Hypothalamic Glucose Sensor

Similarities to and Differences From Pancreatic β-Cell Mechanisms

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Glucose-responsive neurons in the ventromedial hypothalamus (VMH) are stimulated when glucose increases from 5 to 20 mmol/l and are thought to play an essential role in regulating metabolism. The present studies examined the role of glucose metabolism in the mechanism by which glucose-responsive neurons sense glucose. The pancreatic, but not hepatic, form of glucokinase was expressed in the VMH, but not cerebral cortex, of adult rats. In brain slice preparations, the transition from 5 to 20 mmol/l glucose stimulated ~17% of the neurons (as determined by single-cell extracellular recording) from VMH but none in cortex. In contrast, most cells in both VMH and cortex were silent below 1 mmol/l and active at 5 mmol/l glucose. Glucosamine, 2-deoxyglucose, phloridzin, and iodoacetic acid blocked the activation of glucose-responsive neurons by the transition from 5 to 20 mmol/l glucose. Adding 15 mmol/l mannose, galactose, glyceraldehyde, glycerol, and lactate to 5 mmol/l glucose stimulated glucose-responsive neurons. In contrast, adding 15 mmol/l pyruvate to 5 mmol/l glucose failed to activate glucose-responsive neurons, although pyruvate added to 0 mmol/l glucose permitted neurons to maintain activity. Tolbutamide activated glucose-responsive neurons; however, diazoxide only blocked the effect of glucose in a minority of neurons. These data suggest that glucose-responsive neurons sense glucose through glycolysis using a mechanism similar to the mechanism of pancreatic β-cells, except that glucose-responsive neurons are stimulated by glycerol and lactate, and diazoxide does not generally block the effect of glucose. *Diabetes* 48:1763–1772, 1999

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Received for publication 22 January 1999 and accepted in revised form 25 May 1999.

2-DG, 2-deoxyglucose; K-ATP channels, ATP-dependent potassium channels; LGK, liver glucokinase; NE, norepinephrine; pGK, pancreatic glucokinase; R-ACSF, regular artificial cerebrospinal fluid; RT-PCR, reverse transcriptase–polymerase chain reaction; VMH, ventromedial hypothalamus.

Neurons in the ventromedial hypothalamus (VMH) have long been hypothesized to play a major role in metabolic regulation, including body weight (1,2). More recent studies have implicated neurons in the VMH as critical for counterregulatory responses, since sympathetic responses to systemic glucopenia are blocked by lesions in the VMH (3) and by infusion of glucose into the VMH (4), whereas local attenuation of glucose metabolism in the VMH will stimulate a systemic sympathetic response (5). Similarly, infusion of glucose directly into the VMH has an immediate effect on the sympathetic efferent firing rate, and lesions of the VMH block these effects (6). Other studies have demonstrated satiety effects of glucose and the stimulation of feeding by 2-deoxyglucose, also apparently mediated by hypothalamic neurons (7). Thus it is of particular interest that a subpopulation of neurons in the VMH, termed glucose-responsive, are electrically activated by the transition from 5 to 20 mmol/l glucose, and furthermore, that glucose-responsive neurons are not found in other brain areas such as cortex (8).

The mechanisms by which changes in physiological concentrations of glucose regulate hypothalamic neuronal activity are therefore of considerable interest. Although neurons outside the hypothalamus are relatively insensitive to physiological changes in glucose concentrations, neurons throughout the brain become hyperpolarized and silent when glucose concentrations fall much below 1 mmol/l (9). Thus neurons in the hypothalamus, like many neurons throughout the brain, become active during the transition from 0 to 20 mmol/l glucose in vitro (10). The mechanism that underlies the inhibition of neuronal activity at 0 mmol/l glucose has been hypothesized to involve activation of ATP-dependent potassium (K-ATP) channels (11). In support of this hypothesis, tolbutamide, which blocks the K-ATP channel in β-cells, can depolarize hypothalamic neurons at 0 mmol/l glucose (10). Furthermore, mannoheptulose can block the depolarization in hypothalamic neurons, which occurs in the transition from 0 to 20 mmol/l glucose (10). Together with the reports that the pancreatic form of glucokinase is expressed in hypothalamus (12–14), these studies suggest the hypothesis that hypothalamic neurons sense glucose using a mechanism similar to the mechanism by which pancreatic β-cells sense glucose, that is, by increasing ATP-to-ADP ratios leading to depolarization mediated by a blockade of K-ATP channels (15).

Nevertheless, the application of the model of pancreatic β-cells to VMH neurons is not straightforward. First, the details of the mechanism by which β-cells sense glucose are complex and still unresolved (15,16). For example, stimulation of β-cells is probably not simply due to an increase of ATP by oxidative phosphorylation (16), since, for example, pyruvate at low glucose levels produces little stimulatory effect in pancreatic β-cells even though pyruvate is metabolized (16–19). Similarly, there is little evidence that ATP levels change substantially in pancreatic β-cells when glucose levels increase in the physiological range (20). Furthermore, neurons throughout the brain express K-ATP channels (21) and are stimulated by the transition from 0 mmol/l to >1 mmol/l glucose (9), whereas responses to the transition from 5 to 20 mmol/l glucose are reported to be primarily a hypothalamic phenomenon (8). Thus the mechanism that mediates neuronal stimulation during the transition from 0 to 20 mmol/l glucose may be different from the mechanism that mediates neuronal stimulation from 5 to 20 mmol/l glucose. For example, K-ATP channels may play an important role in the depolarization that occurs in the transition from 0 to 0.2 mmol/l glucose (9), whereas pancreatic glucokinase is optimally effective at glucose levels from 5 to 20 mmol/l (22). The purpose of the present study was to further assess the similarities and differences of hypothalamic glucose-sensing mechanisms compared with pancreatic glucose-sensing mechanisms, using methods similar to those previously used to study pancreatic mechanisms (17).

RESEARCH DESIGN AND METHODS

Animals. A total of 152 male Sprague-Dawley rats, 200–300 g body wt, were used in this study and were purchased from Charles River. Rats were housed using reversed light/dark cycle, with ad libitum access to food and water.

