Brain glucose-sensing mechanisms: ubiquitous silencing by aglycemia vs. hypothalamic neuroendocrine responses

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Mobbs, Charles V., Lee-Ming Kow, and Xue-Jun Yang. Brain glucose-sensing mechanisms: ubiquitous silencing by aglycemia vs. hypothalamic neuroendocrine responses. Am J Physiol Endocrinol Metab 281: E649–E654, 2001.—Interest in brain glucose-sensing mechanisms is motivated by two distinct neuronal responses to changes in glucose concentrations. One mechanism is global and ubiquitous in response to profound hypoglycemia, whereas the other mechanism is largely confined to specific hypothalamic neurons that respond to changes in glucose concentrations in the physiological range. Although both mechanisms use intracellular metabolism as an indicator of extracellular glucose concentration, the two mechanisms differ in key respects. Global hyperpolarization (inhibition) in response to 0 mM glucose can be reversed by pyruvate, implying that the reduction in ATP levels acting through ATP-dependent potassium (K-ATP) channels is the key metabolic signal for the global silencing in response to 0 mM glucose. In contrast, neuroendocrine hypothalamic responses in gluoresponsive and glucose-sensitive neurons (either excitation or inhibition, respectively) to physiological changes in glucose concentration appear to depend on glucokinase; neuroendocrine responses also depend on K-ATP channels, although the role of ATP itself is less clear. Lactate can substitute for glucose to produce these neuroendocrine effects, but pyruvate cannot, implying that NADH (possibly leading to anaplerotic production of malonyl-CoA) is a key metabolic signal for effects of glucose on gluoresponsive and glucose-sensitive hypothalamic neurons.

THE BRAIN IS UNIQUELY DEPENDENT on the availability of glucose, not only because it is among the most metabo- 

ically active tissues (normalized for weight, the brain consumes roughly 10 times more oxygen than the body as a whole), but in contrast to most tissues, essentially all of this metabolism is derived from plasma glucose rather than alternative substrates such as free fatty acids (35). Not surprisingly, therefore, protective mechanisms have evolved that allow neurons to sense when glucose availability falls to dangerously low levels and to reduce neuronal activity, thus reducing neuronal metabolic demand. The mechanisms by which neurons sense and respond to profound hypoglycemia are of considerable interest, because impairments of these mechanisms might exacerbate long-term neuronal damage due to hypoglycemia or stroke (as occurs in diabetes), whereas manipulation of these protective mechanisms might conversely provide protection during hypoglycemia or stroke. Although profound hypoglycemia appears to reduce neuronal activity throughout the nervous system, physiological changes in blood glucose may also either stimulate or inhibit the activity of rare neuroen- 

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intake, and metabolic rate. The nature of these neuroendocrine glucose-sensing mechanisms is also of considerable interest, because impairments of these mechanisms might lead to impairments in counter-regulatory responses, as occurs in diabetes, or other metabolic impairments such as those associated with obesity. Recent studies have demonstrated similarities as well as important differences between the ubiquitous and the neuroendocrine glucose-sensing mechanisms of the brain, as well as similarities and differences between these mechanisms and the mechanisms by which pancreatic β-cells sense glucose. As described in the following sections, appreciation of the differences between these mechanisms can clarify observations that otherwise could appear to be paradoxical.

GLOBAL INHIBITION OF NEURONS THROUGHOUT THE NERVOUS SYSTEM AT 0 mM GLUCOSE: ROLE OF K-ATP CHANNELS

Profound hypoglycemia (below ~1 mM glucose) produces loss of consciousness, involving a global but reversible loss of electrical activity throughout the brain, as indicated by electroencephalogram (4, 30). Similarly, in vitro preparations have demonstrated that reducing glucose concentrations to 0 mM reversibly inhibits neuronal activity throughout the nervous system, including neurons from hippocampus (11), midbrain (19), hypothalamus (3, 39), and cortex (Fig. 1). It should be noted that, in vitro, only ~30% of neurons become inhibited after 3 min of aglycemia in hypothalamus (3) and cortex (39), whereas by 15 min of aglycemia, almost all neurons (at least that we have tested over several years) in both cortex and hypothalamus become electrically inhibited (Fig. 1); activity can be restored in most neurons if aglycemia is not maintained for more than ~20 min (Fig. 1).

