Brain Glucose Sensing, Counterregulation, and Energy Homeostasis

Neuronal circuits in the central nervous system play a critical role in orchestrating the control of glucose and energy homeostasis. Glucose, beside being a nutrient, is also a signal detected by several glucose-sensing units that are located at different anatomical sites and converge to the hypothalamus to cooperate with leptin and insulin in controlling the melanocortin pathway.

The current epidemics of obesity and the associated increased incidence of Type 2 diabetes represent major threats for human health in both developed and developing countries. The difficulty in treating or preventing these diseases stems, in part, from the relative lack of knowledge of the molecular mechanisms controlling glucose and energy homeostasis. We know that these homeostatic processes rely on the properly coordinated function of several organs: the liver, white and brown adipose tissues, muscle, and the brain. The function of these organs is coordinated by a multiplicity of hormonal and neuronal signals that are triggered by changes in blood glucose levels induced following nutrient absorption and generated to reflect the amount of energy stored as fat and probably also as glycogen.

Glucose is an important regulatory signal that controls the secretion of hormones by various endocrine cells and activates neurons in the peripheral and central nervous systems. Because the brain derives its metabolic energy almost exclusively from glucose and thus requires that glycemic levels do not fall markedly below ~5 mM, critical glucose-sensing systems are located in the central nervous system and control glucose homeostasis, feeding behavior, and energy storage and expenditure. The purpose of this review is to discuss how glucose regulates autonomic and central nervous mechanisms to control glucose and energy homeostasis.

**Glucose as a Signal to Control Glucose Homeostasis, Feeding Behavior, and Energy Expenditure: Overview**

Glucose triggers many neuronal and endocrine responses from the time it appears in the oral cavity to its rise in the systemic circulation (FIGURE 1). In the mouth, glucose stimulates nervous reflexes, in part initiated by activation of taste receptors and of their afferent fibers (2), which project to the brain stem and are in relation to the nucleus of the tractus solitarius (NTS), the reticular formation, the parabrachial nucleus (PBN), and the dorsal motor nucleus of the vagus (DMNX) (13, 15) (FIGURE 2). Activation of this reflex is responsible for the cephalic phase of insulin secretion, which plays an essential role in glucose tolerance (2).

In the intestine, glucose stimulates the secretion of the gluco-incretin hormones, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1) by, respectively, the intestinal endocrine K- and L-cells (32) and activates autonomic and enteric neurons (70, 115). Activation of neurons may be through glucose binding to the SGLT3 isoform of the Na+/glucose cotransporters, which are involved in signal transduction rather than glucose transport and are expressed in cholinergic neurons in the submucosal and myenteric plexuses (34); some enteric neurons express the ATP-sensitive K+ channel (K_ATP channel) subunits Kir 6.2 and SUR1 (56), suggesting that they sense glucose by a mechanism similar to that of the pancreatic beta-cells (see below). Appearance of glucose in the portal vein stimulates sensors, which activate vagal afferents that project to the NTS and lateral hypothalamic nuclei (1, 107) (FIGURE 2). Glucose sensing by the hepatoporal sensor leads to several adaptive responses, such as stimulation of glucose storage in liver and a subset of peripheral tissues, including the soleus, the heart, and brown adipose tissue; inhibition of counterregulation; termination of food intake; and stimulation of first phase insulin secretion (94, 113).

In hepatocytes, glucose uptake triggers several responses that increase the glucose storage capacity of the liver as well as the conversion of glucose into fat. First, glucose is an allosteric inhibitor of glycogen phosphorylase and glucose-6-phosphate, an activator of glycogen synthase (26). Thus increased glucose uptake and phosphorylation augment glycogen storage. Second, the glucose metabolite xylulose-5-phosphate, generated through the pentose-phosphate shunt, is an activator of the protein phosphatase A2 (PP2A), which dephosphorylates the cytoplasmically located transcription factor ChReBP (carbohydrate response element binding protein) and allows its translocation into the nucleus to activate the
expression of genes involved in glycolysis (l-pyruvate-kinase) and fatty acid synthesis [AcylCoA synthase (ACC) and fatty acid synthase] (33, 48). Glucose also activates the LXR nuclear receptor, thus linking glucose utilization to cholesterol metabolism (76).