To prepare brain slices, each rat was anesthetized by intraperitoneal injection of urethane (1.6 g/kg). Brains were promptly removed without decapitation and placed in ice-cold regular artificial cerebrospinal fluid (R-ACSF) buffer (see below). Thin coronal slices (400 μm) containing the VMH or cerebral cortex were produced by cutting the brain with a Vibratome (Lancer, Series 1000). Three slices were obtained per hypothalamus, and each slice was cut bilaterally to produce six hypothalamic slice preparations per rat. Slices were stabilized by submerging in R-ACSF (in mmol/l: NaCl 124, $NaHPO_4$ 26, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, CaCl 2.4, and D-glucose 10) in which sucrose replaced an equal molar concentration of NaCl for a 1-h recovery period at room temperature as previously described (23), followed by transferring those slices into R-ACSF. R-ACSF refers to the buffer with 10 mmol/l glucose; in producing test solutions based on R-ACSF (referred to as ACSF), which involved altering the concentration of glucose or other components, osmolarity was maintained constant by adjusting the NaCl concentration. All solutions were saturated with 95% O_2 and 5% CO_2, and maintained at pH 7.4 and 34°C.

Reagents. Glucosamine, 2-deoxyglucose (2-DG), mannose, galactose, glyceraldehyde, iodoacetic acid, tolbutamide, phloridzin, diazoxide, and pyruvic acid (sodium salt) were all purchased from Sigma (St. Louis, MO).

Electrophysiology. Neuronal responses were studied by extracellular single-unit recording, largely as previously described (24). Briefly, the slice was laid on a net in a recording chamber (2 ml volume) that was continuously perfused with R-ACSF at the rate of 2–3 ml/min. Glass micropipettes were made, filled with R-ACSF, and tested before recording to ensure a resistance of 5–10 MΩ. Single-cell extracellular recording was made with glass micropipettes filled with R-ACSF from neurons in the VMH or cerebral cortex. Action potentials were recorded by conventional electrophysiological equipment, amplified by an Axoclamp 2A amplifier (Axon Instruments), and the firing rate histograms were compiled and displayed. Data were recorded on videotapes for further analysis.

To detect glucose-stimulated neurons, the initial R-ACSF glucose concentration was 10 mmol/l. An electrode was targeted to the VMH and slowly lowered until an active cell was detected by an extracellular recording exhibiting repeating action potentials. If the increased neuronal activity was stable for at least 5 min, the R-ACSF was then switched to 5 mmol/l glucose ACSF (maintaining constant osmolarity). If the active cell became silent within 5 min, the ASCF was then switched to 20 mmol/l. If the activity of the cell was detectable within 5 min, the cell was provisionally assumed to be a glucose-stimulated neuron (as described below, a subset of these glucose-stimulated neurons are labeled glucose-responsive when they meet further criteria). The ACSF was then switched again to 5 mmol/l glucose, and if the cell once more became silent, further assessment of the neuron was carried out with test agents. Tested agents were applied by switching the recording chamber perfusion to an ACSF containing the test solution. The average perfusion time of the test reagent was ~5 min for each agent. Where appropriate, effects were corroborated by χ^2 nonparametric tests, comparing number of neurons responding (greater than twofold increase in electrical activity within 3 min of exposure to test compound, compared with baseline over the previous 5 min) to number of neurons not responding (failing to reach the criterion increase in electrical activity).

Reverse transcriptase–polymerase chain reaction for glucokinase. Nested primers complementary to the first exon of pancreatic glucokinase (pGK) or liver glucokinase (LGK) or complementary to the third glucokinase exon (common to both pGK and LGK) were used to amplify total RNA from VMH, liver, or cerebral cortex, using protocols as previously described (25). Primer sequences are available upon request. The resulting bands were sequenced using Sequenase. To further assess localization, the band corresponding to pGK was labeled by amplified primer extension, and in situ hybridization was carried out using previously reported methods (26).

RESULTS

Cells in VMH, but not cortex, express the pancreatic form, but not the liver form, of glucokinase. RNA from VMH produced bands corresponding to the appropriate molecular weight for the pancreas-specific form and the glucokinase sequence common to both the liver and the pancreas (Fig. 1*A*); this identity was confirmed by direct sequenc-

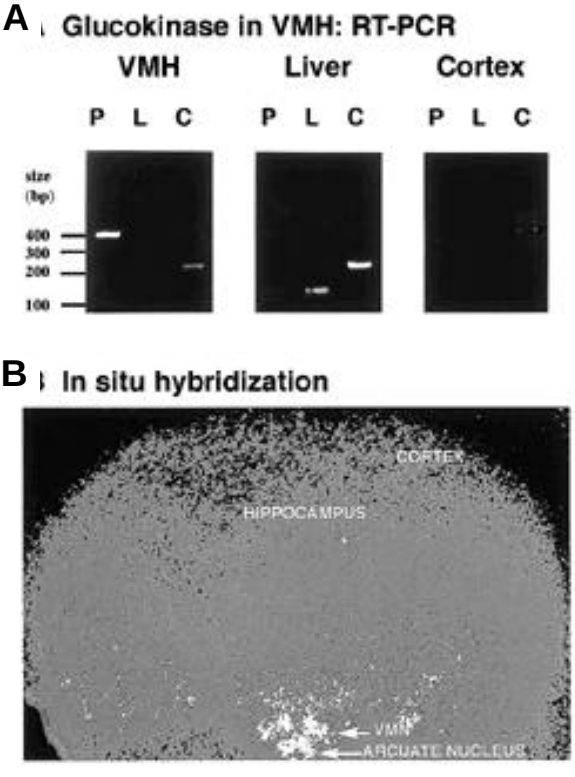

FIG. 1. Hypothalamus expresses the pancreatic-specific form, but not the liver-specific form, of glucokinase. *A*: DNA bands produced by RT-PCR starting with RNA from VMH, liver, or cerebral cortex (see METHODS). P, pancreas-specific glucokinase primers used to amplify mRNA with PCR; L, liver-specific glucokinase primers; C, primers for an exon that is common to both forms of glucokinase. The identity of each amplified band was confirmed by direct sequence analysis. *B*: In situ hybridization using the glucokinase probe generated from bands produced by RT-PCR. The cDNA probe was labeled with ^{32}P, and whole brain section at the hypothalamic level was visualized on film. Grayscale indicates intensity of signal from lowest (black) to highest (white).

ing of the band. In contrast, amplification of hypothalamic RNA by primers specific for LGK produced no signal (Fig. 1*A*). RNA from liver produced bands corresponding to liver-specific exon and the exon common to both forms of glucokinase, but not corresponding to the pancreas form. RNA from cerebral cortex failed to produce bands corresponding to any form of glucokinase. Using the pGK band to produce a specific probe, localization was further assessed by in situ hybridization. Corroborating results of the reverse transcriptase–polymerase chain reaction (RT-PCR), pancreatic glucokinase was detected in the VMH (including both the ventromedial nucleus and arcuate nucleus), but not other brain areas including hippocampus and cerebral cortex (Fig. 1*B*).