A typical example of the general silencing of neurons that occurs at 0 mM glucose is shown in Fig. 1. This typical cortical neuron is spontaneously active at 20 mM glucose, and a reduction to 5 mM glucose does not influence the activity of this neuron (neurons that respond to a change from 20 to 5 mM glucose are largely confined to the hypothalamus (14, 39, and see further discussion)). When glucose is further reduced to 0 mM glucose, activity continues for ~3–15 min, whereupon neurons become silent (sometimes preceded by a brief period of excitation, as in Fig. 1). When glucose is restored to 20 mM shortly after the neuron becomes silent, neuronal activity is gradually restored over ~15 min. This process can be repeated, indicating that the silencing is not a reflection of direct toxicity. It should be noted that, because the majority of spontaneously active neurons respond to the transition from 0 to 20 mM glucose by increasing activity (being typically silent at 0 mM and active at 20 mM), it may be confusing to describe such neurons as “gluoresponsive,” because to use the term gluoresponsive to describe neurons that are activated from 0 to 20 mM glucose implies that neurons throughout the brain are gluoresponsive. The terms gluoresponsive and “gluose-sensitive” were coined to refer to the highly specialized neuroendocrine neurons that are excited and inhibited, respectively, by a more physiological transition of glucose concentrations (27) and that are thought to regulate metabolic processes; true gluoresponsive neurons appear to be largely confined to the hypothalamus (14, 39), consistent with data that lesions that impair neuroendocrine regulation or metabolic processes are largely confined to the hypothalamus (see further discussion).

Because global silencing of neuronal electrical activity during profound hypoglycemia is thought to serve a neuroprotective role during energy deficiency (13), the mechanism by which this response to profound hypoglycemia occurs is of great interest in the context of neuroprotection. The mechanism by which 0 mM glucose produces global silencing or hyperpolarization of neurons appears to involve a reduction in intracellular

Fig. 1. Cortical neurons are not gluoresponsive but are reversibly inhibited at 0 mM glucose. In vitro brain slices were prepared as described (39). Trace shows the electrical activity of a single cortical neuron recorded over an hour, during which glucose concentrations were manipulated. Under these conditions, many cortical neurons are spontaneously active at 20 mM glucose (0–5 min in the figure). Changing glucose concentration in the medium from 20 to 5 mM (5–12 min in the figure) has no effect on electrical activity in cortical neurons, indicating that these neurons are not gluoresponsive, because this same transition in glucose concentration will inhibit gluoresponsive neurons in the ventromedial hypothalamus, representing ~20% of the neurons in that area (39). However, reduction of glucose concentration to 0 mM (12–23 min) will lead to loss of electrical activity (in this case after ~11 min), preceded by a brief depolarization at 22 min. After neurons become silent, restoring glucose to 20 mM (at 25 min) gradually restores electrical activity. This process can be repeated (35–47 min), indicating that electrical silencing by aglycemia does not entail irreversible damage.
glucose metabolism (and a decrease in ATP) leading to an outward potassium current mediated by disinhibition of ATP-dependent potassium (K-ATP) channels in neurons, a mechanism that appears to be operative in several brain regions, including hippocampus (38), midbrain (19), and hypothalamus (2, 39). Certainly components of K-ATP channels are expressed ubiquitously in neurons throughout the brain (13), consistent with the global silencing of neurons that occurs at 0 mM glucose. A role for ATP in the hyperpolarization of neurons at 0 mM glucose is corroborated by the observation that pyruvate can substitute for glucose in restoring neuronal activity at 0 mM glucose in hippocampus (11) and hypothalamus (39).

