Perspectives in Diabetes
Neuronal Glucosensing
What Do We Know After 50 Years?

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Glucosensing neurons are specialized cells that use glucose as a signaling molecule to alter their action potential frequency in response to variations in ambient glucose levels. Glucokinase (GK) appears to be the primary regulator of most neuronal glucosensing, but other regulators almost certainly exist. Glucose-excited neurons increase their activity when glucose levels rise, and most use GK and an ATP-sensitive K$^+$ channel as the ultimate effector of glucose-induced signaling. Glucose-inhibited (GI) neurons increase their activity at low glucose levels. Although many use GK, it is unclear what the final pathway of GI neuronal glucosensing is. Glucosensing neurons are located in brain sites and respond to and integrate a variety of hormonal, metabolic, transmitter, and peptide signals involved in the regulation of energy homeostasis and other biological functions. Although it is still uncertain whether daily fluctuations in blood glucose play a specific regulatory role in these physiological functions, it is clear that large decreases in glucose availability stimulate food intake and counterregulatory responses that restore glucose levels to sustain cerebral function. Finally, glucosensing is altered in obesity and after recurrent bouts of hypoglycemia, and this altered sensing may contribute to the adverse outcomes of these conditions. Thus, although much is known, much remains to be learned about the physiological function of brain glucosensing neurons. *Diabetes* **53:2521–2528, 2004**

More than 50 years ago, Jean Mayer proposed a "glucostatic hypothesis" whereby hypothalamic "glucoreceptors" sense fluctuations in available glucose by ". . . the passage of potassium ions into glucoreceptor cells along with the glucose phosphate. . . ," which ". . . is translated into an electric or neural mechanism" (1) as a means of regulating food intake. It was >11 years before Oomura et al. (2) and Anand et al. (3) actually demonstrated the presence of such specialized glucosensing neurons in the rat brain. They showed that activity of glucose-excited (GE) neurons increases while glucose-inhibited (GI) neurons decrease their activity as ambient glucose levels rise. In the following years, our knowledge has greatly expanded of the regulatory steps that allow neurons to sense ambient levels of glucose, where such glucosensing neurons reside, and, to a lesser extent, the ways in which they participate in a variety of physiological functions. Here we will address these issues and provide evidence for the hypothesis that glucokinase (GK) (hexokinase IV) is the predominant regulator of neuronal glucosensing, much as it is in pancreatic β-cells (4) and α-cells (5,6). We will also make the case that these important neurons respond to more than glucose and are actually metabolic sensors that receive and integrate a variety of metabolic, neural, and hormonal signals from the periphery, which enables them to act as critical regulators of energy homeostasis.

GLUCOSENSING NEURONS INTEGRATE A VARIETY OF TIME- AND CONCENTRATION-DEPENDENT PATTERNS OF GLUCOSE CHANGE

Perhaps because it depends on a constant supply of glucose to fuel its metabolic demands (7), the brain has evolved specialized glucosensing neurons to monitor and respond to the availability of glucose. Unlike most neurons, which use glucose to fuel their metabolic demands, these specialized neurons use the products of intracellular glucose metabolism to regulate their activity and transmitter release. There are also peripheral glucosensors in the portal vein (8), gut (9), and carotid body (10), some of which communicate with central glucosensing neurons, which provides a means of integrating peripheral and central signals relating to glucose availability (11). Central glucosensing neurons pass these integrated signals on to
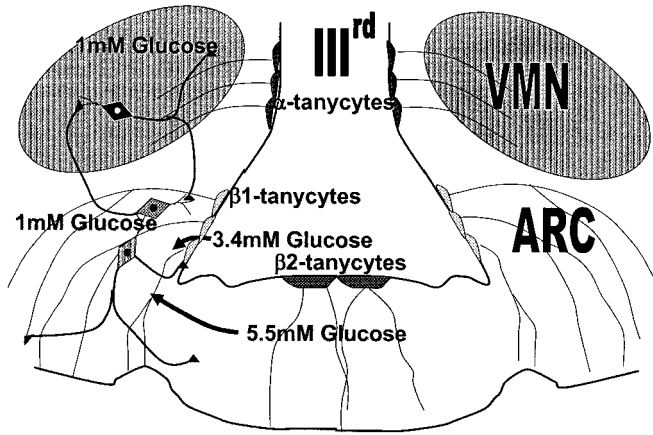
FIG. 1. Location of ARC and VMN neurons relative to blood, CSF, and brain glucose levels. ARC neurons are potentially exposed to glucose from the CSF, which diffuses across the β1-tanycytes lining the IIIrd cerebral ventricle; blood glucose, which diffuses across the fenestrated capillaries in the median eminence; and glucose, which is transported across the blood-brain barrier. Some of these neurons synapse with VMN neurons, which are exposed primarily to glucose transported from blood and diffusing from CSF.