Elevation in systemic glycemia stimulates insulin secretion by pancreatic beta-cells, which induces glucose utilization in liver and glucose uptake in fat and muscle and suppresses glucagon secretion. In contrast, a fall in glycemia below ~5 mM stimulates glucagon secretory activity by mechanisms that will be discussed below.

In the central nervous system, glucose regulates the activity of glucose-sensitive neurons present in the brain stem and the hypothalamus. These neurons have a critical role in regulating glucose and energy homeostasis through secretion of endocrine pancreas hormones, regulation of liver glucose production, feeding behavior, and energy expenditure, as reviewed below (FIGURE 2).

Central Glucodetection

Sites and mechanisms of glucose sensing in the brain

Sites of gluco-detection. The earliest demonstration that the brain is involved in glycemic control was provided by Claude Bernard, who showed, in the dog, that lesioning the hypothalamus induced hyperglycemia. In 1953, Jean Mayer proposed that cells located in the hypothalamus could be specialized to monitor plasma glucose variations and postulated that these cells translate variations in glucose concentrations in electrical or chemical signals that control feeding behavior (65). In the early 1960s, two groups identified, by electrophysiological analysis of hypothalamic slices, neurons able to modulate their firing activity in response to changes in extracellular glucose levels (6, 89). These are glucose-excited (GE; previously called glucose-responsive) neurons, which increase their firing rate with elevation in extracellular glucose concentrations, or glucose-inhibited (GI; previously called glucose-sensitive) neurons, which are activated by a decrease in extracellular glucose concentration or by cellular glucoprivation (101, 128). Both types of neurons are widely distributed in the brain but highly represented in hypothalamic nuclei and the brain stem, regions involved in the control of energy homeostasis and food intake. At the hypothalamic level, GE neurons are found mostly in the ventromedial hypothalamic nucleus (VMH), the arcuate nucleus (AN), and the PVN (36, 108, 121), and GI neurons are mostly located in the LH, median AN, and PVN. Both types of neurons are also found in the brain stem, in particular in the NTS, the area postrema (AP), and the DMNX (1, 30, 77, 129).

Recently, the presence in the AN of GE and GI neurons responsive to glucose over either a low (0–5 mM) or a high glucose concentration range (5–20 mM) have been described; the latter are referred to as HGE (high GE) or HGI (high GI) neurons, respectively (39, 92).

Evidence for the presence of glucose-regulated neurons has also been obtained by intravenous or intracerebroventricular injections of the glucose antimetabolites 2-deoxyglucose (2-DG) or 5-thio-glucose (5-TG). The glucoprivic signal generated by these compounds induces metabolic or behavioral responses, and the activated neurons can be identified by...
Mechanisms of gluco-detection. There is considerable evidence that the mechanisms of glucose sensing by cells in the central nervous system may rely on different mechanisms, and one may be similar to that of the pancreatic beta-cells (104, 126). Glucose signaling in these cells requires glucose uptake by the low-affinity glucose transporter type 2 (GLUT2), glucose phosphorylation by glucokinase, and the consequent metabolism of glucose to increase the intracellular ATP-to-ADP ratio. This leads to the closure of $K_{ATP}$ channels, membrane depolarization, and the entry of $Ca^{2+}$, which triggers insulin secretion. In this pathway, the rate-controlling step is the phosphorylation of glucose by glucokinase, and glucose uptake is a permissive step (64). The $K_{ATP}$ channel also plays a fundamental role since it links changes in glucose metabolism to plasma membrane electrical activity (10). Thus many studies have evaluated the role of GLUT2, glucokinase, and the $K_{ATP}$ channel subunit SUR1, SUR2, and Kir6.2 in central glucose sensing.

Because GE neurons increase their firing activity when extracellular glucose rises, they may share similarity to beta-cells. The presence of GLUT2 in hypothalamic nuclei where glucose-sensitive neurons are present has indeed been reported (8, 50, 54, 55, 81) as well as the presence of glucokinase (37, 121, 128), which is a critical regulator of VMH glucose sensing (49). In GE neurons, the increase in extracellular glucose leads to an augmentation of the ATP-to-ADP ratio and the closure of the $K_{ATP}$ channels (11, 31, 52, 72, 118), which leads to plasma membrane depolarization and $Ca^{2+}$ entry through voltage-gated channels, thereby increasing neuronal activity and neurotransmitter secretion (5, 79). There is, however, also indication that neuronal gluco-detection could be independent of $K_{ATP}$ channels (39), glucokinase (109) or GLUT2 (50). For instance, the activation by

