Convergence of Pre- and Postsynaptic Influences on Glucosensing Neurons in the Ventromedial Hypothalamic Nucleus

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Glucosensing neurons in the ventromedial hypothalamic nucleus (VMN) were studied using visually guided slice-patch recording techniques in brain slices from 14- to 21-day-old male Sprague-Dawley rats. Whole-cell current-clamp recordings were made as extracellular glucose levels were increased (from 2.5 to 5 or 10 mmol/l) or decreased (from 2.5 to 0.1 mmol/l). Using these physiological conditions to define glucosensing neurons, two subtypes of VMN glucosensing neurons were directly responsive to alterations in extracellular glucose levels. Another three subtypes were not directly glucose-sensing themselves, but rather were presynaptically modulated by changes in extracellular glucose. Of the VMN neurons, 14% were directly inhibited by decreases in extracellular glucose (glucose-excited [GE]), and 3% were directly excited by decreases in extracellular glucose (glucose-inhibited [GI]). An additional 14% were presynaptically excited by decreased glucose (PED neurons). The other two subtypes of glucosensing neurons were either presynaptically inhibited (PIR; 11%) or excited (PER; 8%) when extracellular glucose was raised to >2.5 mmol/l. GE neurons sensed decreased glucose via an ATP-sensitive K^+ (K_{ATP}) channel. The inhibitory effect of increased glucose on PIR neurons appears to be mediated by a presynaptic γ-aminobutyric acid–ergic glucosensing neuron that probably originates outside the VMN. Finally, all types of glucosensing neurons were both fewer in number and showed abnormal responses to glucose in a rodent model of diet-induced obesity and type 2 diabetes. *Diabetes* 50:2673–2681, 2001

Neurons that change their action potential frequency in response to changes in extracellular glucose exist within hypothalamic nuclei involved in the regulation of food intake and energy balance. Previously, glucose-responsive (GR) neurons were defined as those that increase their action potential frequency when extracellular glucose levels were increased from 0 to 10 or 20 mmol/l, whereas glucose-sensitive (GS) neurons were those that decrease under those conditions. Defined in this way, GR neurons make up ~20–40% of the neurons in the arcuate and ventromedial hypothalamic nucleus (VMN), whereas GS neurons are more common in the lateral hypothalamus (1). The mechanism whereby GS neurons sense glucose has never been clearly defined. On the other hand, GR neurons possess an ATP-sensitive K^+ (K_{ATP}) channel that inactivates as the ATP-to-ADP ratio increases during glucose metabolism or in the presence of sulfonylureas (2). In GR neurons, this channel consists of four pore-forming units for K^+ (Kir6.2 [3,4] or Kir6.1 [5]) and four sulfonylurea receptors (SURs) (3,4,6). Insulin and leptin, which provide signals to the brain regarding peripheral metabolic status, activate the K_{ATP} channel on GR neurons (7,8). Additionally, GR neurons are abnormal in genetically obese Zucker (*fa/fa*) rats (8). Furthermore, central glucose sensing is abnormal in rats with diet-induced obesity (DIO). These rats do not activate hypothalamic neurons normally, nor do they have normal sympathetic nervous system activation to centrally infused glucose (9,10). Their low-affinity sulfonylurea binding in the arcuate and VMN is virtually absent (11). Given the abnormalities of glucose sensing in both genetically obese Zucker rats and DIO rats, it seems likely that abnormalities of central glucose sensing might play an important role in the control of energy homeostasis.

Extracellular brain glucose levels are ~30% that of plasma glucose (12). In life, they never fall to 0 mmol/l, nor do they rise to ≥10 mmol/l except under pathological conditions, such as untreated diabetes. For example, at a plasma glucose level of 7.6 mmol/l in a fed rat, extracellular brain glucose was only 2.5 mmol/l. When plasma glucose levels were decreased to 2–3 mmol/l or increased to 15.2 mmol/l, brain glucose levels were 0.16 mmol/l and 4.5 mmol/l, respectively (12). However, the majority of prior studies used nonphysiological levels of extracellular glucose (between 0 and 10 or 20 mmol/l) to characterize GR and GS neurons (1,2). Therefore, it is necessary to