effector systems involved in the regulation of peripheral glucose metabolism. Because of their location in a variety of anatomically discrete brain areas, glucosensing neurons are exposed to different quantitative and temporal patterns of changes in glucose levels (Fig. 1). Glucose undergoes facilitated transport across the blood-brain barrier, resulting in extracellular brain glucose levels that range from ~10 to 30% of blood levels during hypo- to hyperglycemic conditions (12–15). This barrier is composed of cerebral microvessels with their tight junctions and astrocytic foot processes (16) (Fig. 2). Extracellular brain glucose levels rapidly equilibrate with blood levels (12) but vary considerably among brain areas. In the hypothalamus, basal levels are ~1.4 mmol/l (13), whereas they are 1.0 mmol/l (14) in the hippocampus and 0.5 mmol/l in the striatum (14). By contrast, cerebrospinal fluid (CSF) glucose levels

are approximately two-thirds of blood levels and take ~15–30 min to equilibrate (17). Finally, glucosensing neurons within areas such as the hypothalamic arcuate nucleus (ARC) and hindbrain nucleus tractus solitarius lie adjacent to brain areas with fenestrated capillaries and ependymal and tanycyte lining cells (18,19). They are thus exposed to and are likely to monitor and integrate glucose signals with different quantitative and temporal profiles simultaneously from blood, brain, and CSF (Fig. 1). This may explain why ARC GE glucosensing neurons respond to a wide range of glucose levels, from 0.5 to 10 mmol/l (20), whereas ventromedial hypothalamic nucleus (VMN) glucosensing neurons, which are exposed to only brain interstitial and CSF glucose levels, respond to a much narrower range of glucose levels, from 0.1 to 2.5 mmol/l (21–23) (Fig. 1).

In general, glucosensing neurons are located in brain areas involved in the control of neuroendocrine function, nutrient metabolism, and energy homeostasis. Glucosensing neurons within these areas also receive direct and indirect neural input from the periphery and from other brain areas that convey information about the sight, smell, taste, texture, rewarding properties, visceral handling, and resultant blood levels of ingested nutrients (24). Glucosensing neurons in the ventromedial hypothalamus (VMH) (ARC + VMN) are among the best characterized. In the VMN, 14–19% are GE and 3–14% are GI in type (21,22). The lateral hypothalamus contains predominantly GI neurons (25). Additional glucosensing neurons are located in a variety of forebrain and hindbrain areas, many of which have important neuroendocrine and autonomic outputs (22,26–31).

HOW IS GLUCOSE SENSED?

Unlike most neurons, glucosensing neurons use glucose in a concentration-dependent manner as a signaling molecule to regulate their membrane potential and action potential frequency (2,20,32,33). In many ways, GE neurons are the
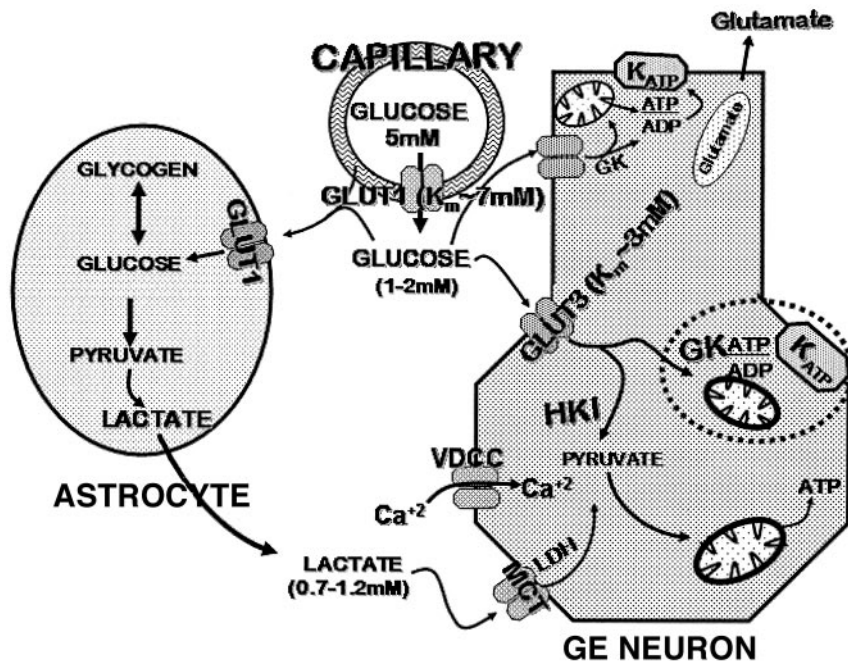

