SA1

Coactivating CREB - coactivating central regulation of energy balance?

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A critical role for the novel CREB coactivator CRTC2 (CREB-regulated transcription coactivator, a.k.a TORC2 (transducer of regulated CREB activity)) in maintaining glucose homeostasis was previously demonstrated in liver, where hormonal and energy-sensing signals mediate their effects on gluconeogenic gene transcription via regulation of CRTC2 phosphorylation.
Here, we report CRTC2 expression in neurons of several hypothalamic areas of the mouse, showing alterations in CRTC2's subcellular localisation and thus activity in response to fasting. Important questions arise: Which metabolic signals regulate hypothalamic CRTC2 activity? Which hypothalamic genes does CRTC2 regulate? What is the physiological role of CRTC2 in the hypothalamus?
Using a range of in vitro, in vivo and imaging studies, we identified glucose as on of the metabolic stimuli regulating hypothalamic CRTC2 activity. More specifically, glucose levels regulate hypothalamic CRTC2 subcellular localisation via AMP Kinase-mediated phosphorylation of CRTC2, thereby controlling CRTC2's occupancy of the insulin receptor substrate 2 (IRS2) promoter. We thus uncover CRTC2 as a novel hypothalamic AMPK target and highlight a role for CRTC2 in linking hypothalamic metabolic sensing pathways with CRE-gene regulation.

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

SA2

AMP-activated protein kinase: part of the glucose-sensing machinery linked to K_{ATP} channels

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AMP-activated protein kinase (AMPK) is an integral part of the system in cells that allows them to sense and respond to metabolic stress and the consequent threat of lowered ATP levels. AMPK responds to various stresses, through sensing a raised cellular AMP/ATP ratio, resulting in increased kinase activity. This initiates multiple cellular outcomes predicated to oppose loss of high-energy phosphates by stimulating ATP producing processes and inhibiting non-essential ATP consuming processes. In single celled eukaryotes AMPK subunit homologs are required for their response to glucose starvation. Some mammalian cells are also capable of sensing alterations in the physiological levels of glucose. The best-known examples of such specialized cells are pancreatic beta cells and certain hypothalamic neurons, with their ability to sense changes in local glucose concentration coupled to the regulation of insulin secretion and to changes in feeding behaviour, respectively.
The archetypal glucose-responsive cell is the pancreatic beta cell, where GLUT2, glucokinase (GK; also called hexokinase IV) and ATP-sensitive K+ (K_{ATP}) channels combine to act as regulators of glucose sensing. In addition, certain glucose-responsive central neurons may use the same molecular mechanisms to recognize and respond to changes in extracellular glucose. In both cases, alteration of physiological glucose levels results in changes in cellular excitability, driven mainly through variation in the activity of K_{ATP} channels. We have attempted to address the question whether AMPK contributes to this glucose sensing and transduction system (i.e through alteration of K_{ATP} channel opening) in these cells. To that end, we have utilized two main approaches: genetic deletion of the AMPKα2 subunit, and pharmacological activation of AMPK activity. The results of deletion of the AMPKα2 subunit from defined hypothalamic neurons and pancreatic beta cells will be presented, showing that their glucose-sensing mechanisms utilize AMPK activity to link glucose metabolism with changes in excitability. We are currently investigating the effects of AMPK activation on the electrical activity of an insulin secreting cell line (CRI-G1). Preliminary data indicate that manipulations designed to increase AMPK activity can alter glucose sensing in these cells, but in a rather unexpected manner.

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SA3

Glucose sensing and metabolic regulation of neuronal excitability in the vagal complex

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A large number of studies have implicated the lower brainstem, and particularly the vagal complex of the medulla oblongata, in the central control of blood glucose levels and in the glucoprivic regulation of feeding. Within the vagal complex the nucleus of the solitary tract which is a major relay station for ascending visceral and cardiovascular homeostatic signals, and the dorsal vagal motor nucleus, which is the source of the vagal efferent fibers projecting to most visceral organs, including liver and pancreas, are of particular interest. Like several hypothalamic nuclei, these structures within the dorsal vagal complex contain glucose-inhibited and glucose-excited neurons. However, relatively little is known about the molecular components involved in glucose sensing and in generating the electrical signal.
Utilizing primarily rodent *in vitro* brainslice preparations we investigate whether specific brainstem cell populations are intrinsically sensitive to physiological changes in glucose availability. We explore which molecular and electrical pathways are responsible for this sensitivity and how it relates to their general metabolic sensitivity. This involves the combination