NEUROENDOCRINE GLUCORESPONSIVE AND GLUCOSE-SENSITIVE NEURONS ARE LARGELY CONFINED TO THE HYPOTHALAMUS

A second motivation to study brain glucose-sensing mechanisms is that specific neuroendocrine hypothalamic neurons that regulate metabolic economy are sensitive to physiological changes in plasma glucose. Glucose has long been proposed to serve as a peripheral signal for hypothalamic regulation of metabolic processes (13). An early motivation for this hypothesis was that the glucose derivative gold-thio-glucose (GTG) produces a lesion largely confined to the ventromedial hypothalamus, leading to obesity and profound metabolic impairments, a lesion that absolutely requires the glucose moiety of GTG and insulin and is blocked by inhibition of glucose transport (6). These data suggested that destruction of glucose-sensitive neurons in the hypothalamus was sufficient to produce profound metabolic impairments. In contrast to the global silencing of neurons that occurs in response to profound hypoglycemia (below 1 mM glucose) (4), neurons from other brain areas, including cortex and hippocampus, do not typically exhibit change in electrical activity during more physiological changes in glucose concentrations (14, 39) (Fig. 1). On the other hand, highly specific subpopulations of hypothalamic neurons become gradually and increasingly active (termed glucoreponsive) or increasingly silent (termed glucose-sensitive), concomitant with a gradual rise in blood glucose from ~3 mM to ~20 mM in rats (34) and humans (14). Elevation of blood glucose induces specific activation of hypothalamic neurons, even when brain blood glucose is elevated by carotid infusion, without elevating peripheral blood levels (7). The physiological relevance of glucoreceptive hypothalamic neurons is suggested by the observation that these neurons cease firing just before a meal begins and then begin to fire again as the meal progresses (28). Glucose-sensitive neurons that become increasingly active as glucose decreases appear to be unique to the hypothalamus (14) and probably include neurons that regulate neuroendocrine counterregulatory responses to moderate hypoglycemia (5). Subpopulations of hypothalamic neurons in in vitro slice preparations also become increasingly active or increasingly silent as bath glucose concentrations increase from ~5 mM to ~20 mM (27, 39) and, as with in vivo studies, neurons sensitive to changes of glucose in this range are not observed in other brain areas such as cortex (39) (Fig. 1). It should be noted that, whereas brain parenchyma glucose concentrations are generally reported to be well below glucose concentrations observed in blood (33), nevertheless, in vitro, ~30% of ventromedial hypothalamic neurons specifically respond to changes in glucose concentrations more similar to blood concentrations (39) than to the much lower levels usually observed in brain parenchyma; the specifically hypothalamic responses are observed primarily in the transition from ~3 to 10 mM glucose (unpublished observations). These observations suggest that local concentrations of glucose to which neuroendocrine hypothalamic neurons are exposed may be higher than glucose concentrations to which most neurons are exposed, possibly due to the presence of unique and specialized but as yet uncharacterized hypothalamic glucose transporters that may also explain the vulnerability of these neurons to GTG.

NEUROENDOCRINE RESPONSES OF HYPOTHALAMIC NEURONS TO PHYSIOLOGICAL CHANGES IN BLOOD GLUCOSE: ROLE OF GLUCOKINASE

In view of the apparent importance of neuroendocrine glucose-sensitive hypothalamic neurons in metabolic control (5, 13), the mechanisms mediating effects of glucose on these neurons are of considerable interest. An obvious hypothesis would be that physiological changes in plasma glucose are sensed in neuroendocrine hypothalamic neurons through a mechanism similar to that by which 0 mM glucose generally silences most neurons, for example, involving the activation of K-ATP channels. Indeed, it is now clearly established that effects of changes in glucose between 5 and 20 mM on hypothalamic neuronal activity are mediated through glucose metabolism (39), as is the case for the global silencing of neuronal activity at 0 mM glucose. On the other hand, K-ATP channels are ubiquitous (13), and most cells, including the majority of neurons, are insensitive to physiological changes in plasma glucose (see Fig. 1). In contrast, pancreatic β-cells are, like hypothalamic neurons, secretory cells whose excitation is modulated by changes in glucose concentrations in the physiological range. Therefore, a guiding hypothesis has been that the glucose-sensing mechanisms of hypothalamic neurons and pancreatic endocrine cells share common features. It is now well established that glucose stimulates insulin secretion through a mechanism involving glucose metabolism in the β-cell (20). Whereas K-ATP channels are thought to play an important role in glucose-induced insulin secretion, a key element in this mechanism is the presence of glucokinase. Unlike other hexokinases, whose properties are such that glucose metabolism is maximum at ~0.2 mM so that higher levels of glucose do not produce higher levels of metabolism, the specific properties of glucokinase allow cells that express glucokinase, including pancreatic β-cells, to metabolize glu-

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cose in proportion to plasma levels of glucose when plasma glucose is in the physiological range. Numerous lines of evidence have led to the consensus, as stated by Matchinsky et al. (20), that “Glucokinase serves as the glucose sensor in the case of beta cells.” Such considerations led several investigators to hypothesize that glucokinase serves as a “glucose transduction mechanism” in the hypothalamus (26), that “a possible mechanism for linking the effects of small changes in glucose to ATP generation (in glucose-sensitive hypothalamic neurons) is the interposition of a ‘glucokinase-type’ enzyme in a role similar to that which it has in glucose-sensing pancreatic β-cells” (34), or that the specificity of glucose-sensitive hypothalamic neurons must arise from “a glucose transporter and/or glucokinase, or an as yet undiscovered hexokinase...” (13).