Neurons in VMH, but not cortex, are stimulated by the transition from 5 to 20 mmol/l glucose, and 5 mmol/l glucose is permissive for activity in most neurons from both areas. Because neurons throughout the brain become silent at 0 mmol/l glucose (9), we hypothesized neurons that respond to the transition from 5 to 20 mmol/l use a hypothalamic-specific mechanism relevant to neuroendocrine metabolic regulation, whereas the transition from 0 mmol/l glucose to >1 mmol/l glucose leads to activation by a more general neuronal mechanism that perhaps functions to generally protect the brain during hypoglycemia (9,11). Because the purpose of the present study was to assess the neuroendocrine-relevant mechanism, we compared hypothalamic and cortical responses to the transition from 5 to 20 mmol/l and the transition from 0 to 20 mmol/l glucose.

The results of this study are in Fig. 2. As indicated in METHODS, neurons were first detected by their activity at 10 mmol/l glucose. Pilot studies indicated that a subpopulation (~17%) of neurons in the VMH would become silent when glucose concentration was decreased to 5 mmol/l and would become active after raising the glucose concentration to 20 mmol/l (Fig. 2*A*). In contrast, the majority of neurons in the VMH, and all the neurons recorded in the cortex (a total of 23), exhibited no change in firing rates when glucose increased from 5 to 20 mmol/l (Fig. 2*B* and *C*). We observed no difference in activity of any neurons between 3 and 5 mmol/l glucose, so the transition from 5 to 20 mmol/l was used to maintain comparability with previous studies in hypothalamus (27,28) and pancreas (17). Pilot studies indicated that 0 mmol/l glucose would not only silence most neurons in both VMH and cortex, but after ~10 min at 0 mmol/l glucose, these neurons would also sometimes exhibit a rapid burst of activity followed by silence that was irreversible by restoration of 20 mmol/l glucose. We interpreted this result to suggest that prolonged exposure to 0 mmol/l glucose could, perhaps not surprisingly, produce permanent neuronal damage or even cell death, which would make studies of mechanism (which require repeated returns to the low dose of glucose) difficult to interpret. However, by stepping glucose concentration to 1 mmol/l instead of 0 mmol/l, we were able to observe that at this concentration, most neurons in both VMH and cortex became silent, and the transition from 1 to 5 mmol/l permitted resumption of activity in these neurons (Fig. 2*B* and *C*). In contrast, the transition from 1 to 5 mmol/l glucose was insufficient to permit activity of neurons (observed only in the VMH) that were stimulated by a transition from 5 to 20 mmol/l (Fig. 2*A*). Therefore, subsequent studies were confined to assessing the mechanisms by which VMH neurons are stimulated by the transition from 5 to 20 mmol/l glucose.

The presence of glucokinase suggests that VMH neurons sense glucose through its metabolism. Using a series of inhibitors that inhibit glucose metabolism at specific steps, we assessed which steps were necessary for VMH neurons to sense the transition from 5 to 20 mmol/l. Similarly, using a series of intermediate metabolites, we assessed which steps were sufficient for VMH neurons to sense the transition from 5 to 20 mmol/l. A total of 638 neurons from the VMH were recorded in the present study, among which 109 neurons (17.1%) were silent at 5 mmol/l glucose and active at 20 mmol/l glucose. In the present report, the term glucose-responsive neuron is used to refer to these 109 neurons that were stimulated by the transition from 5 to 20 mmol/l glucose, but not by the transition from 1 to 5 mmol/l, in accordance with previous nomenclature (27). Thus the term "glucose-responsive" is meant to specifically exclude the more general

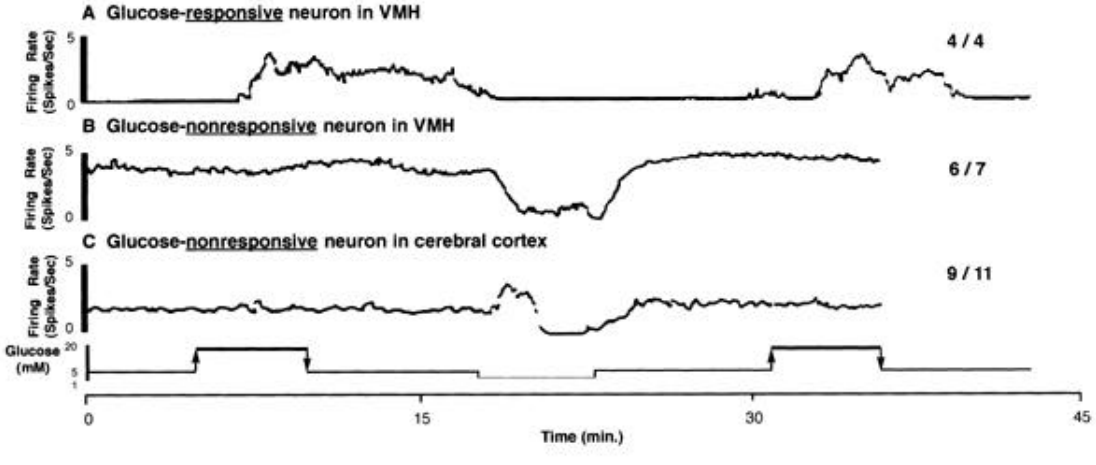

FIG. 2. Neurons in VMH, but not cerebral cortex, are stimulated by the transition from 5 to 20 mmol/l glucose; 5 mmol/l glucose is permissive for activity in most neurons from both areas. *A*: Neuron from VMH stimulated by the transition from 5 to 20 mmol/l glucose. *B*: Neuron from VMH not responsive to the transition from 5 to 20 mmol/l glucose, but decreasing in activity when glucose concentration decreases to 1 mmol/l. *C*: Neuron from cortex not responsive to transition from 5 to 20 mmol/l glucose, but, like similar neurons from VMH, decreasing in activity at 1 mmol/l glucose. In the numbers in the upper right corner of each panel, the denominator indicates the number of cells tested according to the protocol indicated for that panel, and the numerator indicates the number of cells that behaved as indicated in that panel.