of patch-clamp electrophysiology with single-cell RT-PCR and immunocytochemistry. Our results indicate that brainstem responses to hypoglycemia are elicited at higher glucose levels than in hypothalamic nuclei. They also demonstrate the involvement of glucokinase and the activation of ATP-sensitive K^+channels in the hypoglycemia response of glucose-excited neurons and suggest a variety of different mechanisms, including, but not limited to, inhibition of 2-pore-domain K^+channels, in glucose-inhibited cells. We are using transgenic mouse models to ascertain the involvement of specific ion channels and to characterize the responses of specific cell populations.

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SA4

Arousal reward and metabolism: the hypocretin connection

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The hypothalamus encompasses multiple neuronal circuits regulating arousal, energy homeostasis and goal-oriented behaviors. Recent observations have linked sleep perturbances with leptin and ghrelin pathways and subsequent metabolic imbalances. Thus, hypothalamic circuits sharing both sleep and metabolic functions, such as the Hypocretin (Hcrt) and Melanin-Concentrating Hormone (MCH) systems, are strong candidates for mediating these imbalances. Hcrt containing neurons receive significant input from NPY and POMC neurons in the arcuate nucleus, are regulated by leptin and hyperpolarized by glucose; hence Hcrt neurons provide a powerful sensor of metabolism that is integrated into stable wakefulness. Optogenetic manipulation of Hcrt neurons is sufficient to induce sleep-to-wake behavioral transitions. Activation of Hcrt neurons can reinstate drug and food seeking behavior in extinguished animals, demonstrating a role for this peptidergic system in motivation and goal-oriented behaviors. Here, we propose new roles for these peptidergic systems as sensors and effectors of arousal state, and discuss their implications in the plasticity of complex hypothalamic networks regulating sleep and energy balance. Finally, we suggest that new tools for remote control of neuronal circuit activity provide an effective way of testing related questions by interrogation of these circuits with unprecedented specificity and temporal resolutions.

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SA5

Sugar sensing by arousal and appetite neurons

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Hypocretin/orexin neurons of the hypothalamus are essential for normal wakefulness, feeding behaviour, and reward seeking. Their electrical activity is directly silenced by glucose, providing a potentially important link between energy state and behavioural coordination. However, the cellular mechanisms underlying this electrical silencing are not well understood. Our new experiments show that the orexin/hypocretin glucosensors display a novel sugar selectivity, detecting mannose, D-glucose, and 2-deoxyglucose, but not galactose, L-glucose, fructose or alpha-methyl-D-glucoside.
Furthermore, conventional glucose metabolism does not appear critical for orexin cell glucosensing: the effects of extracellular glucose on orexin cells are not mimicked by intracellular glucose or lactate, and are not affected by glucokinase inhibitors. We also show that orexin cell glucosensing also exhibits an unusual temporal profile.
About 70% of orexin neurons are inhibited by glucose only transiently, self-restoring their firing despite high sugar levels. The remaining 30% of cells display sustained inhibition that follows the time-course of glucose application. The transient and sustained glucosensor cells display significant differences in their ion channel expression. The implications of these findings for the control of feeding and cognitive arousal will be discussed.

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SA6

Leptin and the biological basis of obesity

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The cloning of the ob gene and its gene product leptin has led to the elucidation of a robust physiological system that maintains fat stores at a relatively constant level. Leptin is a peptide hormone secreted by adipose tissue in proportion to its mass. Recessive mutations in the leptin gene are associated with massive obesity in mice and some humans establishing a genetic basis for obesity. Leptin circulates in blood and acts on the brain to regulate food intake and energy expenditure. When fat mass falls, plasma leptin levels fall stimulating appetite and suppressing energy expenditure until fat mass is restored. When fat mass increases, leptin levels increase, suppressing appetite until weight is lost. This system maintains homeostatic control of adipose tissue mass.
The cloning of the ob gene and hormone leptin has led to several new insights. The identification of leptin has uncovered a new endocrine system regulating body weight. This system