FIG. 2. Hypothetical model of a GE neuron and its relationship to adjacent astrocytes. Glucose is transported across the microvessel by GLUT1 and enters either the astrocyte by GLUT1 transport or the extracellular space, where it is transported into a GE neuron by GLUT3. GK is localized together with adjacent mitochondria beneath the plasma membrane containing a K$_{ATP}$ channel. ATP formed in this microenvironment binds to the K$_{ATP}$ channel, which inactivates (closes) the channel leading to membrane depolarization, entry of calcium through a voltage-dependent calcium channel (VDCC), and, in many cases, increased neuronal activity. Local changes in ambient glucose concentrations at the axon terminal can also inactivate K$_{ATP}$ channels with release of neurotransmitters such as glutamate. Hexokinase I (HKI) regulates the formation of ATP for general metabolic functions of the neurons. Glucose transported into astrocytes is stored as glycogen. Glycogenolysis produces lactate that is transported by an MCT1 transporter into the extracellular space and then into the neuron through MCT1. This lactate is converted to pyruvate by LDH and oxidized in the mitochondria with resultant ATP production. Under low ambient glucose conditions, this astrocyte-derived lactate can raise neuronal ATP levels sufficiently to close the K$_{ATP}$ channel, leading to neuronal activation.

brain analogs of the pancreatic β-cell, whereas GI neurons have some similarities to α-cells. GE neurons and β-cells are activated and GI neurons and α-cells are inhibited by rising glucose levels (5,20–23,34). We currently know a great deal about the glucose-dependent mechanism regulating activity and transmitter release in GE neurons and β-cells but know relatively less about the way glucose alters activity in GI neurons and α-cells.

Our current hypothetical model for glucosensing in GE neurons is presented in Fig. 2. Although some glucosensing and nonglucosensing neurons express GLUT2 and GLUT4 (23), the majority use the high-capacity high-affinity glucose transporter 3 (GLUT3) as their primary transporter (23). Intracellular glucose is phosphorylated by hexokinase I and, in many glucosensing neurons, by the pancreatic form of GK (hexokinase IV), the rate-limiting step in β-cell glucosensing (34). Glucose metabolism increases the ratio of ATP to ADP. This causes ATP to bind to the ATP-sensitive K^+ (K_{ATP}) channel composed of a Kir6.2 pore-forming unit for potassium and a sulfonylurea receptor (35). Binding of either ATP or sulfonylureas inactivates (closes) the channel and depolarizes the cell membrane. Depolarization is followed by influx of extracellular calcium through a voltage-dependent calcium channel (22,23) and is often associated with increased action potential frequency. Glucose-induced closure of the K_{ATP} channel at nerve terminals on some presumptive GE neurons can also release neurotransmitters independently of action potentials propagated from the cell body (36). Although the K_{ATP} channel is a necessary component of glucosensing in most GE neurons (37), it is unlikely to be the sole determinant of GE neuronal glucosensing because it is present in many other neurons that have no apparent glucosensing capability (38). In those neurons, activation of the K_{ATP} channel may play a neuroprotective role by hyperpolarizing the membrane as protection against the neurotoxic amounts of glutamate released during severe hypoglycemia and hypoxia (39). Although the K_{ATP} channel appears to be the final common pathway involved in GE glucosensing, much less is known about GI neuronal glucosensing. A Cl^- channel (21), the Na^+-K^+ ATP pump (40), and an ATP-responsive K^+ channel (41) have all been proposed as possible final common pathways, but the actual effector of GI glucosensing is unknown.