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ACSF, artificial cerebrospinal fluid; CFTR, cystic fibrosis transmembrane regulator; DIO, diet-induced obesity; DR, diet-resistant; GABA, γ-aminobutyric acid; $GABA_A$, GABA type A; GE, glucose-excited; GI, glucose-inhibited; GR, glucose-responsive; GS, glucose-sensitive; K_{ATP}, ATP-sensitive K^+ channel; PED, presynaptically excited by decreased extracellular glucose; PER, presynaptically excited when extracellular glucose was raised; PIR, presynaptically inhibited when extracellular glucose was raised; RMP, resting membrane potential; SUR, sulfonylureas receptor; VMN, ventromedial hypothalamic nucleus.

reevaluate and redefine the function of purported glucosensing neurons under more physiological conditions to determine their relevance to physiological glucose sensing. Our hypothesis is that VMN glucosensing neurons are important mediators of the central regulation of glucose homeostasis. If this is true, then they should be most responsive to changes in glucose centered around a steady state midpoint of ~2.5 mmol/l, and their function should be altered in DIO-prone rats whose central glucose sensing is altered.

RESEARCH DESIGN AND METHODS

Male 14- to 21-day-old Sprague-Dawley rats selectively bred for the traits of developing DIO or being diet-resistant (DR) when placed on a diet moderately high in fat and calories (13) were obtained from colonies at the VA Medical Center in East Orange, New Jersey. At this age, the DIO trait is not expressed, so they are referred to here as DIO-prone. They were housed with their dams on a 12:12-h light:dark cycle at 22–23°C and given low-fat diet (Purina Rat Chow 5001) and water ad libitum. On the day of the experiment, rats were anesthetized with ketamine/xylazine (80:10 mg/kg i.p.) and transcardially perfused with ice-cold oxygenated (95% O_2/5% CO_2) perfusion solution composed of the following (in mmol/l): 2.5 KCl, 7 $MgCl_2$, 1.25 NaH_2PO_4, 28 $NaHCO_2$, 0.5 $CaCl_2$, 7 glucose, 1 ascorbate, and 3 pyruvate (osmolarity adjusted to ~300 mOsm with sucrose, pH 7.4). Brains were rapidly removed and placed in ice-cold (slushy) oxygenated perfusion solution. Sections (350 μm) through the hypothalamus were made on a vibratome (Vibroslice; Camden Instruments). The brain slices were maintained at 34°C in oxygenated high-Mg^{2+} low-Ca^{2+} artificial cerebrospinal fluid (ACSF; [in mmol/l]: 126 NaCl, 1.9 KCl, 1.2 KH_2PO_4, 26 $NaHCO_3$, 2.5 glucose, 9 $MgCl_2$, and 0.3 $CaCl_2$; osmolarity adjusted to ~300 mOsm with sucrose; pH 7.4) with 0.2 mmol/l 2,3-butanedione monoxime for 30 min and allowed to come to room temperature. Slices were then transferred to normal oxygenated ACSF (2.4 mmol/l $CaCl_2$ and 1.3 mmol/l $MgCl_2$) for the remainder of the day.

Viable neurons were visualized and studied under infrared differential-interference contrast microscopy using a Leica DMLS microscope equipped with a 40× long working–distance water-immersion objective. Current-clamp recordings (standard whole-cell recording configuration) from neurons in the VMN were made using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Data were stored on a digital-analog tape recorder (Biologic, Claix, France) for further analyses. During recording, brain slices were perfused at 10 ml/min with normal oxygenated ACSF. Borosilicate pipettes (1–3 MΩ; Sutter Instruments, Novato, CA) were filled with an intracellular solution containing (in mmol/l): 128 K-gluconate, 10 KCl, 4 KOH, 10 HEPES, 4 $MgCl_2$, 0.5 $CaCl_2$, 5 EGTA, and 2 Na_2ATP; pH 7.2. Osmolarity was adjusted to 290–300 mOsm with sucrose. Input resistance was calculated from the change in membrane potential in response to small 500-msec hyperpolarizing pulses (−10 to −20 pA) given every 3 s. The reversal potentials for changes in membrane conductance in response to glucose were derived from the voltage response to hyperpolarizing current steps as described previously (7). Briefly, hyperpolarizing current pulses varying from −10 to −120 pA were applied at 10- or 20-pA increments. At each increment, four pulses were applied. The duration of each pulse was 500 ms, and pulses were applied every 3 s. The membrane potential response during the last 5 ms of each pulse (when $dV/dt = 0$) was measured, and the average of the four pulses at each amplitude was calculated. The membrane potential response was measured only after the membrane response to altered extracellular glucose had stabilized, and this value was compared with controls that were measured immediately before changing extracellular glucose. Extracellular glucose levels were altered and chemicals added as described in the figures. All chemicals were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted. Data are expressed as means ± SE.