**FIGURE 2.** Afferent and efferent pathways activated by interconnected glucose sensing structures

A: afferent vagal pathways can be activated at the level of the gut or hepatoportal vein area. These vagal afferents project to the NTS. Blood glucose variations can also be detected by neurons present in the dorsal vagal complex, which comprises the NTS, the AP, and the DMNX as well as neuron in the basal hypothalamus. The glycemic signals detected directly or indirectly at the brainstem level can be transmitted to the hypothalamus, either directly or indirectly via the basolateral medulla (BLM), containing the A1/C1 catecholaminergic group of cells or the parabrachial nucleus (PBN). At the hypothalamic level, this information is integrated to form the appropriate response to the glucose change. This can involve other communication between the hypothalamus and cerebral structures such as the amygdala or the cortex. B: efferent pathways activated by the glucose signal integrated at the hypothalamus activate sympathetic and parasympathetic efferences. Sympathetic efferencies from the hypothalamus project to the intermediolateral cell column (in green) and receive also inputs from the PBN and the BLN. Parasympathetic efferencies (in red) comprise projections from the BLM and the dorsal vagal complex complex (NTS, AP, and DMNX). These parasympathetic efferencies are modulated by a network of interaction between hypothalamus and BLM and the dorsal vagal complex. The sympathetic innervation is responsible, among many other controls, for stimulating glucagon secretion, inhibiting insulin secretion, activating the adrenal secretion of epinephrine, and activating of thermoregulation in brown adipose tissue. Parasympathetic efferencies stimulate insulin secretion and inhibit hepatic glucose production. C: coronal sections at the indicated brain levels. In blue: the nuclei where glucose or glucoprivic sensitive neurons are located.
glucose of HGE neurons seems to depend on the glucose-regulated activity of a TRP (transient response potential) channel (39).

In GI neurons, the mechanism linking a decrease in glucose concentrations to increased firing activity is less clear, but suppression of firing activity may be controlled by the increase in the ATP-to-ADP ratio, which leads to an increase in Na-K-ATPase activity (88, 108) and/or an opening of ATP-regulated chloride channels (101, 109), which hyperpolarize the plasma membrane. For GI orexin neurons, it has been shown that tandem-pore K⁺ channels could mediate their inhibition by glucose (23) (FIGURE 3).

**Physiological functions regulated by central glucose sensing**

A major goal of current research is to identify the cells in the central nervous system that are sensitive to glucose, elucidate the molecular mechanisms by which they sense glucose, and identify the particular

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**FIGURE 3. Integrated detection of hypoglycemia to control counterregulation and several molecular mechanisms of glucose sensing**

A: utilization of glucose and its storage induce a decrease in blood glucose (a) that could be detected by portal vein glucose sensors via a GLUT2-dependent glucose sensing unit. This glycemic signal is transmitted by vagal afferences to the brain stem and hypothalamus (b). The decrease in blood glucose concentration is also detected at the brain level (c), in particular in the dorsal vagal complex and the VMH. In the NTS and DMNX, glucose sensing probably involves GLUT2-expressing astrocytes. Glucose levels can also be detected directly by hypothalamic neurons expressing other glucose transporter isoforms. For instance, VMH neurons in GLUT2-null mice are normally activated by intraperitoneal 2-DG injections, in contrast to neurons in the dorsal vagal complex. The activated neurons then activate vagal afferences to stimulate glucagon and epinephrine secretion (d), which restore normoglycemia by combined action on several hormone secretion and glucose production by liver or uptake by muscle or fat (e). B: decrease in blood glucose concentration triggers change in firing rate of glucose-sensitive neurons through several possible mechanisms. In the model of astrocyte-neurons metabolic coupling, a fall in glycemia leads to a reduced transport of glucose through endothelial GLUT1 and a reduction in GLUT2-dependent glucose uptake by astrocytes. This leads to reduced lactate production and transfer to neurons through the monocarboxylate transporters MCT1/4 and MCT2 and a consequent reduction in pyruvate formation in neurons and decreased ATP production. In glucose-inhibited neurons, the reduced ATP-to-ADP ratio induces the closure of chloride channels and/or a reduction in the activity of the Na-K pump, depolarization of the plasma membrane, activation of voltage-sensitive Ca²⁺ channels, and synaptic neurotransmitter secretion. In glucose-excited neurons, the decrease in ATP in glucose-excited neurons, the decrease in ATP-to-ADP ratio leads to activation of the K ATP channels and plasma membrane hyperpolarization, and thus reduction in neuronal firing activity. There is also evidence that glucose can be directly sensed by certain neurons following uptake through either GLUT2 or GLUT3, and possibly also through other glucose transporters (see text). These various glucose detection systems are probably involved in both counterregulation and feeding control.
physiological function they control. So far, this knowledge is only sketchy, and we will review some of the physiological functions known to be controlled by central glucose-sensing units and the evidence from gene knockout mice that specific genes, also present in the pancreatic beta-cells, may be important for these responses to glucose.

