Feature Review

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Brain glucose sensing in homeostatic and hedonic regulation

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Glucose homeostasis as well as homeostatic and hedonic control of feeding is regulated by hormonal, neuronal, and nutrient-related cues. Glucose, besides its role as a source of metabolic energy, is an important signal controlling hormone secretion and neuronal activity, hence contributing to whole-body metabolic integration in coordination with feeding control. Brain glucose sensing plays a key, but insufficiently explored, role in these metabolic and behavioral controls, which when deregulated may contribute to the development of obesity and diabetes. The recent introduction of innovative transgenic, pharmacogenetic, and optogenetic techniques allows unprecedented analysis of the complexity of central glucose sensing at the molecular, cellular, and neuronal circuit levels, which will lead to a new understanding of the pathogenesis of metabolic diseases.

Glucose-sensing cells form a topographically distributed but highly integrated metabolic control system

Glucose-sensing cells form a distributed system that monitors glucose concentrations at different anatomical sites, including taste buds in the tongue, intestinal and pancreatic endocrine cells, as well as glucose-sensing neurons of the peripheral and central nervous systems (CNS) [1,2]. Information sampled by these cells is integrated to control glucose homeostasis through modulation of glucose utilization by the liver, white and brown fat, and muscle, as well as by glucose production in the liver, and the homeostatic and hedonic components of feeding behavior. An essential goal of this glucoregulatory system is the maintenance of normoglycemia to ensure a constant supply of glucose to the brain, which almost exclusively utilizes this nutrient as a source of metabolic energy. Therefore, it is not surprising that a plethora of glucose-sensing cells are located in the CNS where they control autonomic nervous activity and feeding behavior.

In addition to the control of feeding initiation and termination, glucose sensing by the nervous system also controls thermogenesis, β cell proliferation, insulin and glucagon secretion, and motivated glucose-seeking behavior [1,3,4]. Defects in all of these regulatory aspects of glucose affect energy homeostasis and contribute to the development of obesity and diabetes. Because the incidence of these diseases is increasing at an alarming rate in developed and developing countries, there is an obvious need to characterize the diversity of the central glucose-sensing mechanisms, the cells involved, the neuronal circuits they are integrated in, and the physiological functions they control. Recent technological developments (Box 1), including new genetic approaches allowing pharmacological or optical activation, or silencing of neuronal populations in living, freely-behaving mice, and more classical electrophysiological techniques, open up a new era towards a more precise understanding of the role of central glucose sensing in health and metabolic diseases.

Central glucose-sensing neurons control autonomic nervous activity

Brain glucose-sensing cells are found in many locations, but are especially enriched in the hypothalamus and brainstem. In the hypothalamus, glucose-sensing neurons are mainly found in the arcuate nucleus (ARC) and in the ventromedial (VMN) and lateral (LH) nuclei; in the brainstem they are found in the dorsal vagal complex (DVC) which consists of the area postrema (AP), the nucleus tractus solitarius (NTS), and the dorsal motor nucleus of the vagus (DMNX), as well in the ventral part of the medulla (basolateral medulla, BLM) (Figure 1). Glucose-sensing neurons are divided in glucose-excited (GE) and glucose-inhibited (GI) neurons, which increase their firing rates when extracellular glucose concentrations increase or decrease, respectively [1,5–8]. Evidence also indicates that astrocytes and tanycytes, which are specialized glial cells located in the lower part of the third ventricle, are also involved in glucose sensing and may control hypothalamic neuron activities [9] (Figure 2).

A key role of these central glucose-sensing cells is to control the activity of both the sympathetic (SNS) and parasympathetic (PNS) branches of the autonomic nervous system (ANS). The physiological functions controlled by glucose-dependent regulation of the PNS include the stimulation of β cell proliferation and of insulin secretion, and the secretion of glucagon when glycemia falls below 5 mM. The SNS activity stimulates glucagon secretion and inhibits insulin secretion [10], increases thermogenesis in brown adipose tissue (BAT) [11], stimulates epinephrine secretion by the adrenals, enhances lipolysis in white adipose tissue (WAT), and regulates hepatic glucose output [12,13] (Figure 1).

Tracing experiments using pseudorabies viruses (Box 1) have described the specific pathways that link hypothalamic

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Box 1. Neuroinvestigation methodologies

Pseudorabies virus (PRV): also known as Suid herpesvirus 1(SuHV), PRV is a disease-causing virus in swine. A less-virulent strain, Bartha SuHV1, is used in neuroscience to trace neural pathways. In retrograde tracing experiments, the PRV (or a genetically modified form, which encodes the gene for a marker protein, i.e., GFP) is injected in the organ of interest where it infects nerve terminals. The PRV is then transported in a retrograde manner and is transferred *trans*-synaptically to connecting neurons. By precise timing of animal sacrifice, first-, second-, and third-order neurons linking the organ under study with central neuronal populations can be visualized using PRV or marker protein immunodetection. Selective analysis of sympathetic innervation requires simultaneous sectioning of the parasympathetic nerve innervating the organ studied and, reciprocally, sectioning of the sympathetic nerve is necessary to specifically study parasympathetic innervation.

