase PACAP mRNA. Furthermore, mice with genetic VMN-specific deletion of leptin receptor also show reduced PACAP in the VMN, along with a modestly obese phenotype. To further investigate the direct action of leptin on VMN PACAP neurons we are examining endogenous PACAP and leptin receptor expression, and c-Fos induction using dual in situ hybridisation histological techniques. Together, these data reveal a highly plastic system which may be essential for maintaining normal energy homeostasis and modulating the central actions of leptin.


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

Noradrenaline differentially regulates neuronal excitability in hypothalamic arcuate neurones in vitro

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The hypothalamic arcuate nucleus (ARC) is a key central neural component of the homeostatic feedback systems responsible for maintaining energy balance. Function-specific neural populations within the ARC respond to signals of central and peripheral origin indicating shifts in energy status, including noradrenergic inputs originating from brainstem nuclei. We have utilized whole cell patch clamp recording techniques in vitro, to investigate the role of noradrenaline (NA) in regulating neuronal excitability in these neurones.

Adult male Wistar rats were humanely killed by cervical dislocation, in accordance with UK guidelines, and whole-cell recordings obtained from ARC neurones in hypothalamic slices as described previously (van den Top et al., 2004). Brief (5-15s), bath application of NA (40μM) induced membrane depolarisation and increased electrical excitability in 51% (88/172) of ARC neurones, including orexigenic NPY/AgRP neurones (n=9), responses that persisted in TTX (n=12) suggesting a direct effect. NA-induced excitation was associated with increases (n=7; reversal potential -84.1 ± 5.3mV), decreases (n=5; reversal potential -24 ± 2.9mV) or no change (n=10) in conductance indicating inhibition of resting potassium and activation of non-selective cation conductances underpin these responses. Depolarising responses to NA were mimicked by phenylephrine (10μM; n=14), completely blocked by prazosin (200nM; n=16) and partly reduced by the α₁a-adrenoceptor antagonist RS 100329 hydrochloride (100nM; n=14) suggesting excitation was mediated through α₁a-adrenoceptors, including α₁a. 15% (26/172) of ARC neurones, including 4/9 putative anorexigenic cocaine-and-amphetamine regulated transcript (CART)-expressing neurones, responded to NA with hyperpolarisation and reduced excitability, the remaining CART neurones responding with excitation. 7.5% responded to NA with biphasic inhibitory/excitatory responses. NA-induced inhibition was characterised by an increase in conductance, reversal potential close to that for potassium (-83±7mV), that persisted in TTX. NA-induced inhibition was mimicked by UK-14,304 (10μM; -12) and suppressed by idazoxan (200nM; n=4), indicating a mechanism involving activation of α₂-adrenoceptors coupled to a potassium conductance.

Taken together these findings suggest an orexigenic role for NA in the ARC, through activation of α₁ on NPY/AGRP and in part through inhibition of anorexigenic CART-expressing neurones. The functional significance of differential regulation of CART neurones requires further clarification.


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Thyrotropin-releasing hormone excites hypocretin (orexin) cells of the hypothalamus

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The neuropeptide thyrotropin-releasing hormone (TRH) was originally described as the hormone that regulates the hypothalamus-pituitary-thyroid axis, but its additional role as a neurotransmitter is now widely recognised. Central effects of TRH include the regulation of energy balance and cognitive arousal (1), though the mechanisms involved are not yet clear. A few areas in the brain that express TRH send their projections to the lateral hypothalamus, e.g. the dorsomedia hypothalamus (2). The latter is critical for the temporal organisation of food entrainment of circadian rhythms. On the other hand, the lateral hypothalamus is the only brain region to contain hypocretin (orexin) cells. These cells are essential for cognitive arousal and feeding behaviour; their loss leads to obesity and narcolepsy, whereas their activation promotes wakefulness (3).

Considering that both TRH and hypocretin have a role in the regulation of sleep and feeding, and that some of the afferents to the lateral hypothalamus (where hypocretin cells are located) arise from TRH-expressing areas, we tested the effects of TRH on the activity of hypocretin cells.
Poster Communications

Primer sequence

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<th>Primer</th>
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<th>Expected size (bp)</th>
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</tr>
<tr>
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Figure 1. Expression of nucleoside transporters in rat astrocytes in the primary culture. Figure shows an ethidium bromide stained gel. Primer sequences and expected sizes of products are presented in the Table 1. Negative controls including both reverse transcriptase minus samples (RT-) and water samples were negative (data not shown).