A great deal of evidence suggests that GK is the primary regulator of glucosensing in many GE and GI neurons. Hexokinase I is an unlikely regulator of glucosensing, since it is saturated at physiological brain glucose concentrations and is subject to feedback inhibition by its primary product, glucose-6-phosphate (42). On the other hand, the brain expresses the pancreatic form of GK (42–45), and this enzyme is not subject to end product inhibition (34). GK mRNA and/or its immunoreactive protein are selectively localized in several brain areas involved in glucosensing (6,22,31,46,47). GK mRNA is expressed in ~70% of GE and ~40% of GI neurons (23) and appears to be the critical regulatory step in their glucosensing ability (20,22,23,43,47,48), as it is for insulin release in β-cells and glucagon release in α-cells (5,6,34). Pharmacological inhibition of GK activity decreases activity in GE and increases activity in GI neurons (20,22,23,43,48), and knockdown of GK mRNA in cultured VMH neurons by transfection of small interfering mRNAs for GK almost completely abolishes demonstrable GE and GI neurons (49). Finally, under conditions in which VMH GK mRNA expression is increased, rats have a reduced counterregulatory response to systemic glucoprivation (22,30,50), possibly due to a leftward shift in the sensitivity of GK to glucose. Thus, mounting evidence, although clearly incomplete, suggests that GK does function as the primary regulator of glucosensing in many GE and GI neurons.

This issue is far from settled, however. First, it is unclear how an enzyme with a putative K_m of 8–10 mmol/l (42) might regulate glucosensing at the low concentrations and small (0.1–0.2 mmol/l) incremental changes in brain glucose levels. It is unlikely that GK activity is modified by the GK regulatory protein as it is in the liver. Although found in brain (51,52), only 10% of GE and no GI neurons express the mRNA for this protein (23). Alternatively, GK activity might be altered in the brain as it is in the β-cell by binding to intracellular structures (53) or by interaction with phosphofructo-2 kinase/fructose-2,6-bisphosphate, an enzyme that is also present in the brain (54). However, these possibilities remain untested, largely because of the extremely low abundance of brain GK expression and enzymatic activity. Similarly, the high K_m and low abundance of brain GK relative to hexokinase I, as well as the fact that intracellular ATP is extremely well buffered and difficult to change, even in the face of increased glucose utilization (55), makes it unlikely that GK would have a significant effect on intracellular ATP levels in glucosensing neurons. Compartmentalization of GK beneath the plasma membrane in a microenvironment near individual K_{ATP} channels might enable GK to regulate their activity (56). Whereas this is an attractive hypothesis, such compartmentalization has not been demonstrated for GK in neurons to date.