**Central glucose sensing and counterregulation.** A fall in blood glucose level below ~5 mM induces a rapid counterregulatory response to restore normoglycemia. This involves the activation of glucagon secretion from pancreatic alpha cells and of catecholamines from adrenal glands (27, 75, 112), as well as the activation by the autonomic nervous system of hepatic glucose production (FIGURE 3A) (93).

The sites of hypoglycemia detection are multiple and can be activated either by hypoglycemia or by peripheral or central glucoprivation. Classically, experimental counterregulation is activated by insulin-induced hypoglycemia or by injection of 2-DG or 5-thioglucose (5-TG). In both conditions, afferent neurons located in the abdominal regions relay the information to the brain stem and the hypothalamus, which can also be directly activated by hypoglycemia or the glucoprivic signal. Some of the glucemia-sensitive abdominal afferent neurons are located in the hepatoportal vein area as shown by the possibility to suppress hypoglycemia-induced glucagon secretion by portal vein glucose infusion (47). The role of central glucose sensing in the control of counterregulation can be similarly evidenced by intracarotid glucose infusion, which blocks hypoglycemia-induced secretion of counterregulatory hormones and endogenous glucose production (16, 41), or by intracerebroventricular injection of 2-DG, which stimulates glucagon and catecholamine secretion and endogenous glucose production to induce hyperglycemia (see below and FIGURE 3A).

The involvement of hypothalamic nuclei, in particular the VMH, has been assessed in lesion studies and by pharmacological or genetic interference with glucose detection systems (41). For instance, glucagon secretion can be induced by direct injection of 2-DG in the VMH (18), or, in contrast, hypoglycemia-induced glucagon secretion can be suppressed by direct VMH injection of glucose (17). Interestingly, the corticotrophin-releasing factors CRF and urocortin 1, which are agonists of CRF receptor 1 and 2, respectively, either increase or suppress, respectively, hypoglycemia-induced counterregulation by directly modulating the firing rate of the glucose-sensitive VMH neurons (28, 69).

There is also strong evidence that brain stem nuclei play an important role in the control of glucagon secretion. For instance, when the cerebral aqueduct, which allows circulation of cerebrospinal fluid between the third and fourth ventricle, is obstructed, 5-TG induces a glucoregulatory response only when injected in the fourth but not in the third ventricle (95). In addition, whereas 5-TG injections in different nuclei of the hypothalamus fail to induce a glucoregulatory response, its injections into the NTS and the basolateral medullary regions containing the A1/C1 catecholaminergic neurons, which project to different sites of the hypothalamus, induce a strong response (40, 96, 98). Also, in decerebrated rats, the hyperglycemic response to an intraperitoneal injection of 2-DG is preserved (35), and c-fos immunostaining revealed that the activated neurons are present in the NTS, the DMNX, and the catecholaminergic neurons of the basolateral medulla (99).

Thus glucose-sensing units involved in the physiological control of counterregulation are present at multiple locations in the hepatoportal vein region, the brain stem, and hypothalamus. These sites are synaptically connected and form a network for monitoring blood glucose levels. This information is integrated at the central level to control the counterregulatory response by activating afferent autonomic nerves that stimulate glucagon secretion and the secretion of epinephrine by the adrenals, and also block insulin secretion (FIGURE 3A).