RESULTS

Responses of VMN neurons in DR rats. Using physiological conditions to define GR and GS neurons, it became apparent that there were several subtypes of neurons in the VMN that fit the original description of such cells (Table 1). Two subtypes of VMN glucosensing neurons were directly responsive to alterations in extracellular glucose levels. Another three subtypes were not inherently glucose-sensing themselves, but rather are presynaptically modulated by changes in extracellular glucose.

TABLE 1
Frequency of occurrence of subtypes of VMN neurons in DR and DIO-prone rats that respond to changes in extracellular glucose

	DR rats		DIO-prone rats
	Primary	Presynaptic	
Decreased glucose			
Excited	3 (3); GI	14 (14); PED	3 (6)
Inhibited	14 (14); GE	0	3 (6)
Increased glucose			
Excited	0	8 (9); PER	1 (3)
Inhibited	0	10 (11); PIR	1 (3)

Data are n subtype (% VMN neurons that subtype represents in DR and DIO-prone rats). A total of 100 neurons in DR rats and 53 neurons in DIO rats were tested for a response to a decrease in extracellular glucose levels from 2.5 or 5 to 0.1 mmol/l. Altogether, 92 neurons in DR rats and 36 neurons in DIO rats were tested for a response to an increase in extracellular glucose levels from 2.5 to 5 or 10 mmol/l. VMN neurons in DR rats are primarily glucosensing if the response to glucose is postsynaptic. Presynaptic refers to neurons in DR rats in which the observed changes were abolished under conditions of high Mg^{2+} and low Ca^{2+}, which remove presynaptic transmission. Determination of primary versus presynaptic glucose responses in DIO rats was impossible because of either high steady-state activity or low occurrence. In DR rats, VMN neurons that were directly excited by decreased glucose are referred to as GI neurons, whereas those that were directly inhibited are GE neurons. VMN neurons that were presynaptically excited by decreased glucose are referred to as PED neurons. VMN PER and PIR neurons are presynaptically excited or inhibited, respectively, when extracellular glucose was raised.

The most common subtype of inherently glucosensing VMN neuron will be referred to herein as glucose-excited (GE) because its action potential frequency changed in parallel with changes in extracellular glucose. Of the VMN neurons, 14% (14 of 100) fit this criterion. Under control conditions (2.5 mmol/l glucose), their resting membrane potential (RMP) was −43 ± 2 mV (n = 14), and their input resistance was 876 ± 99 MΩ (n = 10). GE neurons were reversibly hyperpolarized by 6.0 ± 1 mV, and their action potential frequency was decreased when extracellular glucose levels were decreased from 2.5 to 0.1 mmol/l (n = 11) (Fig. 1*A*) or from 5 to 0.1 mmol/l (n = 3). Input resistance was reduced by 29.5 ± 3.2% when glucose levels were decreased from 2.5 to 0.1 mmol/l (n = 8), indicating that conductance was increased. This conductance increase reversed at −95 ± 3 mV (theoretical K_{eq} = −99 mV), suggesting that a K^+ channel was activated (n = 4) (Fig. 1*B*). GE neurons showed no further excitation as glucose levels were increased to >2.5 mmol/l (n = 4). Moreover, a reduction in extracellular glucose from 2.5 to 1.0 mmol/l was not sufficient to inhibit GE neurons. However, these same neurons were inhibited when the glucose level was subsequently reduced to 0.5 mmol/l (n = 4). The sulfonylurea drug tolbutamide reversed the inhibitory effects of 0.1 mmol/l extracellular glucose (n = 4) (Fig. 1*A*). Finally, the inhibitory effect of 0.1 mmol/l glucose persisted under conditions of high Mg^{2+} (3.1 mmol/l) and low Ca^{2+} (0.3 mmol/l) (n = 2), which remove presynaptic transmission (Fig. 1*C*) (14,15). Thus, the inhibitory effect of decreasing extracellular glucose within the physiological range appears to be mediated by a K_{ATP} channel on the cell body of VMN GE neurons.