Even if GK is the primary regulator of activity in many glucosensing neurons, a substantial proportion of these do not express measurable amounts of GK (23). Because of the symbiotic relationship between astrocytes and neurons, astrocyte-derived lactate might regulate activity in these neurons. Astrocytes readily take up and store transported glucose as glycogen and release lactate into the extracellular space (57) (Fig. 2). In glucosensing neurons, lactate appears to be transported by monocarboxylate (MCT)-1, where it is converted by lactate dehydrogenase (LDH) to pyruvate, which is then oxidized in mitochondria to provide ATP (23,58). In fact, some VMH glucosensing neurons express LDH-A and -B and MCT1 (23) and alter their activity when extracellular lactate levels are changed (43). Despite this ability to transport and use astrocyte-derived lactate as a source of signaling, it is clear that glucosensing neurons can sense and respond to glucose in the complete absence of lactate (22,23). Further, even though changes in extra-neuronal lactate clearly can alter neuronal activity, the mechanism for this signaling capacity has not been identified. Finally, the fact that glucosensing and nonglucosensing neurons appear to express MCT and LDH in equivalent abundance makes it likely that astrocyte-derived lactate is more often used as an alternate substrate to support neuronal metabolism than as a signaling molecule to regulate activity (22,23,59).
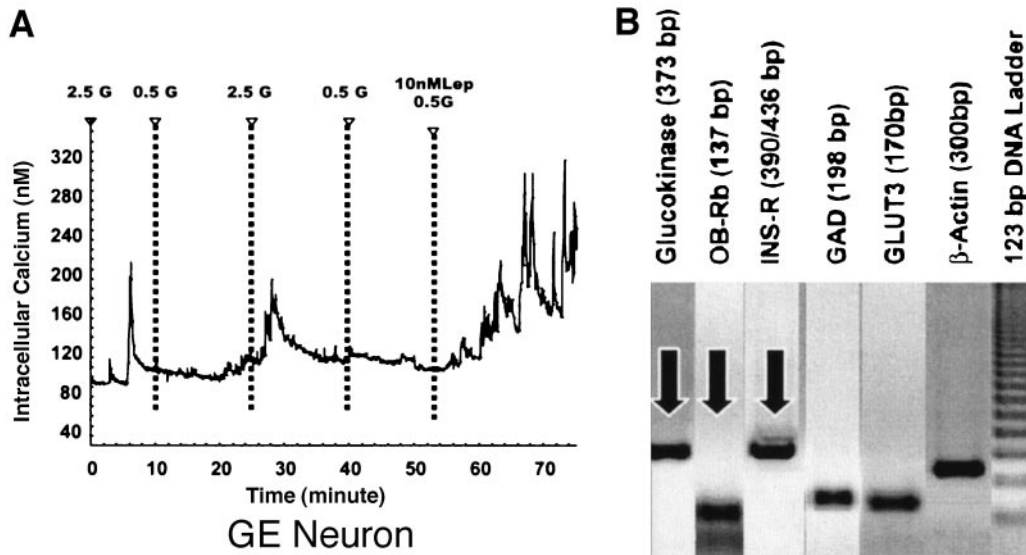
FIG. 3. Leptin (Lep) increases intracellular calcium oscillations in a dissociated VMN GE neuron that expresses the OB-Rb receptor. Addition of 10 nmol/l leptin to a GE neuron that was inactive at 0.5 mmol/l glucose (G) leads to activation (increased intracellular Ca^{+2} oscillations). Single-cell RT-PCR of the cytoplasmic contents of this neuron demonstrated that it expressed mRNA for GK, the signaling form of the leptin receptor (OB-Rb), the insulin receptor (INS-R), GLUT3, and GAD (which defines it as GABAergic) (23,96).

Glucose transport is also not a likely regulator of neuronal glucosensing. GLUT3, the ubiquitous neuronal transporter, is fully saturated at most levels of brain glucose and is thereby unlikely to provide a gate-keeping function for glucosensing (23,60). Although it is expressed in up to one-third of glucosensing neurons, GLUT2 is also unlikely to be a critical determinant of neuronal glucosensing in most cases, since an equal number of nonglucosensing neurons also express GLUT2 (23). On the other hand, 75% of GE and 60% of GI neurons coexpress the insulin receptor and the insulin-dependent transporter GLUT4 (23). But, it is unlikely that insulin-mediated glucose transport is required for neuronal glucosensing in most cases, since all GE and GI neurons respond to alterations of ambient glucose levels in the complete absence of insulin (20,21,23). However, this might become important at low glucose concentrations, where insulin-mediated glucose transport could supplement low intracellular glucose levels and lead to increased activity in GE neurons (20). Finally, although some GE neurons reduce their activity in the presence of phlorizin, an inhibitor of the sodium-glucose–linked glucose transporter (SGLT) (43), only 25% of GE and 10% of GI neurons express the mRNA for SGLT1 (23). However, other members of this family are found in the brain (61) and might provide an alternate method of glucosensing in glucosensing neurons without GK.

Taken together, current evidence favors a primary regulatory role for GK in the majority of brain glucosensing neurons. In GE neurons, the K_{ATP} channel appears to provide the transduction mechanism whereby changes in the intracellular ATP/ADP ratio are translated into changes in membrane potential and neuronal activity. Unfortunately, we still have no firm working model of how GI neurons sense glucose. We have shown that almost 40% of GI neurons use GK as a gatekeeper and that depolarization is associated with closure of an as yet unidentified Cl^- channel (21,23). This Cl^- channel may be the final pathway in GI neurons, as is the K_{ATP} channel in GE neurons. In those neurons that do not express GK, glucosensing might be mediated by lactate or glucose transport via GLUT2, GLUT4, or SGLT.