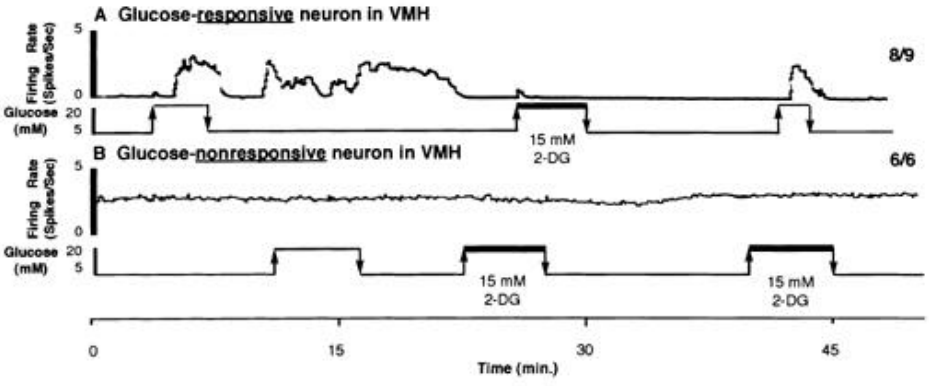

FIG. 3. 2-DG blocks the effect of glucose on glucose-responsive neurons (*A*) but does not affect glucose-nonresponsive neurons (*B*).

class of neurons observed throughout the brain that fall silent at very low levels of glucose. Thus neurons whose activity was not influenced by the transition from 5 to 20 mmol/l glucose, although their activity was inhibited by reducing glucose concentrations to 1 mmol/l or below, were referred to as glucose-nonresponsive neurons; the effect of glucose on these neurons is regarded as permissive. The phrase "stimulated by glucose" refers specifically to a stimulation of firing rate by the transition from 5 to 20 mmol/l glucose.

2-DG blocks the effect of glucose on glucose-responsive neurons. 2-DG blocks glucose metabolism by various mechanisms, including competition with glucose uptake, competition with glucose for phosphorylation by hexokinases (not specifically for glucokinase), and possibly other mechanisms. Because 2-DG blocks glucose-stimulated insulin secretion (17), the present study assessed if 2-DG would similarly block the stimulatory effects of glucose on glucose-responsive neurons. The stimulation of glucose-responsive neurons by glucose was blocked by the simultaneous administration of 15 mmol/l 2-DG (Fig. 3*A*; $n = 9$). However, the effect of 2-DG was reversible, since subsequent elevation of glucose from 5 to 20 mmol/l stimulated the same neurons (Fig. 3*A*). The same concentration of 2-DG had no effect on the resting activity of glucose-nonresponsive neurons ($n = 6$) (Fig. 3*B*).

Glucosamine blocks the effect of glucose on glucose-responsive neurons. At appropriate concentrations, glucosamine has been reported to inhibit glucokinase, but not other metabolic processes (29). In vitro glucosamine inhibits glucose-stimulated insulin secretion, which has been interpreted to suggest an important role for glucokinase in the glucose-sensing mechanism of the β-cell (29). In the present study, glucosamine, when added simultaneously with 20 mmol/l glucose, blocked the stimulation of glucose-responsive neurons by glucose (Fig. 4*A*; $n = 7$); the same neurons regained their responsiveness to glucose within 15 min after the removal of glucosamine (Fig. 4*A*). Furthermore, the same concentration of glucosamine did not block the stimulatory effect of norepinephrine in the same glucose-responsive neurons (Fig. 4*A*) nor did glucosamine have any effect on the resting activity of glucose-nonresponsive neurons (Fig. 4*B*; $n = 14$). It should be noted that while this result is consistent with an important role for glucokinase in glucose-responsive neurons, other interpretations are possible, including, for example, that effects of glucosamine may be mediated by other mechanisms besides inhibition of glucokinase.

Phloridzin blocks the effect of glucose on glucose-responsive neurons. Phloridzin, which blocks glucose transport, has been reported to block the pancreatic response to glucose (17). In the present study, phloridzin also blocked the stimulation of glucose-responsive neurons by glucose (Fig. 5*A*), and this effect was not reversed 15 min after removal of the phloridzin. The same dose of phloridzin had a small inhibitory effect on the activity of glucose-nonresponsive neurons, which was reversed 15 min after removal of the inhibitor (Fig. 5*B*).

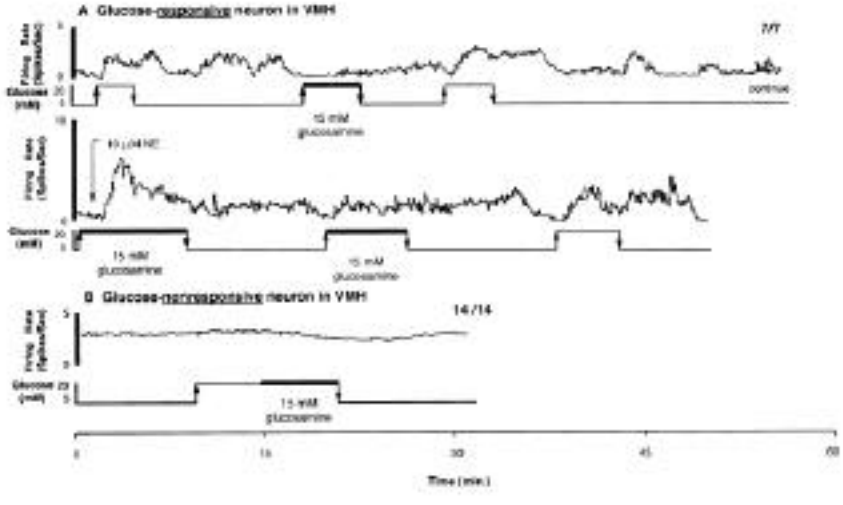

FIG. 4. Glucosamine blocks the effect of glucose on glucose-responsive neurons (*A*), but does not affect glucose-nonresponsive neurons (*B*). When glucosamine was washed out, responsiveness to glucose was restored. This same neuron continues to the next line, demonstrating that when glucosamine was administered simultaneously with norepinephrine (NE), NE still stimulated the neuron. The inhibitory effect on stimulation by glucose, and reversal of inhibition by washout, were then reversed.

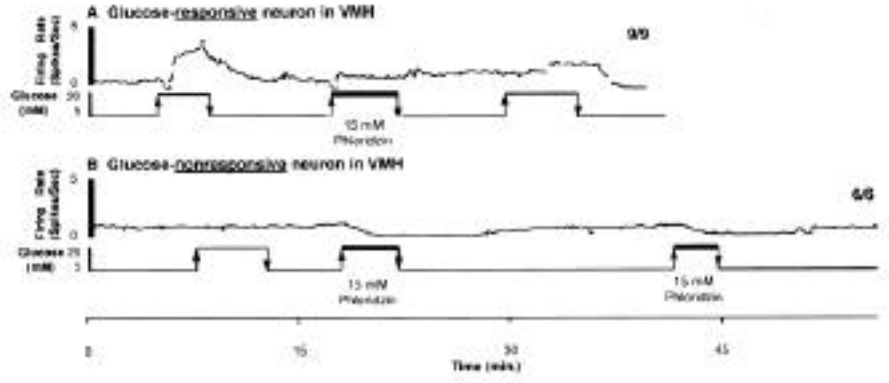

FIG. 5. Phloridzin blocks the effect of glucose on glucose-responsive neurons (*A*) but does not affect glucose-nonresponsive neurons (*B*).