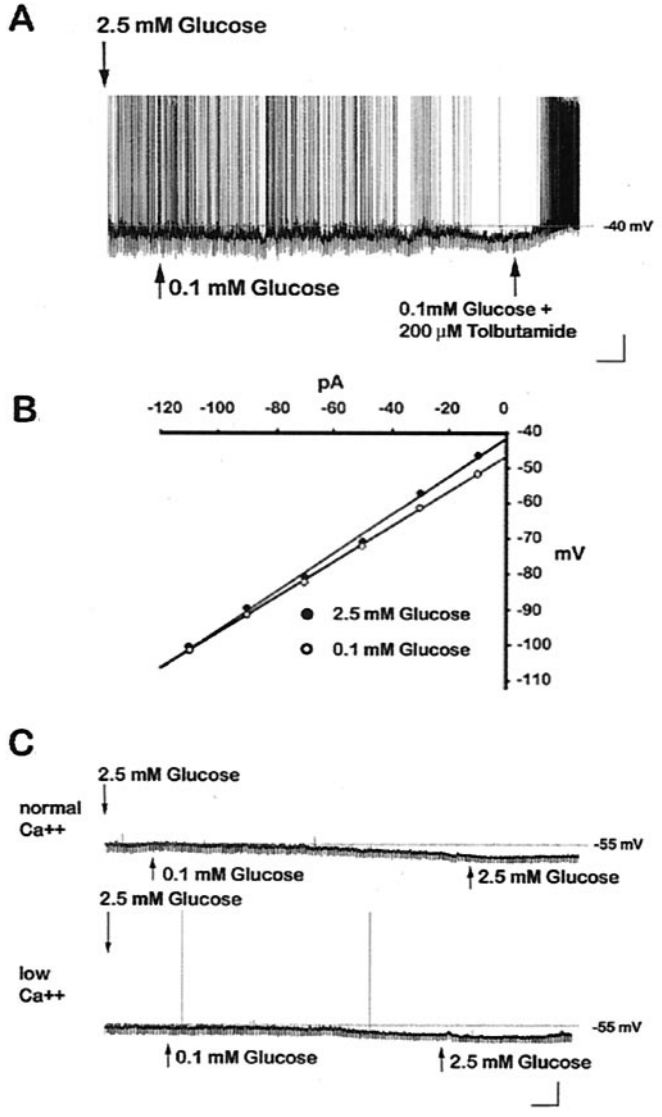

FIG. 1. Electrophysiological characterization of VMN GE neurons in DR rats. *A* and *C*: Standard whole-cell current-clamp recordings of spontaneous electrical activity in VMN neurons in brain slices. RMP is indicated by the dotted line and is noted to the right of each trace. The downward deflections represent the membrane voltage response to a constant current pulse. *A*: Decreasing extracellular glucose levels from 2.5 to 0.1 mmol/l causes hyperpolarization, decreased action potential frequency, and decreased input resistance. The sulfonylurea drug tolbutamide (200 μmol/l) reverses the inhibitory effect of 0.1 mmol/l glucose. *B*: Membrane potential response to increasing hyperpolarizing currents from −10 to −110 pA in 20-pA steps. Current-voltage relations indicate that the conductance induced by decreasing extracellular glucose from 2.5 to 0.1 mmol/l reverses at the theoretical potassium equilibrium potential (−99 mV). *C*: This panel shows consecutive traces from the same neuron. The inhibitory response to decreasing extracellular glucose is similar in normal and low-Ca^{2+} ACSF (top and bottom trace, respectively). The vertical scale in *A* and *C* is 10 mV, and the horizontal scale is 1 min.