Several lines of evidence are consistent with the hypothesis that the pancreatic form of glucokinase plays the same key role as “glucose sensor” in true glucoreceptive and glucose-sensitive hypothalamic neurons as it plays in pancreatic β-cells. First, several groups have reported expression of the pancreatic form of glucokinase in neurons largely confined to the hypothalamus (12, 16, 26, 39). Furthermore, inhibitors of glucokinase block responses of hypothalamic neurons to changes in physiological concentrations of glucose (9, 39), and inhibitors of glucokinase also promptly induce feeding when infused into the third ventricle (9). Finally, individuals heterozygous for a disrupted glucokinase gene exhibit impairments in neuroendocrine function thought to be regulated by hypothalamic neurons, including counterregulatory responses, food intake, body weight, and thermogenesis (10) (unpublished observations).

GLOBAL RESPONSES TO PROFOUND HYPOGLYCEMIA AND HYPOTHALAMIC RESPONSES TO CHANGES IN GLUCOSE AT PHYSIOLOGICAL CONCENTRATIONS INVOLVE DISTINCT DEPENDENCIES ON ATP

Although both the global mechanism and the neuroendocrine mechanism mediating responses to glucose involve intracellular sensors of glucose metabolism, neurons throughout the brain appear to express K-ATP channels (13), whereas the pancreatic form of glucokinase appears to be expressed mainly in the hypothalamus (12, 26, 39). Therefore, neuroendocrine and global responses to glucose may differ in their dependency on glucokinase (13), consistent with the different glucose concentrations that activate those respective mechanisms. It has also been suggested that K-ATP channels play a major role in the regulation of glucoreponsive hypothalamic neurons by glucose (3). However, with the notable exception of a paper recently published and to be discussed here (22), much of the data that implicate K-ATP channels in mediating hypothalamic responses to glucose has examined only the role of K-ATP channels in the silencing of these neurons at 0 mM glucose (3), which we have described as a general and global mechanism, not specific to hypothalamic neurons and probably unrelated to neuroendocrine regulation. Although these neurons have been referred to as glucoreponsive (3), use of this term to describe neuronal silencing at 0 mM glucose differs from the commonly accepted meaning, based on its original use, in which glucoreponsive applies to specific responses to physiological changes in glucose concentration thought to be relevant to neuroendocrine metabolic regulation (27).

The distinction between global inhibition by aglycemia and neuroendocrine responses to physiological levels of glucose can be illustrated by comparing these two responses to glucose in hypothalamic neurons with hypothalamic neuroendocrine responses to leptin and insulin. In general, leptin, insulin, and glucose each produce a neuroendocrine signal indicating nutritional adequacy; thus, not surprisingly, these factors generally produce similar neuroendocrine effects on hypothalamic neurons. For example, hypothalamic neuropeptide Y (NPY) neurons are activated by fasting (25), due in part to reduction in the inhibitory effects of insulin (31), leptin (24), and glucose (23) on NPY neurons. Similarly, in vitro hypothalamic neurons that are inhibited by physiological increases in glucose are generally inhibited by leptin, and hypothalamic neurons that are stimulated by physiological increases in glucose are generally stimulated by leptin (32), observations that we have corroborated (unpublished observations). These results might appear to be inconsistent with reports that leptin (36) and insulin (37) activate K-ATP channels (thus inhibiting activity) in hypothalamic neurons described as glucose receptive (thus stimulated by glucose). However, this apparent inconsistency may be resolved by recognizing that, in these latter studies, glucose-receptive neurons were defined as neurons whose activity-increased glucose concentration was increased from 0 to 20 mM. As described above, almost all neurons, even neurons activated by glucopenia (i.e., glucose-inhibited or glucose-sensitive neurons) become silent at 0 mM glucose; thus activation of neurons by the transition from 0 to 20 mM glucose does not indicate how these neurons would respond to more physiological changes in glucose concentration. Therefore, the hypothalamic neurons inhibited by leptin and insulin (36, 37), although like most neurons inhibited at 0 mM glucose, are in all likelihood actually glucose sensitive and not glucoreponsive and would be activated by increasing glucose concentration from 0 to ~3 mM, but would then again be inhibited by increasing glucose from 3 to 10 mM glucose, consistent with other reports (32).