Iodoacetic acid blocks the effect of glucose on glucose-responsive neurons. The previous results suggested that transport and phosphorylation of glucose are necessary for the activation of VMH glucose-responsive neurons by the transition from 5 to 20 mmol/l glucose. To assess the requirement for later steps in glycolysis, the effect of iodoacetic acid (which, at appropriate doses, is reported to specifically inhibit glyceraldehyde phosphate dehydrogenase [30]) was assessed. At the dose used, iodoacetic acid blocked the stimulation of glucose-responsive neurons by glucose, and this effect was not reversed 15 min after removal of the iodoacetic acid (Fig. 6*A*; $n = 5$). In contrast, at the dose used, iodoacetic acid did not prevent the stimulatory effect of norepinephrine (Fig. 6*A*) and had no effect on the resting activity of glucose-nonresponsive neurons (Fig. 6*B*; $n = 5$). While this result is consistent with an important role for glyceraldehyde phosphate dehydrogenase in the glucose-sensing mechanism of glucose-responsive neurons, it is possible that the inhibitor may also act on other enzymes.

Early glycolytic metabolites stimulate glucose-responsive neurons. The studies with inhibitors, described above, indicated that glycolytic steps up to and including the dehydrogenation of glyceraldehyde-3-phosphate are necessary for the stimulation of VMH neurons by the transition from 5 to 20 mmol/l glucose. To assess which metabolites are sufficient to stimulate VMH neurons at 5 mmol/l glucose, the following studies assessed the effects of substituting 15 mmol/l of early glycolytic metabolites on the VMH firing rate. The addition of 15 mmol/l mannose, galactose, and glyceraldehyde to 5 mmol/l glucose were able to stimulate glucose-responsive neurons (Fig. 7*A*; $n = 6$), whereas these metabolites had no effect on glucose-nonresponsive neurons (Fig. 7*B*; $n = 11$). Furthermore, the addition of 15 mmol/l glycerol also stimulated glucose-responsive neurons (Fig. 8*A*; $n = 4$), but again glycerol did not influence the resting activity of glucose-nonresponsive neurons (Fig. 8*B*; $n = 4$). These results were of interest because, although consistent with the role of early glycolytic intermediates in sensing glucose, neither galactose nor glycerol (which are not phosphorylated by glucokinase) can stimulate pancreatic β-cells (17,31). Thus in this respect, presumably downstream from the glucokinase step, glucose-responsive VMH neurons differ from pancreatic β-cells.

Pyruvate does not stimulate glucose-responsive neurons but is metabolized. A remarkable feature of the stimulation of pancreatic β-cells by glucose is that despite the essential role of glucose metabolism, pyruvate does not stimulate pancreatic β-cells, even though pyruvate is readily metabolized (15–19). To assess if glucose-stimulated VMH neurons also exhibit this signature property of β-cells, the ability of pyruvate to stimulate VMH neurons was assessed.

Addition of 15 mmol/l pyruvate to 5 mmol/l glucose failed to stimulate either glucose-responsive neurons (Fig. 9*A*; $n = 10$) or glucose-nonresponsive neurons (Fig. 9*B*; $n = 13$). Nevertheless, pyruvate was effectively metabolized, since the addition of 20 mmol/l pyruvate did support electrical activity when glucose was reduced to 0 mmol/l glucose (Fig. 9*C*; $n = 4$). Extending this observation, in the presence of 0 mmol/l glucose, 20 mmol/l pyruvate maintained electrical activity. When pyruvate was withdrawn, neurons became electrically silent; restoring pyruvate once more restored electrical activity (Fig. 9*D*; $n = 4$). These observations are consistent with extensive data demonstrating that neurons can effectively use pyruvate as a sole (and in some cases preferential) source of carbon for metabolism (32).

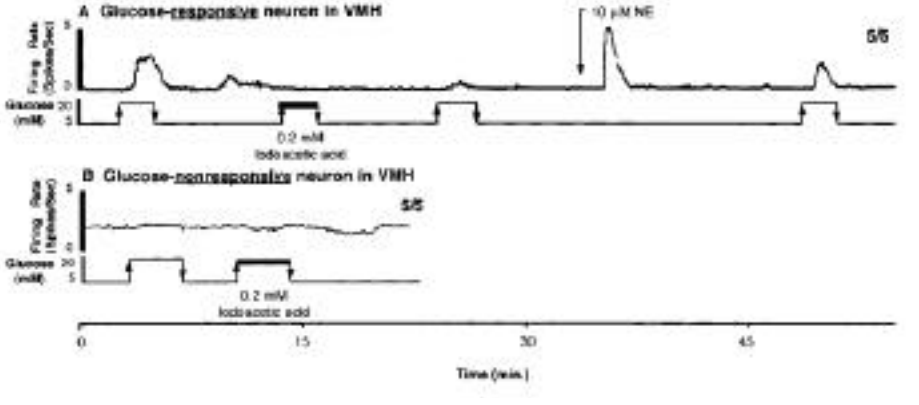

FIG. 6. Iodoacetic acid blocks the effect of glucose on glucose-responsive neurons (*A*) but does not affect glucose-nonresponsive neurons (*B*). However, iodoacetic acid does not block the excitatory effects of NE.

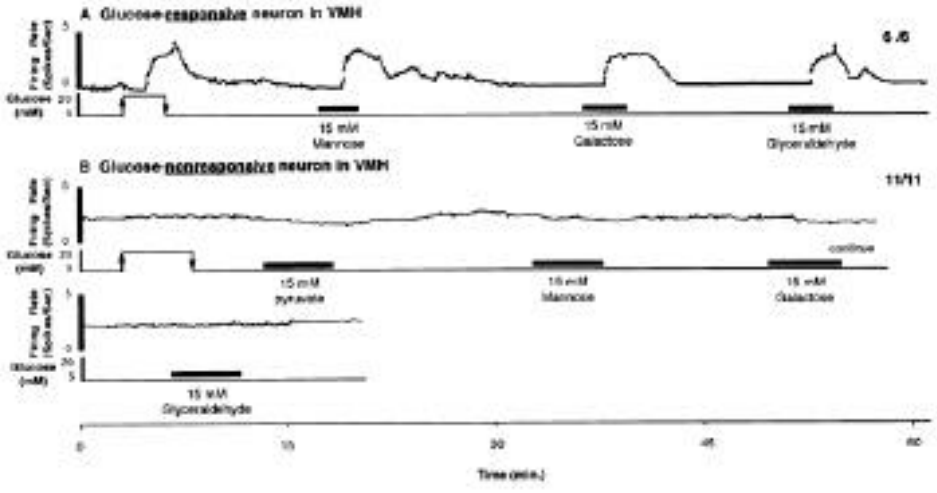

FIG. 7. Early glycolytic metabolites stimulate glucose-responsive neurons (*A*) but do not affect glucose-nonresponsive neurons (*B*).