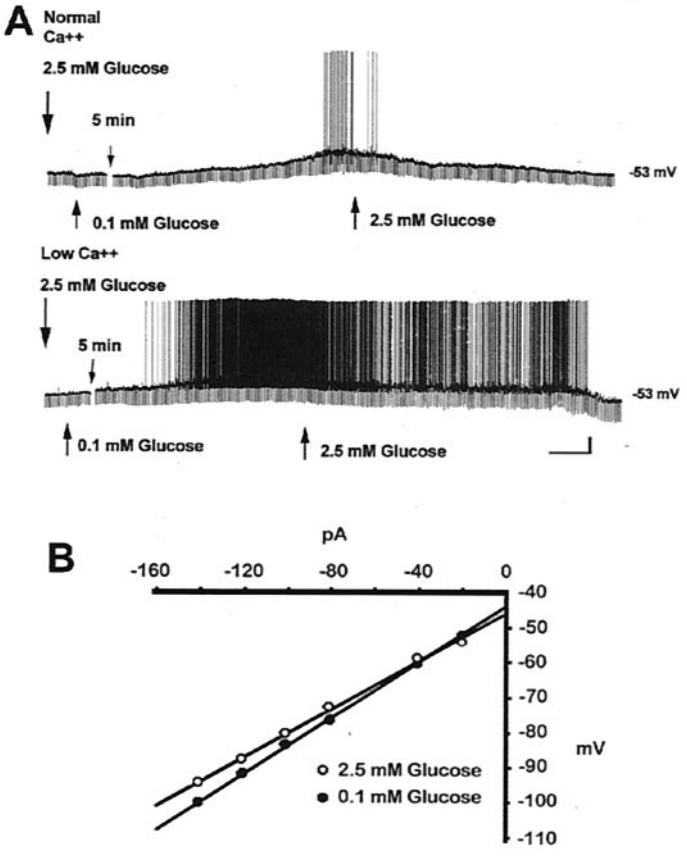

FIG. 2. *A*. Electrophysiological characterization of VMN GI neurons in brain slices from DR rats. Shown are standard whole-cell current-clamp recordings of spontaneous electrical activity in VMN neurons. Each panel shows consecutive traces from the same neuron. The downward deflections represent the membrane voltage response to a constant current pulse. VMN GI neurons depolarize and increase their action potential frequency and input resistance when extracellular glucose is decreased from 2.5 to 0.1 mmol/l (top trace). This excitatory response to decreasing extracellular glucose is similar in normal and low-Ca^{2+} ACSF (top and bottom trace, respectively). The vertical scale is 10 mV, and the horizontal scale is 1 min. *B*: Membrane potential response to increasing hyperpolarizing currents from −20 to −140 pA in 20-pA steps. Current-voltage relations indicate that the decreased conductance in response to decreasing extracellular glucose reversed close to the theoretical equilibrium potential for Cl^- (−57 mV).

The second, and less common, subtype of inherently glucosensing VMN neuron will be referred to as the glucose-inhibited (GI) neuron because its action potential frequency varied inversely with extracellular glucose levels (1). Only 3 of 100 VMN neurons were found to be GI. Their RMP in 2.5 mmol/l glucose was −48 ± 2 mV, and their input resistance was 590 ± 125 MΩ. These GI neurons reversibly depolarized by 4 ± 3 mV and increased their action potential frequency as extracellular glucose decreased from 2.5 to 0.1 mmol/l (Fig. 2*A*, top trace). This was associated with a 32 ± 11% increase in input resistance (n = 3), indicating a decrease in conductance. This response persisted under conditions of high Mg^{2+} and low Ca^{2+} in all three neurons, suggesting that they are inherently glucosensing neurons (Fig. 2*A*, bottom trace). Unlike the GE neurons, which reversed at approximately −90 mV, the conductance decrease of these GI neurons reversed at −50 ± 5 mV (n = 3; theoretical Cl^- equilibrium potential = −57 mV) in both normal- and high-Mg^{2+}/low-Ca^{2+} ACSF. It would be desirable to show that alteration of the Cl^- gradient caused an appropriate shift in the reversal potential for the response to glucose in these GI neurons. However, the paucity of GI neurons in the VMN (3 of 100) makes this extremely difficult and would significantly hinder timely presentation of this information. Nevertheless, these data are consistent with the hypothesis that decreasing extracellular glucose levels increased the action potential frequency and decreased conductance in GI neurons by inactivation of a Cl^- channel (Fig. 2*B*).

Three additional subtypes of VMN glucosensing neurons did not have inherent glucosensing properties. Rather,

their action potential frequencies and membrane properties were altered by presynaptic inputs in response to changes in extracellular glucose. Of these three noninherently glucosensing subtypes of VMN neurons, the first subtype responded to decreased extracellular glucose levels, whereas the other two subtypes responded to increased extracellular glucose levels. That is, 14% (14 of 100) of the VMN neurons reversibly depolarized by 2.4 ± 0.5 mV (RMP = −44 ± 1 mV) in response to a decrease in glucose levels from 2.5 to 0.1 mmol/l and increased their action potential frequency (Fig. 3*A*, top trace). In this case, input resistance was reduced by 16 ± 2.7% (from −934 ± 99 MΩ in 2.5 mmol/l glucose; $n = 10$), indicating increased membrane conductance. However, this response was lost when presynaptic transmission was removed ($n = 3$) (Fig. 3*A*, middle trace). These neurons will be referred to as presynaptically excited by decreased glucose (PED) neurons. The current voltage relations for the increased conductance in response to decreased extracellular glucose in these PED neurons were parallel between −50 and −100 mV ($n = 3$) (Fig. 3*B*). This suggests that neither K^+ nor Cl^- channels were involved. In current-clamp studies of spontaneously active neurons, action potentials obscured accurate measurement of the voltage response to positive current injection. Thus, we were unable to investigate the current-voltage relations above the RMP (approximately −50 mV) and could not determine whether their current-voltage relations remained parallel or intersected at a more positive voltage.