**Central GLUT2, glucokinase, K_{\text{ATP}}, channels and AMPK in counterregulation response.** To link particular glucose-sensing cells with the physiological response they control, molecular markers of these cells are required. The involvement of GLUT2 in brain glucose sensing has thus been studied in mice with inactivation of the GLUT2 gene and which express a transgenic GLUT1 transporter in their pancreatic beta-cells to restore normal glucose-stimulated insulin secretion (ripglut1;glut2^{−/−} mice) (114). In these mice, plasma glucagon levels in the fed state are twice as high as in control mice but can be normalized by injection of the ganglionic blocker hexamethonium or chlorisondamine (22). This suggests that, in the absence of GLUT2, there is an increased autonomic tone to the pancreatic alpha cells that induces an exaggerated glucagon secretion. Hyper- and hypoglycemic clamps, which normally inhibit or stimulate, respectively, plasma glucagon level fail to affect glucagonemia in the ripglut1;glut2^{−/−} mice. Furthermore, intraperitoneal or intracerebroventricular 2-DG injections failed to stimulate glucagon secretion in these mutant mice. Thus central GLUT2-dependent glucose sensors are involved in the counterregulatory response (62). Analysis of c-fos-positive cells in response to 2-DG injections indicates a decreased number of activated cells in the brain stem, in particular in the NTS and the DMNX of GLUT2-null mice. At the level of the hypothalamus, a reduced stimulation of c-fos-positive cells was observed in the PVN but not in the VMH. Thus the central GLUT2-dependent glucose sensors seem to be important for the physiological regulation of counterregulation and to be associated with brain stem structures known to contain glucose-sensitive neurons, the NTS and DMNX. In
contrast, VMH c-fos response to the glucoprivic signal was independent of GLUT2 expression. Since the VMH is also important for counterregulatory response, this suggests that there are different mechanisms to monitor hypoglycemia and trigger glucagon secretion.

In genetic complementation experiments, in which a GLUT2 cDNA was expressed in Ripglut1;glut2−/− mice under the control of the glial acidic fibrillary protein (GFAP; glial-specific) or the synapsin (neuron-specific) promoter, counterregulation was restored only when GLUT2 was expressed in glial cells (62). This was shown in hypoglycemic clamps and following intraperitoneal 2-DG injections and was associated with an almost complete restoration of the pattern of c-fos labeling in the brain stem. Therefore, GLUT2-dependent glucose-sensing units controlling counterregulation may consist of astrocytes and neurons. According to a previously published hypothesis (60), this metabolic coupling may rely on astrocytes taking up glucose through GLUT2, catalyzing it to lactate, and transferring lactate to neurons via monocarboxylate transporters for production of ATP through further degradation by the Krebs cycle (FIGURE 3B).

The hypothesis of a coupling between astrocytes and neurons for glucose sensing is in agreement with other publications showing that methyl sulfoximide, an astrocyte-specific inhibitor of glycolysis, blocks increase in c-fos labeling following intracarotid or brain stem 2-DG injections (43, 131) and that the release of lactate from neighboring glial cells could be involved in glucose response of hypothalamic neurons (3, 51). In the brain stem, lactate is sensed as a metabolic signal that can regulate the activation of glucose-sensitive neurons in the AP and NTS, as detected by c-fos labeling studies, and blocking monocarboxylate transporter by injection in the fourth ventricle of the inhibitor α-cyano-4-hydroxycinnamate leads to elevations in blood glucose concentrations (19, 90, 91).

A role for hypothalamic glucokinase in glucagon secretion was shown by demonstrating that intracerebroventricular injection of the glucokinase inhibitor alloxan, at cytotoxic concentrations, increases this enzyme mRNA expression and inhibits the hyperglycemic response induced by intraperitoneal 2-DG injection. This effect is transient, and the hyperglycemic response to glucoprivation is restored when glucokinase expression is normalized (102, 122).

The involvement of K\textsubscript{ATP} channel in central glucose recognition and counterregulation has been evidenced by the intracerebroventricular or direct VMH injection of the channel inhibitor glibenclamide, which blocks the counterregulatory response (38) to a hypoglycemic clamp or induced by intracerebroventricular 5-TG injection. Inactivation of K\textsubscript{ATP} channel by Kir6.2 gene knockout also leads to impaired glucagon response, and this is correlated with suppressed glucose-regulated firing activity of VMH neurons (72). In contrast, activation of ATP-sensitive K\textsuperscript{+} channels in the VMH amplifies counterregulatory hormone responses to hypoglycemia in normal and recurrently hypoglycemic rats (66).