Lactate stimulates glucose-responsive neurons. The failure of pyruvate to stimulate glucose-stimulated neurons is consistent with observations in pancreatic β-cells (15,16, 18,19). It has also been reported that lactate fails to stimulate pancreatic β-cells (15,33), which appears to be correlated with the inability of pancreatic β-cells to metabolize lactate, since in pancreatic β-cells, lactate fails to stimulate insulin secretion and fails to increase ATP-to-ADP ratios (33,34); in HIT cells, lactate does stimulate insulin secretion, but whether lactate is metabolized in these cells remains to be established (35). Consistent with these results, lactate dehydrogenase activity is very low in pancreatic β-cells (36). In contrast, neurons metabolize lactate preferentially, consistent with the expression in neurons of the heart form of lactate dehydrogenase, which preferentially converts lactate to pyruvate (37). Therefore, it was of interest to assess if lactate could mimic the effect of glucose to stimulate VMH glucose-stimulated neurons.

In marked contrast to pyruvate and in contrast to pancreatic β-cells, addition of 15 mmol/l lactate to 5 mmol/l glucose was at least as effective as glucose to excite glucose-responsive neurons (Fig. 10*A*; $n = 6$). In contrast, addition of 15 mmol/l lactate to 5 mmol/l glucose had no effect on glucose-nonresponsive neurons (Fig. 10*B*; $n = 12$).

Tolbutamide stimulates glucose-responsive neurons, but diazoxide usually fails to block effect of glucose on glucose-responsive neurons. Blockade of K-ATP channels appears to be both sufficient and necessary for the stimulation of pancreatic β-cells by glucose (15), since blockade of the K-ATP channels by sulfonylureas will stimulate insulin secretion even at low levels of glucose, and, conversely, opening the K-ATP channels by diazoxide will block effects of glucose. A similar mechanism has been proposed in the stimulation of VMH neurons by glucose (10). Nevertheless, there appear to be major differences in K-ATP channels in VMH neurons and K-ATP channels in pancreas (10). For example, compounds that open the K-ATP channel in pancreatic β-cells apparently fail to open K-ATP channels in VMH (38). To assess the relevance of these results to the mechanism by which glucose-responsive neurons are stimulated by glucose, the effect of tolbutamide and diazoxide was assessed in these neurons.

Addition of 500 µmol/l tolbutamide to 1 or 5 mmol/l glucose stimulated all glucose-responsive neurons tested (Fig. 11*A*; $n = 8$). In contrast to the reliable effects of tolbutamide in glucose-responsive neurons, diazoxide only blocked the effect of glucose in three-eighths of the glucose-responsive neurons (Fig. 11*A* and *B*; $n = 8$). To assess the specificity of the inhibitory effect of diazoxide, norepinephrine was added after an inhibitory effect of diazoxide was observed. When norepinephrine was added after diazoxide, norepinephrine was no longer able to stimulate neuronal activity (Fig. 11*B*; $n = 3$). This result suggested that the inhibitory effect of diazoxide, when observed, was not specific to the glucose-sensing system. This conclusion was corroborated by the observation that diazoxide inhibited the activity of half of the glucose-nonresponsive neurons tested and that inhibition could be reversed by tolbutamide (Fig. 11*C*; $n = 12$). On the other

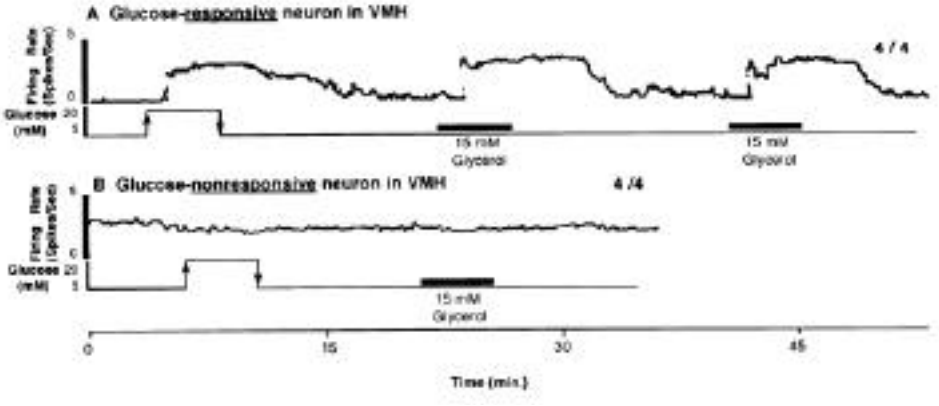

FIG. 8. Glycerol stimulates glucose-responsive neurons (*A*) but does not affect glucose-nonresponsive neurons (*B*).

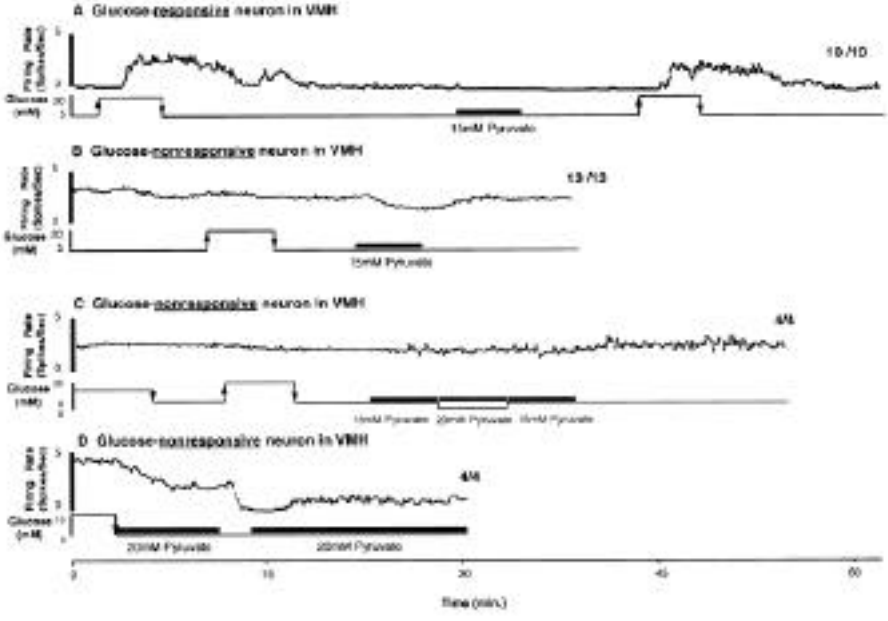

FIG. 9. Pyruvate added to 5 mmol/l glucose does not stimulate glucose-responsive neurons (*A*) or glucose-nonresponsive neurons (*B*). However, pyruvate maintains electrical activity in the presence of 0 mmol/l glucose (*C*). Pyruvate also restores electrical activity when added to 0 mmol/l glucose (*D*).