As mentioned above, the last two subtypes of VMN neurons altered their action potential frequency in response to increased extracellular glucose concentrations that were >2.5 mmol/l as a result of presynaptic inputs (Table 1). The first of these was reversibly inhibited as extracellular glucose levels were increased. Of the neurons, 11% (10 of 92) hyperpolarized by 6 ± 2 mV (RMP = −44 ± 2 mV) and decreased their action potential frequency when extracellular glucose was increased from 2.5 to 10 mmol/l ($n = 8$) (Fig. 4*A* and *B*) or from 5 to 10 mmol/l ($n = 2$) (Fig. 4*D*). Input resistance was 759 ± 80 MΩ in 2.5 mmol/l glucose and decreased by 22.2 ± 4.8% when extracellular glucose was increased to 5 or 10 mmol/l ($n = 7$). The γ-aminobutyric acid type A ($GABA_A$) receptor antagonist bicuculline reversed the inhibitory effect of 10 mmol/l glucose ($n = 5$) (Fig. 4*A*). The inhibitory effect of increased extracellular glucose was also abolished under conditions of high Mg^{2+} and low Ca^{2+} ($n = 2$) (Fig. 4*B*). Furthermore, these neurons were also inhibited by tolbutamide ($n = 2$) (Fig. 4*C*). This suggests that this subtype of VMN neuron is not inherently glucosensing. Instead, it appears to receive synaptic input from a GABAergic glucosensing neuron. Tolbutamide mimics the inhibitory effect of high glucose, suggesting the involvement of a K_{ATP} channel on the presynaptic locus. Thus, these neurons were presynaptically inhibited as glucose levels were raised (PIR neurons). Two PIR neurons were also inhibited when extracellular glucose was decreased from 2.5 or 5 to 0.1 mmol/l, and they were stimulated by tolbutamide (as shown in Fig. 4*D*). On the other hand, one PIR neuron was excited as glucose levels were reduced from 2.5 to 0.1 mmol/l. These differences are likely to reflect differences in the maintenance of individual synaptic inputs after the actual slice procedure. That is, presynaptic neurons that provide the VMN with inhibitory input as glucose levels rise may synapse on a variety of different VMN neurons, including GE and GI neurons as well as noninherently glucosensing neurons, as suggested above. The in vivo occurrence of such synapses is difficult to determine in this in vitro brain slice preparation because these inputs may not always be intact, depending on the exact slice location.