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Hypothalamic cytokine signalling pathways increase following recurrent hypoglycaemia

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Counterregulatory responses (CRR) to acute hypoglycaemia may become impaired following exposure to recurrent hypoglycaemia. Current evidence suggests that the predominant sites for hypoglycaemia sensing and/or triggering CRR are in the hypothalamus. We hypothesised that impaired CRR develops through coordinated changes in gene expression in hypothalamic pathways.

We created a rodent model of impaired CRR in catheterised (inserted surgically under isoflurane anaesthesia 1 week prior to studying) Sprague Dawley rats. One group (recurrent hypo [RH], n = 4) underwent 3 days of sc regular insulin injections (10, 8 and 6 units/kg on days 1 to 3) followed by a day 4 hyperinsulinaemic (20 mU/kg/min) hypoglycaemic clamp. A second group (acute hypo [AH], n = 4) had 3 days of sc saline followed by a day 4 hypoglycaemic clamp, while a third control group also had 3 days sc saline followed by clamped euglycaemia ([EU], n = 4). At 150 min, whole hypothalami were removed, frozen for later RNA extraction etc and expression analysis using Affymetrix rat 230 genome 2.0 chip. Genes showing significant change (using Genespring GX- Agilent) were subjected to pathway analysis (Ingenuity Systems).

We identified 116 and 103 genes which significantly increased or decreased expression respectively in AH compared with EU, a surprisingly high number considering that these groups were treated identically except for 120 min of exposure to different plasma glucose values on day 4 (fig below). Pathway analysis suggested significant decreases (p<0.05) in AH in glucocorticoid (GC), interleukin (IL) 6 and acute phase response signalling. Looking then at the comparison between acute and recurrent hypoglycaemia (AH vs RH), 143 genes increased and 158 decreased. As a striking comparison when compared with AH, pathways with significant increases in RH (p<0.05) included GC, IL-6, IL-10, IL-2 and acute phase response pathways.

Finally, given this analysis suggested coordinated changes in hypothalamic cytokine/ inflammatory signalling and previous data suggesting a role in the hypothalamus for the key inflammatory cytokine IL-1-beta in controlling peripheral metabolism, we quantified IL-1B expression with RT-PCR. In keeping with the broad patterns seen in microarray data, hypothalamic IL1B expression was significantly higher in RH than AH (figure below).

In summary, our data show that (1) even 120 min of hypoglycaemia results in robust coordinated changes in hypothalamic gene expression and (2) that marked differences exist in gene expression between acute and recurrent hypoglycaemia in cytokine/ inflammatory pathways. We speculate that that non-neuronal cells- astrocytes and/or microglia- may contribute to modulating neuronal glucose-sensing in the hypothalamus by locally modulating cytokine/ inflammatory signalling.
Maternal diet during pregnancy and/or lactation plays a role in inducing the offspring metabolic phenotype. We examined the phenotypic outcome in offspring if they were fed a diet mismatched between pre- and postnatal life. Pregnant MF-1 mice were assigned to either control (C, 18% casein) or protein-restricted (PR, 9% casein) diet. PR dams were further sub-divided into those fed the PR diet throughout pregnancy (PRP) or both pregnancy and lactation (PRPL). Weaned offspring were then fed a high fat (HF, 45% Kcal fat) diet or standard chow (C, 21% Kcal fat) to adulthood. This generated six experimental groups based on dam/offspring dietary consumption: C/C, C/HF, PRP/C, PRP/HF, PRPL/C and PRPL/HF. Food intake and body weight were monitored and blood pressure was recorded by tail cuff plethysmography before animals were sacrificed. Hypothalamic tissues and fat depots were then collected for gene expression analysis by real time-PCR. Body weight and food intake was analyzed by mixed model analysis. All other data was analyzed by ANOVA with the appropriate post hoc test.