Optogenetics: allows precise light control of neuronal activity with good spatial and temporal resolution. This technique is based on the use of light-sensitive proteins termed opsins. Some, such as the channelrhodopsin2 (ChR2) of the green algae *Chlamydomonas reinhardtii*, are cation channels that are activated by blue light (473 nm). Expression of ChR2 in specific neuronal population can be achieved by viral delivery systems or by transgenesis. Viruses, often AAV, are engineered so that the *Chr2* gene can be expressed under a cellular specific promoter or can be expressed only in cells expressing the Cre recombinase (from transgenic mice expressing Cre under a cell-specific promoter). In this latter condition, the *Chr2* gene in the AAV is preceded by lox-STOP-lox (LSL) sequence that ensures that the *Chr2* gene is expressed only in the Cre-expressing cells. Alternatively, ChR2 can come from a transgenic mouse (*Rosa26*-LSL-ChR2), which allows expression of ChR2 under the control of the ubiquitously expressed *Rosa26* region following Cre-dependent removal of the STOP cassette. ChR2 can then be activated in brain slices by direct illumination or *in vivo* by pulses of light delivered through implanted optical fibers or diodes. Stimulation of ChR2 by illumination leads to cation influx and neuronal depolarization. Other opsins, such as halorhodopsin, a light-activated chloride pump, or archaerhodopsin, a light-activated proton pump, can instead be used to inhibit neuronal activity.

DREADD (designer receptor exclusively activated by designer drugs) system: DREADDs are genetically modified G protein-coupled receptors that are solely activated by synthetic ligands. DREADDs are used in neurosciences to specifically activate or inhibit neuronal populations. Engineered muscarinic receptors rendered responsive to the ligand clozapine-*N*-oxide (CNO) can be used to either increase neuronal activity (e.g., hM3Dq), via Gq-mediated signaling, or to silence neurons (e.g., hM4Di) via Gi-mediated signaling. As for optogenetics, a viral recombination system is used to express DREADDs in a selective manner. Interestingly, each animal becomes its own control because the naïve and CNO injected conditions can be directly compared.

TRAP (translational ribosome affinity purification): obtaining cell-specific gene expression profiles is not a simple task in neuroscience because of the cellular diversity and the architectural complexity of the brain. TRAP is a method that combines cell type-specific expression of a transgene consisting of the ribosomal protein L10 fused to GFP. After cell transfection, this fusion protein will integrate into ribosomes without interfering with their activity. Following cell lysis the ribosomes with the translated mRNAs can be immunopurified with anti-GFP antibodies, and the translating mRNAs can be profiled by microarray or RNASeq analysis.

and brainstem nuclei premotor and motor neurons of both branches of the ANS innervating the pancreas, BAT, and WAT [14–17] (Figure 1). Preganglionic motor neurons of the PNS are located in the DMNX, and these are in synaptic contact with neurons of several brain regions, including the NTS, the PVN and the LH, as well as the zona incerta (ZI) and the VMN. The motor neurons of the SNS are located in the intermediolateral (IML) cell columns of the spinal cord, and their premotor neurons are found in the NTS and the BLM. These premotor neurons are themselves innervated by neurons from several nuclei including the PVN and LH, indicating that activation of the PNS or SNS by glycemic variations must be under the control of GE and GI neurons located in the mentioned brain nuclei. The challenge of current research is to identify these GE and GI neurons, and to determine how the signals they generate are integrated to control glucose and energy homeostasis through ANS activity regulation.

Glucose-sensing mechanisms

The mechanisms of glucose sensing by GE and GI neurons are extremely diverse [18] and still not completely characterized. A model for glucose sensing by GE neurons is that of the pancreatic β cells, which depends on glucose metabolism. In this model, signaling is initiated by glucose uptake via the glucose transporter GLUT2, followed by glucose phosphorylation by glucokinase (GCK), an enzyme that catalyzes the rate-controlling step in glucose-stimulated insulin secretion. The crucial next steps involve a rise in the ATP/ADP ratio, which induces closure of K_{ATP} channels, membrane depolarization, and entry of Ca^{2+} through voltage-gated Ca^{2+} channels in the β cell to trigger insulin granule exocytosis. This GLUT2/GCK/K_{ATP} signaling pathway is a model for activation of GE neurons, and each of these proteins is expressed in the brain with, however, only partial colocalization. GLUT2 is expressed in small populations of cells present in several brain locations, with a high presence in the brainstem and in the thalamic area, but a relatively low number in the hypothalamus [19]. GCK expression is high in the hypothalamus, in particular in the ARC and VMN, and the enzyme is also expressed in the brainstem [20]; by contrast, the K_{ATP} channel is expressed in most neurons [21]. Therefore, the varied cellular distribution of these proteins suggests that they define different types of glucose-sensing cells, although some hypothalamic neurons have been found to express both GLUT2 and GCK [22]. Moreover, GE neurons can also be activated following glucose uptake by SGLT1, an electrogenic Na^+/glucose cotransporter that induces membrane depolarization [23].

Activation of GI neurons by hypoglycemia was originally proposed to be secondary to a decrease in ATP production, which leads to reduced sodium extrusion by the Na^+/K^+ ATPase, and then plasma membrane depolarization and closure of a chloride channel [24]. More recently, it has been proposed that activation of GI neurons from the ventromedial hypothalamus (VMH, which usually includes the VMN and ARC) involves activation of the AMP-dependent protein kinase (AMPK), increased production of nitric oxide (NO) and cGMP to further activate AMPK, eventually suppressing the chloride channel activity of the cystic fibrosis transmembrane regulator (CFTR) [25]. A similar pathway, which involves AMPK activation and closure of a K^+-leak current, instead of activation of the CFTR, has been identified in NTS GLUT2 neurons [26]. Finally, orexinergic neurons of the LH respond to glucose by a metabolism-independent mechanism, possibly involving a cell surface glucose receptor that eventually leads to activation of a K^+-leak current [27].