hand, tolbutamide added to 5 mmol/l glucose had no effect on the resting activity of glucose-nonresponsive neurons (Fig. 11*D*; $n = 12$) that were active at 5 mmol/l glucose. In contrast, addition of tolbutamide to 0 mmol/l glucose produced excitation in half of the glucose-nonresponsive neurons that were silent at 0 mmol/l glucose (Fig. 11*E*; $n = 12$), consistent with previous reports (39). These results are consistent with the hypothesis that K-ATP channels are present in glucose-nonresponsive neurons as well as glucose-responsive neurons.

DISCUSSION

The present studies examined the mechanism by which glucose-responsive neurons are stimulated by the transition from 5 to 20 mmol/l glucose—a transition that also stimulates pancreatic β-cells. Neurons that are stimulated by this transition appear to be largely confined to the hypothalamus, as previously reported (8,40). In contrast, neurons in which glucose exerts a permissive effect and that become more active in the transition from 1 to 5 mmol/l glucose are found throughout the brain (41). The present studies indicate that the mechanisms by which glucose-responsive neurons are stimulated by the transition from 5 to 20 mmol/l glucose are largely similar but not identical to the mechanisms by which pancreatic β-cells are stimulated by the transition from 5 to 20 mmol/l glucose and the mechanisms by which glucose exerts a permissive effect on resting neuronal activity throughout the brain. Although all three effects of glucose involve metabolism, there are unique aspects to each mechanism, as described below.

Assessment of effects of pyruvate was particularly revealing. As with pancreatic β-cells (17), early metabolic products of glycolysis could mimic effects of glucose to excite glucose-responsive neurons. Consistent with these results, compounds that inhibited early steps of glycolysis (up to the dehydrogenation of glyceraldehyde) blocked the excitation of these neurons by glucose. Nevertheless, pyruvate failed to excite glucose-responsive neurons. This result was unequivocal: across a range of concentrations, the addition of pyruvate to 5 mmol/l glucose never excited glucose-responsive neurons, despite clear evidence that pyruvate was being metabolized, consistent with numerous reports that pyruvate is metabolized by neurons (32). Pyruvate also fails to excite pancreatic β-cells (19) even though β-cells metabolize pyruvate—a key observation that is not yet fully understood (16).

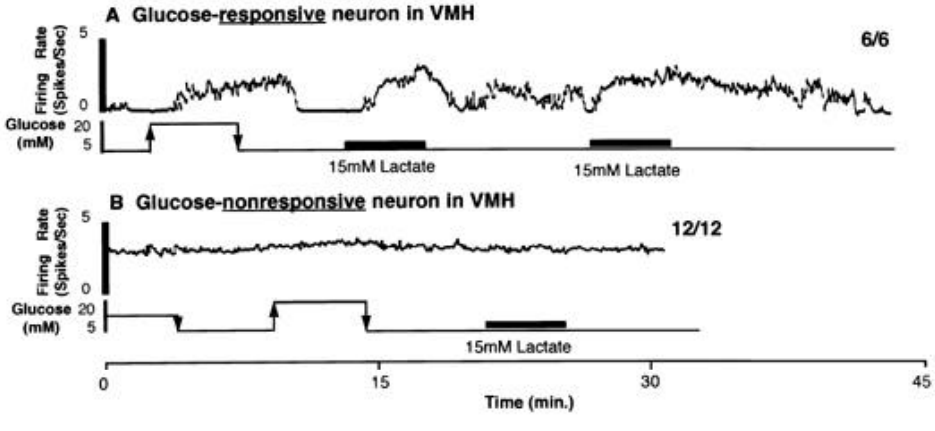

FIG. 10. Lactate stimulates glucose-responsive neurons (*A*) but does not affect glucose-nonresponsive neurons (*B*).