AMP-activated protein kinase (AMPK) is an important “fuel gauge” that is present in every tissue, including neurons and astrocytes (29, 117). Decrease in fuel availability increases cellular AMP, an allosteric activator of this enzyme, which is also activated by phosphorylation by the kinases LKB1 or CAMKII. Activated AMPK switches on catabolic pathways such as fatty acid oxidation and switches off anabolic pathways such as lipogenesis or gluconeogenesis. In peripheral tissues, these actions are important to help restore energy depletion (45). In the AN/VMH and PVN regions, AMPK activity is increased in response to insulin-induced hypoglycemia (44) or reduced by peripheral or intracerebroventricular glucose injections (73). The role of this enzyme in the counterregulatory response to hypoglycemia has been evaluated by direct microinjection of the AMPK-activator AICAR in the VMH. This markedly increased endogenous glucose production during a hypglycemic clamp without increasing epinephrine or glucagon secretion. This suggests that, in the VMH, AMPK may regulate hepatic glucose production through activation of the autonomic nervous system (68). Activation of AMPK in the VMH also improves the counterregulatory response in animals with defective counterregulation due to previous exposure to hypoglycemic or glucoprivic signals (4, 67). Also, blocking hypothalamic AMPK with compound C or by expressing a dominant negative form of the kinase strongly reduced counterregulation to insulin-induced hypoglycemia (44).

Together, the above studies indicate that central GLUT2, glucokinase, K\textsubscript{ATP} channel, and AMPK may each play a role in hypoglycemia detection and counterregulatory response. However, we are still lacking a precise mapping of the cells where these genes need to be expressed for the response to hypoglycemia. It is known that K\textsubscript{ATP} channels and AMP-kinase are expressed in almost all regions of the brain, although activation of the AMPK by glucoprivation seems to be restricted to the basolateral hypothalamus and the PVN. With regard to GLUT2 and glucokinase, their expression is more restricted in the brain but concordant probably only in a subset of cells. Thus the hypoglycemia-sensing process is clearly very complex and probably involves interactions between different sensing cells monitoring glucose levels by different means. Deciphering the complexity of this hypoglycemia-activated neuronal circuit is required to understand the molecular events leading to hypoglycemia-associated autonomic failure (HAAF), which is the main limiting factor in insulin therapy of Type 1 or Type 2 diabetes (27).

Central glucose sensing in the regulation of food intake and energy expenditure. The control of body weight depends on the balance between food intake and energy storage and expenditure. Hypothalamic as
as brain stem nuclei play a critical role in integrating the information on absorbed food, on the amount of energy stored in the form of fat and on blood glucose levels to regulate feeding, energy storage, or expenditure. This information is transmitted to the brain by hormones produced by gut endocrine cells, such as ghrelin, CCK, GLP-1, or peptide YY$_{3-36}$ by fat (leptin) or pancreatic beta-cells (insulin), but also through autonomic nervous connections activated by lipids, amino acids, and glucose (63, 105, 115, 123). The sites of convergence of these signals are neuronal circuits in the hypothalamus and brain stem (58, 115) (Figure 2), in particular, neurons in the AN, which produce the orexigenic neuropeptides NPY and Agrp or the anorexigenic peptides POMC and CART. These can be directly regulated by leptin, insulin, ghrelin, lipids, and glucose (80, 86, 87, 106, 116). The AN neurons in turn regulate neurons located either in the PVN or the LH. Whereas the PVN neurons express anorexigenic peptides such as TRH and CRH, LH neurons express the orexigenic peptides orexins and melanin concentrating hormone (MCH) (106). The balance between the output from the different hypothalamic nuclei, forming the so-called melanocortin pathway, regulates peripheral tissues metabolism and higher brain structure to control feeding behavior, glucose, and energy homeostasis.