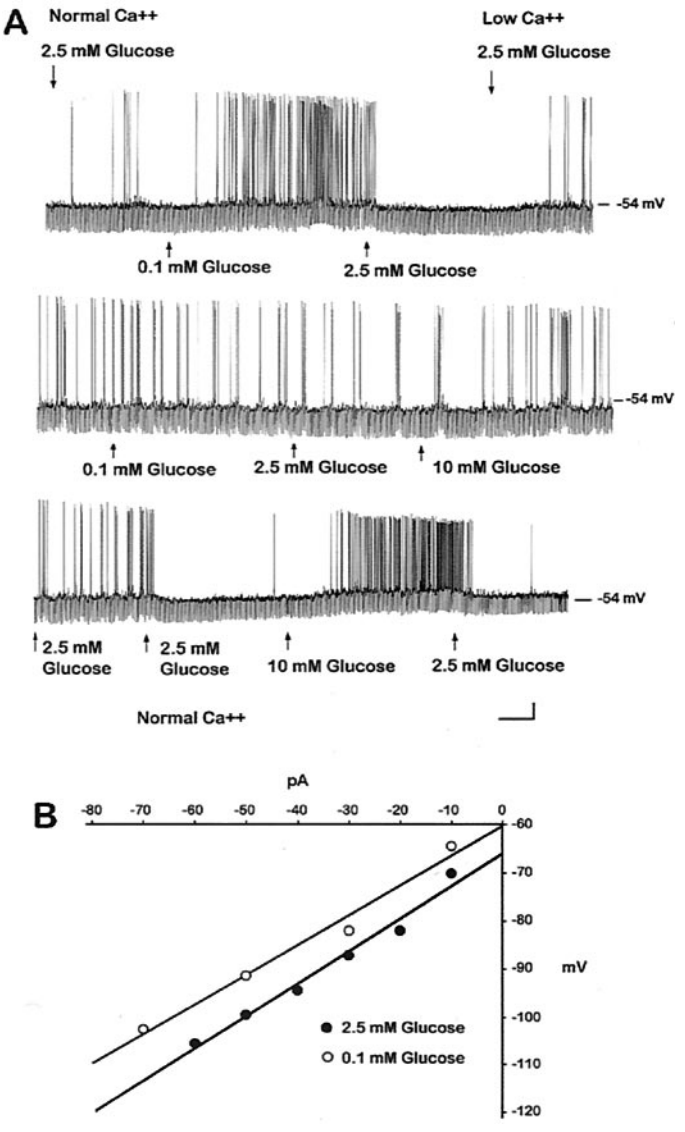

FIG. 3. Electrophysiological characterization of those VMN glucosensing neurons in DR rats that are presynaptically excited as extracellular glucose is decreased (PED neurons). *A*: Standard whole-cell current-clamp recordings of spontaneous electrical activity in VMN neurons in brain slices. Each panel shows consecutive traces from the same neuron. RMP is noted to the right of each trace. The excitatory response to decreasing extracellular glucose from 2.5 to 0.1 mmol/l in VMN PED neurons (top trace) was abolished in low-Ca^{2+} ACSF (middle trace). Input resistance decreased in response to decreased extracellular glucose. This PED neuron also depolarized, increased action potential frequency, and decreased input resistance in response to increased extracellular glucose (bottom trace). Similarly, this latter response was abolished in low-Ca^{2+} ACSF (middle trace). The vertical scale is 10 mV, and the horizontal scale is 1 min. *B*: Membrane potential response to increasing hyperpolarizing currents from −10 to −70 pA in 10-pA steps. Current-voltage relations indicate that the increased conductance in response to decreasing extracellular glucose did not reverse at the theoretical equilibrium potential for either K^+ (−99 mV) or Cl^- (−57 mV).