GLUCOSENSING NEURONS AS METABOLIC SENSORS

Their location within brain areas critically involved in the regulation of energy homeostasis such as the ARC also suggests that these neurons might do more than just sense glucose. Besides glucose, glucosensing neurons respond to a variety of metabolites such as lactate, ketone bodies, and free fatty acids (43,48,62,63). Glucosensing neurons also express receptors for and respond to peripheral hormones that convey signals relating to fat stores such as leptin (64) (Fig. 3) and insulin (20). Both leptin (64) and insulin (65) decrease action potential frequency in GE neurons at high glucose concentrations by activating the K_{ATP} channel. Long-chain acyl-CoA also activates this channel (66) as well as inhibits GK activity (67). Thus, glucosensing neurons are really "metabolic sensors" in which a variety of metabolic, hormonal, transmitter, and peptide signals related to metabolic status are summated at the level of the membrane potential to alter neuronal activity.

Anabolic ARC neuropeptide Y (NPY) and catabolic proopiomelanocortin (POMC) neurons are prototypic examples of such metabolic sensing neurons because of their important roles as regulators of energy homeostasis. NPY neurons are GI and POMC neurons are GE (68,69), and both express GK mRNA (22,44). Both have receptors for and respond to peripheral hormones such as leptin and insulin (20,70,71). These hormones, as well as a variety of metabolic substances, are transported across the blood-brain barrier (72,73) but can also freely diffuse from capillaries in the adjacent median eminence. Thus, as they are for glucose, ARC GI (NPY) and GE (POMC) neurons are well positioned to sample these hormones and metabolites simultaneously from brain interstitial space, blood, and CSF (Fig. 1). Finally, they have well-established pro-

jections to downstream effector areas controlling neuroendocrine, metabolic, and autonomic functions that regulate energy homeostasis and glucose metabolism (74,75).

WHAT PHYSIOLOGICAL FUNCTIONS ARE REGULATED BY GLUCOSENSING NEURONS?

After 50 years, it is still uncertain whether Mayer was correct in his contention that glucose is an important regulator of food intake (1). In rats (76) and humans (77), some meals begin after a spontaneous 10% drop followed by an upswing in blood glucose levels. However, it is unclear whether these changes in blood glucose levels actually initiate meal taking or are simply an epiphenomenon of a larger central pattern of feeding-related behaviors and metabolic events. Clearly, the reductions in glucose availability experienced during hypoglycemia produce feelings of hunger in humans (78) and stimulate food intake in rats (79). But humans report hunger only after glucose levels fall to dangerously low levels, slightly before impaired cognition occurs (80). Systemic (81) and central (82) administration of 2-deoxyglucose, a competitive inhibitor of glucose metabolism, also elicits food intake in rodents. Such glucoprivic feeding also can be initiated from both hypothalamic (30) and hindbrain sites (29). But it is impossible to know what level of cellular glucoprivation this drug actually produces. Whereas glucoprivation of a sufficient degree clearly increases feeding, data in support of a satiating effect of raised blood or brain glucose levels are conflicting. In rats, acute third ventricular injections of glucose reduced food intake (83), whereas sustained infusions were required to produce reduced body weight gain in another study (84). However, in neither case were the levels of extracellular glucose produced by these manipulations known. In humans, acute hyperglycemia reduced hunger during fasting but not after a nutrient preload (85) in one study, whereas >2 h of hyperinsulinemic-hyperglycemic (15 mmol/l) clamping was required to decrease hunger in another study (86). To reduce food intake, several days of combined elevations of both blood glucose and insulin levels were required in baboons (87) and carotid artery glucose plus insulin infusions were required in rats (88). Thus, although very low or high levels of glucose availability may alter feeding and body weight regulation, it is unclear whether acute changes of glucose within the physiological range have any primary role in regulating normal meal initiation or termination. On the other hand, the level of basal glucose may participate in the state-dependent regulation of food intake as one of many signals integrated by metabolic sensing neurons at the level of their membrane potential.