The role of glucose in the regulation of feeding was evaluated in many studies (65, 78); in particular, it has been shown that initiation of feeding is preceded by a drop in glycemia (57). If this is prevented by glucose infusion, initiation of feeding is suppressed (24). Experimentally, induction of cellular glucoprivation by administration of 2-DG either peripherally (84) or centrally (14, 74) induces food intake. In the central nervous system, evidence points to the hindbrain as the primary site of 2-DG detection controlling feeding. Indeed, whereas intracerebroventricular injection of 2-DG induces food intake, direct injection of the anti-metabolite into the LH or VMH (14), or injection into the lateral ventricles but in the presence of an obstruction of the cerebral aqueduct (95), failed to activate feeding. Furthermore, food intake can be stimulated by direct injections of 5-TG into the BLM, DMNX and NTS (98) where glucose and 2-DG-sensitive neurons are located (30, 77, 98, 129, 130). These neurons are catecholaminergic (99) and project to the hypothalamus, in particular the PVN and the AN. Destruction of these neuronal projections by immunotoxins, suppressed the effect of intraperitoneal 2-DG administration on food intake (96) and on the regulation of NPY and Agrp expression (40). Thus the hindbrain appears to be a critical site of glucodetection in the control of feeding.

GLUT2 glucokinase, $K_{\text{ATP}}$ channels, and AMP-kinase in the control of feeding and energy expenditure. A role for central GLUT2 expression in feeding regulation has been suggested by experiments in which GLUT2 antisense oligonucleotides were injected intracerebroventricularly in rats. This led to a reduction in feeding and body weight and a suppression of the feeding response to 2-DG (119). Also, GLUT2-null mice (rippl1;glut2–/–) show abnormal feeding behavior (12). When fed ad libitum, they eat ~20% more than their control, and, following a 24-h fast, they initiate feeding less rapidly, but after a 48-h period their total food intake is again higher than that of control mice. These defects in feeding response can be traced to a defect in central glucose sensing. Indeed, both intraperitoneal and intracerebroventricular injections of glucose in fasted mice reduced refeeding in control but not in Glut2-null mice; similarly, both intraperitoneal and intracerebroventricular injections of 2-DG in fed mice induced feeding in control but not in mutant mice. Thus, in the absence of GLUT2, glucose or 2-DG are no longer recognized as regulators of feeding, and at least some of the effects of glucose or glucoprivation could be controlled by central sensors. Evaluation of the regulated expression of the orexigenic (NPY, Agrp) and anorexigenic (POMC, CART) neuropeptides during the fast-to-refed transition revealed that absence of GLUT2 prevented the normal regulation of these neuropeptides. This was shown to be the direct consequence of central glucose sensing since intracerebroventricular glucose injection in fasted mice decreased NPY and increased POMC expression in control but not in GLUT2-null mice. During the fast-to-refed transition, the regulation of plasma leptin and insulin levels, as well as glycemia, are the same in control and mutant mice. Thus the defect in the regulation of the melanocortin pathway is due to a defect in glucose recognition, indicating also that the leptin and insulin signals can regulate this neuronal circuit only if glucose is also normally detected. This indicates that a convergence of three signals on the NPY and POMC neurons may be necessary for the normal regulation of feeding.

The location of the central GLUT2-dependent sensors is still unclear. This transporter is present in several hypothalamic and brain stem structures (50, 54, 55), and immunohistochemical detection studies at the light and electron microscopic levels found GLUT2 in many cerebral structures but in dispersed cells rather than associated with specific nuclei or groups of cells (8, 9). GLUT2 was found in neurons, astrocytes, endothelial cells, and tanyocytes of the third ventricles (42, 59, 82). Specific expression in NPY or POMC neurons has, however, not been reported. Thus regulation of orexigenic and anorexigenic peptide expression by GLUT2-dependent sensors may be controlled indirectly, possibly even by sensors located in other brain regions, such as the brain stem. This would be compatible with the location of glucose-sensitive neurons of the brain stem controlling feeding (see above).

It must be emphasized, however, that there may not be a single site of glucose sensing controlling feeding. Indeed, peripheral injections of 2-DG may also activate the hepatoportal sensor (84), which requires the
presence of GLUT2 for its normal function (21) and also participates in the regulation of feeding (83, 103). Thus, as for counterregulation, glucose regulates feeding by acting on several sensors, located at different anatomical sites and connected together, in particular at the level of the brain stem and converging to regulate the melanocortin pathway. Also, there is evidence that the GLUT2-dependent sensors involved in the control of feeding are distinct from those involved in counterregulation (61).

The involvement of glucokinase in the control of feeding was evidenced by showing that its pharmacological inhibition by alloxan injection in the fourth ventricle induced feeding (100). In contrast, intracerebroventricular injection of alloxan at relatively high concentrations (40 µg/rat) increased glucokinase mRNA expression in the hypothalamus and inhibited the stimulation of feeding in response to intraperitoneal 2-DG injection. This effect was transient and related to the level of expression of glucokinase mRNA. It was proposed that the higher expression of this enzyme increases glucose utilization, intracellular ATP levels, and consequently prevents feeding initiation (102, 122).