HF offspring were heavier vs. C animals, regardless of maternal diet during pregnancy. However, PRPL/HF males were lighter vs. C/HF group, but were significantly fatter (p<0.001). The increased adiposity observed in PRPL/HF males was not evident in the PRP/HF group. Daily energy intake was similar for all groups except for the PRP/HF males, whose intake was reduced by 20% vs. the PRP/C or C/HF groups (p<0.001). PRP/HF males had reduced hypothalamic mRNA levels of genes involved in appetite regulation, namely neuropeptide Y (NPY) and the leptin receptor Ob-Rb, vs. PRP/C animals (p<0.001 and p<0.05, respectively). These PRP/HF males also had reduced expression of genes involved in thermogenesis, namely beta-3 adrenergic receptor and uncoupling protein 1, in the interscapulary brown adipose tissue vs. PRP/C animals (p<0.05). These changes in gene expression were not observed in PRPL offspring. Systolic blood pressure in all PR offspring was greater by 16% and 10% in males and females, respectively, vs. C offspring (p<0.05), and increased further (p<0.05) by 15% and 7% in the HF male and female offspring, respectively. Our study shows that maternal protein restriction during pregnancy leads to sex-specific adaptive responses in male offspring, resulting in altered energy homeostasis following post-weaning HF-feeding. Extending maternal protein restriction to include the lactation period resulted in greater adiposity in the HF-fed male offspring. Nevertheless post-weaning HF-feeding exacerbated cardiovascular dysfunction in both male and female offspring, regardless of whether maternal protein restriction was imposed during pregnancy or both pregnancy and lactation.

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**A mismatched pre- and post-weaning diet has window of exposure- and sex-specific effects on energy homeostasis, adiposity and cardiovascular function in mice**

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with adjustment for age, sex and lean mass. tCys was the strongest plasma variable associated with fat mass, stronger than and independent of plasma lipids. Women in the highest tCys quintile had fat mass 6 kg greater than that of women in the lowest quintile (95% CI: 5, 7 kg), with adjustment for plasma lipids, physical activity, and dietary fat, protein, and total energy intakes. Corresponding values for men were 4 kg (95% CI: 3, 5 kg; P<0.001 for ANOVA across quintiles in both genders). A higher baseline tCys and a rise in tCys over 6 y were both associated with greater fat mass at follow-up (P<0.001 by linear regression), with no effect on lean mass.

Discussion

Literature evidence points to tCys as a powerful but ignored determinant of fat mass. Homocystinurics with genetic deficiency of CBS enzyme (and hence decreased cysteine synthesis) are thin and underweight [3], a feature not reported for other types of homocystinuria, in which cysteine synthesis is normal. In contrast, Down syndrome patients, having triple copies of the CBS gene and elevated tCys, are overweight. Dietary cysteine supplements enhance weight gain in cachectic AIDS and cancer patients [4]: an effect generally attributed to improved lean mass, but do we know? Dietary restriction of the cysteine-precursor methionine reduces visceral fat mass in rats [5]. Several early studies on rat adipocytes demonstrate potent antilipolytic and lipogenic actions of cysteine.

Conclusions

Overall, our data and literature evidence suggest that cysteine could be an important modulator of body fat mass in humans, and if so, provides an attractive anti-obesity target.

References


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Serotonin 2C receptor agonists improve type 2 diabetes via central MC4R signaling pathways


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The burden of type 2 diabetes and its associated premature morbidity and mortality is rapidly growing, and the need for novel efficacious treatments is pressing. We have shown that serotonin 2C receptor agonists, typically investigated for their anorectic properties, significantly improve glucose and insulin tolerance in murine models of obesity and type 2 diabetes. Importantly, these improvements in glucose homeostasis occurred at concentrations of agonist which had no effect on ingestive behavior, VO2, locomotor activity, body weight, or fat mass. We determined that this primary effect on glucose homeostasis requires downstream activation of central melanocortin-4 receptors (MC4Rs), but not MC3Rs, and is associated with MC4R-mediated stimulation of sympathetic preganglionic neurons in the spinal cord, increased insulin signaling in liver and skeletal muscle, and inhibition of hepatic gluconeogenesis at the transcriptional level. These findings suggest that pharmacological targeting of 5-HT2CRs may enhance glucose tolerance independently of alterations in body weight, and that this may prove an effective and mechanistically novel strategy in the treatment of type 2 diabetes.

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