Although considerable data indicate that silencing of neuronal activity at 0 mM glucose involves activation of K-ATP channels, less information is available on the role of K-ATP channels in mediating the neuroendocrine effects of glucose in glucoreceptive or glucose-sensitive neurons. True glucoreponsive neurons clearly express K-ATP channels, because these neurons can be stimulated by tolbutamide at 5 mM glucose (39), whereas in nonglucoreponsive neurons, we observed that tolbutamide was effective only to stimulate activity at 0 mM glucose (39). On the other hand,
diazoxide, which activates pancreatic K-ATP channels and blocks insulin secretion, could only block the stimulation of neuronal activity during the transition from 5 to 20 mM in a minority of glucoreponsive neurons (39). Furthermore, whereas pyruvate can substitute for glucose to restore activity in neurons that are silent at 0 mM glucose (11), pyruvate cannot substitute for glucose to stimulate true glucoreponsive neurons (39). Although pyruvate metabolism generates far more ATP than glycolysis generates, these results also call into question the importance of ATP generation on true glucoreponsive neurons. It is particularly significant that pyruvate cannot substitute for glucose to stimulate insulin secretion from pancreatic β-cells (21). The inability of pyruvate to stimulate insulin secretion, as well as other anomalies reviewed in detail (1), has suggested that the role of ATP production, even in insulin secretion, is not as clear as had been previously supposed. Nevertheless, it has recently been reported that genetic ablation of Kir6.2, a pore-forming subunit of K-ATP channels, blocks hypothalamic neuroendocrine responses to physiological changes in glucose concentration (22), similar to neuroendocrine impairments observed in individuals heterozygous for ablation of the glucokinase gene (10) (Yang X-J, and Mobbs CV, unpublished observations). These results support the hypothesis that, as in pancreatic β-cells, K-ATP channels as well as glucokinase are necessary for full hypothalamic neuroendocrine responses to glucose.

On the other hand, the observation that lactate, in contrast to pyruvate, could mimic the neuroendocrine effects of glucose on hypothalamic neurons suggests an alternative to ATP as a neuroendocrine signal of glucose metabolism in hypothalamic neurons (39). When lactate is converted to pyruvate, NADH is produced; because pyruvate cannot mimic neuroendocrine effects of glucose, this observation supports the theory that the graded production of NADH in proportion to physiological changes in plasma glucose may play a key role in mediating effects of glucose on glucoreponsive neurons. Indeed, the metabolic step whose inhibition produced the most profound blockade of glucose-stimulated activation of hypothalamic neurons was the step catalyzed by glyceraldehyde phosphate dehydrogenase, the only step that produces NADH in glycolysis (39). We have also observed that 2-deoxyglucose can stimulate glucose-sensitive neurons, which are inhibited by the transition from 5 to 20 mM glucose, whereas lactate can also substitute for glucose to inhibit neuronal activity, suggesting that glucose-sensitive neurons sense glucose through a mechanism similar to that utilized by true glucoreceptive neurons (unpublished observations). Similarly, glucose stimulation of pancreatic β-cells requires the production and transport to the mitochondria of NADH (8). The mechanism by which NADH transport to the mitochondria leads to an electrical signal is not clear, but the malate shuttle system used in this process would plausibly contribute to an anaplerotic process leading to the production of malonyl-CoA (17), which has been implicated in mediating effects of glucose to activate pancreatic β-cells (29). In this respect, it is of great interest that an increase in hypothalamic malonyl-CoA has been recently implicated as the mechanism by which fatty acid synthase inhibitors produce profound reductions in food intake and body weight as well as enhancement of thermogenesis (15, 18). Whether a glucokinase-dependent NADH/malonyl-CoA mechanism may alter neuronal firing rate by influencing activity of K-ATP channels, possibly independent of the production of ATP, remains to be addressed.

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