Evidence for a role of K_{ATP} channels in the control of feeding comes from the study of mice with inactivation of the Kir6.2 gene, which displays a smaller feeding response to intraperitoneal 2-DG administration than control mice (72).

In the hypothalamus, AMPK may play an important role in the control of whole body energy homeostasis by integrating nutrient and hormonal signals that regulate food intake and energy expenditure. Hypothalamic AMPK is stimulated by orexigenic agents such as ghrelin and 2-DG, whereas it is inhibited by leptin, insulin, and glucose, which inhibit food intake (7, 73, 125). Activation of AMPK in the hypothalamus is sufficient to increase food intake, body weight, as well as the expression of NPY and AgRP in arcuate nucleus and melanin-concentrating hormone in lateral hypothalamus. In contrast, suppression of this activity induces a decrease of these parameters. In vitro, neuronal cell lines that express a dominant negative AMPK significantly decreased low glucose- or 2-DG-induced AgRP expression (53). Thus hypothalamic AMPK activity may participate in the control of glucose of food intake by regulating the expression of neuropeptides from the melanocortin pathway.

In this regulatory mechanism, high glucose inhibits food intake by reducing AMPK activity and, consequently, the phosphorylation of ACC. Dephosphorylated ACC is more active and produces increased amounts of malonyl-CoA, thereby inhibiting mitochondrial carnitine palmitoyltransferase 1 (CPT1) and reducing fatty acid beta-oxidation (45). This suppresses food intake and reduces the expression of hypothalamic NPY and AgRP (46, 85). It has been proposed that the increase in cellular fatty acyl-CoA that results from inhibition of CPT-1 may lead to increased usage of glucose. The increase in glucose metabolism has been proposed to be the key signal to control feeding (124).

Summary and Perspectives

Besides its well described role in controlling insulin secretion by pancreatic beta-cells, glucose is also an important modulator of the activity of several subsets of neurons, which control many physiological responses, including counterregulation, feeding behavior, and energy homeostasis. Importantly, there are multiple sites of glucose detection: in the gut and hepatoportal vein area, in the hindbrain, and in the hypothalamus, and those are connected to each other. These sites form a network for monitoring blood glucose concentration and integrate this information at the brain stem and hypothalamic level to regulate the activity of autonomic nerves and also send signals to higher brain structures to control behavior.

There is evidence for multiple classes of glucose sensors that respond either by activating or suppressing their firing activity in response to increases in extracellular glucose concentrations, and a further distinction can be made depending on the range of glucose concentration considered since HGE and HGI neurons seems to be distinct from the originally described GE and GI neurons. Furthermore, whereas neurons can probably be directly regulated by glucose, there is also strong evidence that glucose response may depend on the initial uptake and metabolism of glucose by astrocytes and the subsequent transfer of lactate to neurons for ATP generation and regulation of firing activity. There is also good evidence that different glucose-regulated neurons may control counterregulation or feeding behavior.

At the molecular level, proteins required for neuronal glucose sensing have been identified, and several also play a major role in pancreatic beta-cell glucose sensing, GLUT2, glucokinase, and the K_{ATP} channel. However, AMP-kinase, Na-K-ATPase, chloride channels, and tandem pore K’ channels have also been suggested to be important for the response to low glucose concentration and TRP channels for response to glucose over high concentration ranges.

Although not discussed in this review, the activity of glucose-sensing neurons is also modulated by a variety of substrates, such as lipids (71, 120), and hormones, such as insulin and leptin (25, 110, 111, 121), leading to an increased degree of complexity in the response of these neurons to changes in whole body energy level.

Thus there is still an enormous amount of work to be done to identify the different neurons and/or astrocytes involved in glucose sensing, the cellular network they form, how they are regulated by other hormonal and nutrient signals, and the specific physiological
functions they control. Identifying the variety of genes required for glucose sensing, mapping their expression, and evaluating their function by combined gene knockout and physiological analysis will be required to be in a position to interfere with defects in counter-regulatory response associated with hypoglycemia-associated autonomic failure and with obesity development.

References