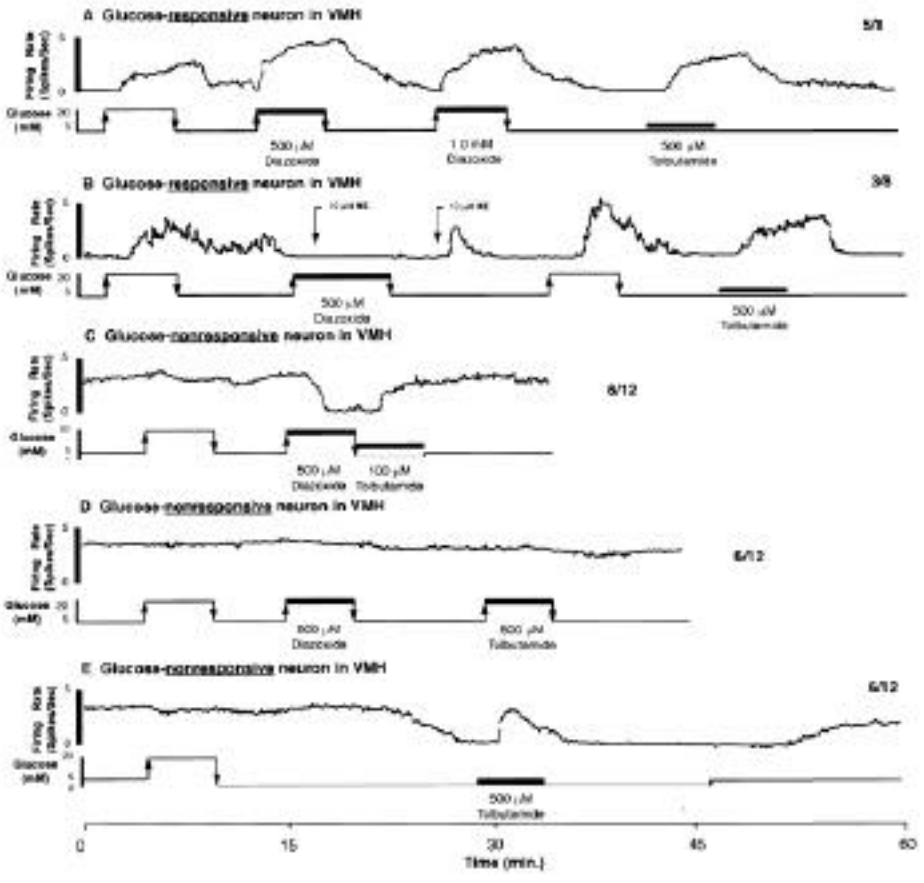

FIG. 11. Tolbutamide stimulates glucose-responsive neurons, but diazoxide usually fails to block the effect of glucose on glucose-responsive neurons. *A*: Diazoxide failed to block the effect of 20 mmol/l glucose on most neurons, but tolbutamide stimulated all tested neurons. *B*: In the three neurons in which diazoxide blocked the effect of glucose, diazoxide also blocked the stimulatory effects of norepinephrine. *C*: Diazoxide inhibited the resting activity of one-half of glucose-nonresponsive neurons tested, and this inhibition was reversed by tolbutamide. *D*: In the other half of the tested glucose-nonresponsive neurons, neither diazoxide nor tolbutamide influenced activity. *E*: Tolbutamide stimulated the activity of glucose-nonresponsive neurons that were silent at 0 mmol/l glucose.

However, the observation that both glucose-responsive neurons and β-cells exhibit this pyruvate insensitivity suggests that the mechanism by which they sense glucose is similar. In contrast, addition of pyruvate to 0 mmol/l glucose permitted activity of neurons that would otherwise become silent at 0 or 1 mmol/l glucose, indicating that this permissive mechanism is quite distinct from that used by β-cells and glucose-responsive neurons.

The present study also indicated several revealing differences in the mechanisms that glucose-responsive neurons and pancreatic β-cells sense glucose. First, galactose and glycerol stimulate glucose-responsive neurons, but they do not stimulate insulin secretion (15). It should be noted, however, that in contrast to pyruvate, galactose and glycerol, which are not substrates of glucokinase and thus are phosphorylated by other kinases, do not appear to be metabolized by β-cells (15). When gene transfer was used to enable β-cells to metabolize glycerol, glycerol was then able to stimulate insulin secretion (31). Thus with regard to glycerol and galactose, the main difference between VMH neurons and β-cells appears to be that glucose-responsive neurons can metabolize these sugars and β-cells cannot. Nevertheless, the ability of these metabolites to excite glucose-responsive neurons strengthens the conclusion that the key intermediate mediating the excitatory effects of glucose is produced in the middle or late stages of glycolysis, as previously suggested (19). Similar results have lead to the hypothesis that the production of NADH by glyceraldehyde phosphate dehydrogenase is a key event in the excitation of pancreatic β-cells by glucose (42), a hypothesis that has recently been strongly supported (43). This hypothesis is particularly compelling in glucose-responsive neurons, since lactate also stimulates these cells, and the conversion of lactate to pyruvate also generates NADH. Indeed, because pyruvate does not stimulate these neurons, but lactate does, the production and subsequent metabolism of NADH would appear to constitute a sufficient pathway to mediate excitation of glucose-responsive neurons. The failure of lactate to stimulate insulin secretion from β-cells may therefore be due to an inability of β-cells to metabolize lactate. Furthermore, overexpression of the isoform of lactate dehydrogenase that stimulates the conversion of pyruvate to lactate (thus consuming an NADH) inhibits glucose-stimulated insulin secretion (44)—a result consistent with the importance of NADH. Thus the differences between β-cells and glucose-responsive neurons in response to individual metabolites can be accounted for simply by the ability of glucose-responsive neurons to metabolize products that β-cells cannot metabolize; otherwise, the dependence on a late step in glycolysis is the same between both cell types.

A more fundamental difference is reflected by the responses (or lack thereof) of glucose-responsive neurons to diazoxide. Like pancreatic β-cells, glucose-responsive neurons were stimulated by tolbutamide, indicating that glucose-responsive neurons express a functional K-ATP channel. However, this response was not specific to glucose-responsive neurons, since tolbutamide could also stimulate some glucose-nonresponsive neurons when these neurons were silent because of low glucose or diazoxide inhibition. This result is consistent with previous reports that both the K-ATP channel and the SUR-1 receptor are found distributed throughout the

brain (21), in contrast with pancreatic glucokinase, which is localized primarily to the hypothalamus. On the other hand, whereas diazoxide robustly blocks insulin secretion, diazoxide only inhibits a minority of glucose-responsive neurons, and even in those neurons, the effect of diazoxide appears to be nonspecific. These results suggest that while some glucose-responsive neurons appear to express SUR-1 (which is sensitive to diazoxide), most do not, and the effect of diazoxide to inhibit these neurons may not be related to the mechanism by which glucose stimulates these neurons.

One conundrum associated with a hypothalamic mechanism that senses the transition from 5 to 20 mmol/l glucose is that it is widely believed that the concentration of glucose in the cerebral spinal fluid is well below 5 mmol/l, whereas glucokinase functions optimally in the range of 5–20 mmol/l glucose. However, it is clear that glucose levels in cerebral spinal fluid increases rapidly as blood glucose levels rise, and at the same time, glucose-responsive neurons respond to that change. One hypothesis to explain these observations is that glucoreceptive neurons release peptides directly into the hypothalamic-pituitary portal system, thus making direct contact with the blood stream and exposing these neurons to plasma levels of glucose. This hypothesis is plausible because both growth-hormone–releasing hormone and β-endorphin are synthesized in the vicinity of the glucoreceptive neurons and are released into the portal system (45,46). Furthermore, both are stimulated by feeding (26,47), at least part of which appears to be mediated by glucose (T.M. Mizuno, H. Makimura, C.V.M., unpublished observations).

In conclusion, the proximal processes by which glucose-responsive neurons sense the transition from 5 to 20 mmol/l glucose appear to be highly similar to those involved in the glucose sensor of pancreatic β-cells, although the precise nature and role of the K-ATP channel remains to be fully established. These results reinforce the hypothesis that a core glucose-sensing mechanism may be broadly characteristic of glucose-sensing neuroendocrine cells, especially those that express the pancreatic form of glucokinase. Thus it will be of great interest to assess other aspects of the pancreatic mechanisms in glucose-responsive neurons, including the role of an anaplerotic/malonyl-CoA pathway (16), as well as oxidative phosphorylation (15).

ACKNOWLEDGMENTS

These studies were supported by NIH/NIDDK Grant DK 50110 (to C.V.M.).

We appreciate the support of Dr. Donald Pfaff, in whose laboratory these experiments were carried out.

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