In addition to feeding, extremes of brain glucose availability also affect sympathoadrenal and neuroendocrine function. Elevations of blood glucose within the physiological range lead to sympathetic activation (89), and this is reproduced by slow intracarotid glucose infusions that activate sympathetic effector areas in the hypothalamus (27). On the other hand, injections of glucoprivic agents into either the VMH (90) or discrete caudal hindbrain sites activate neurohumoral counterregulatory responses (29,30) similar to those seen during systemic hypoglycemia. Aside from these emergency responses that protect the supply of cerebral glucose, it may well be that glucose-mediated neurohumoral and sympathoadrenal activation also participates in the physiological processes involved in glucose homeostasis, but this postulate remains to be proven.

HOW IS GLUCOSENSING ALTERED BY DISEASE?

Because tight control of blood glucose levels with increased insulin administration has become accepted practice in the treatment of type 1 diabetic patients, the incidence of severe bouts of hypoglycemia has increased (91). Such bouts lead to an attenuated ability to sense the symptoms of and mount a full counterregulatory response to subsequent hypoglycemic episodes (50,92). This down-regulation may represent an adaptive response that allows the brain to use glucose more efficiently at low levels. Part of this adaptation might involve hypothalamic glucosensing neurons, since blunting of the counterregulatory response is associated with an upregulation of hypothalamic GK mRNA at 48 h after a single bout of hypoglycemia (22,50). Similarly, 2-deoxyglucose–induced hyperglycemia and hyperphagia are inhibited in association with upregulation of hypothalamic GK mRNA, which follows third ventricular injections of the pancreatic β-cell toxin alloxan (30). This alloxan effect is transient and, when GK levels fall, both glucoprivic food intake and hyperglycemia are restored. Thus, if the upregulation of GK mRNA was translated into increased GK enzyme activity, it could enable the neurons that express GK to maintain ATP production during low levels of glucose, thus lowering the glucose threshold at which counterregulatory responses to hypoglycemia are initiated.

Another example of altered glucosensing is seen in rats that exhibit a polygenic predisposition to develop diet-induced obesity when fed a high-fat diet. Even before they become obese, the K_{ATP} channels of their GE neurons have reduced sensitivity to both ATP and sulfonylureas (93). They also have a reduced number of all subtypes of glucose-sensing neurons in their VMN (21) as well as a variety of defects in whole animal and cellular responses to altered glucose levels (93). Perhaps in compensation for these multiple glucosensing defects, these obesity-prone rats also have increased VMH GK mRNA expression (22). In keeping with their reduced ability to sense glucose, they are also hyporesponsive to the anorectic effects of leptin (94,95), suggesting that, overall, rats with an obesity-prone genotype have an increased threshold for metabolic sensing that might require them to become obese before such signals would be detected by these neurons.

SUMMARY AND CONCLUSIONS: WHAT HAVE WE LEARNED AND WHAT REMAINS TO BE LEARNED?

It is now clear that glucosensing neurons represent a specialized and discretely localized set of cells that sense glucose. Many also respond to a host of metabolic and hormonal signals from the periphery. Many glucosensing neurons are located within brain areas that allow them to sample such signals simultaneously from several different compartments. The integrated output of these neurons is relayed to neurohumoral and autonomic effector areas involved in the regulation of glucose metabolism and overall energy homeostasis. A compelling case can be

made for GK as the predominant regulator of neuronal glucosensing in GE and, to a lesser extent, in GI neurons. In GE neurons, the K_{ATP} channel appears to be the ultimate transduction mechanism for integrating signals generated by both glucose metabolism and activation of receptors for insulin and leptin, as well as possibly responses to fatty acids. Whereas much has been learned, many questions remain unanswered. How does GK with its high K_m and low abundance regulate intracellular ATP levels to control activity of the K_{ATP} channel in GE neurons? What is the signal transduction mechanism by which GI neurons sense glucose? How do glucosensing neurons that do not express GK sense glucose? What are the convergence points within metabolic sensing neurons by which various hormones and metabolites alter neuronal activity? What is the true role of glucose in the physiological control of food intake, autonomic function, and energy homeostasis? These and many other questions provide fertile ground for future research in the area of neuronal glucosensing.

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