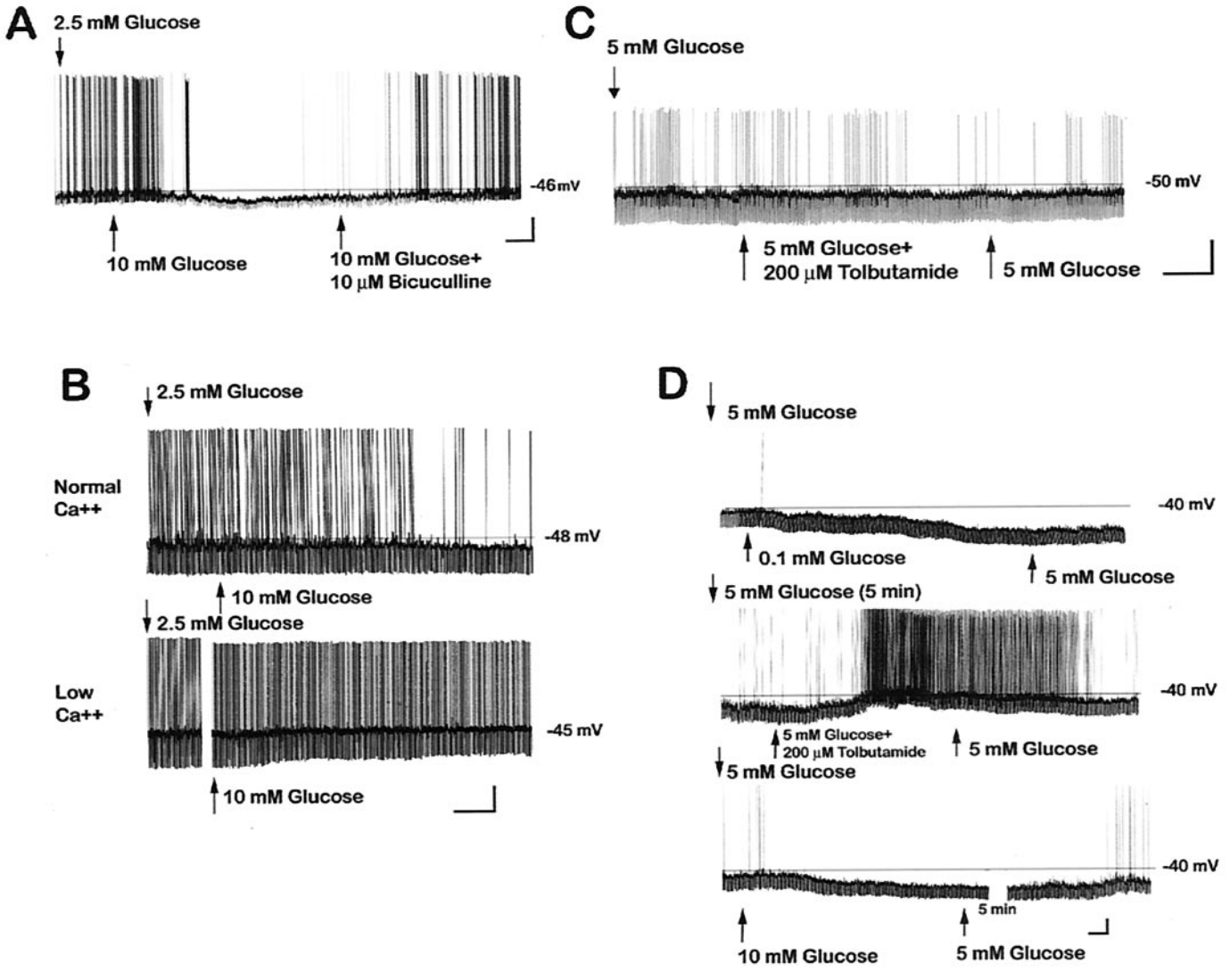

FIG. 4. Electrophysiological characterization of those VMN glucosensing neurons in DR rats that are presynaptically inhibited as extracellular glucose is raised (PIR neurons). Shown are standard whole-cell current clamp recordings of spontaneous electrical activity in VMN neurons in brain slices. RMP is indicated by the dotted lines and noted to the right of each trace. Each panel shows consecutive traces from the same neuron. The downward deflections represent the membrane voltage response to a constant current pulse. *A*: VMN PIR neurons are hyperpolarized and decrease their action potential frequency when extracellular glucose is increased from 2.5 to 10 mmol/l. The inhibitory response to increased extracellular glucose is reversed by the $GABA_A$ receptor antagonist bicuculline (10 μmol/l). *B*: This inhibitory response to increased extracellular glucose (top trace) is abolished in low-Ca^{2+} ACSF (bottom trace). *C*: VMN PIR neurons are also inhibited by tolbutamide (200 μmol/l). *D*: Some VMN PIR neurons hyperpolarize and decrease their action potential frequency when extracellular glucose is decreased to 0.1 mmol/l (top trace) as well as when it is increased to 10 mmol/l (bottom trace). These VMN PIR neurons are excited by tolbutamide (200 μmol/l) (middle trace). The vertical scale is 10 mV, and the horizontal scale is 1 min.

The second subtype of VMN neuron to alter its action potential frequency in response to increasing the extracellular glucose concentration to >2.5 mmol/l was presynaptically excited when extracellular glucose levels were raised (PER neuron). That is, 9% (8 of 92) of the VMN neurons reversibly depolarized by 3 ± 1 mV (RMP = −48 ± 2 mV; n = 8) and increased their action potential frequency when extracellular glucose was increased from 2.5 mmol/l to 5 or 10 mmol/l (Fig. 3*A*, lower trace). Input resistance was 978 ± 132 MΩ in 2.5 mmol/l glucose and decreased by 14.7 ± 4% when extracellular glucose levels were raised. This effect was lost when presynaptic input was abolished under conditions of high Mg^{2+} and low Ca^{2+} (n = 2) (Fig. 3*A*, middle trace). Interestingly, these PER neurons were also excited when extracellular glucose levels were decreased from 2.5 to 0.1 mmol/l (n = 5) (Fig. 3*A*, top and bottom traces). The response to decreased glucose persisted under conditions of high Mg^{2+} and low Ca^{2+} in one of these VMN PER neurons, whereas in another, the response to decreased glucose was abolished when presynaptic transmission was inhibited. The remaining three PER neurons that were also excited as glucose decreased were not evaluated under conditions of high Mg^{2+} and low Ca^{2+}. Thus, some neurons that provide excitatory presynaptic input to VMN neurons when extracellular glucose increases to >2.5 mmol/l synapse on either GI or PED neurons. As mentioned above, this is likely to be caused by differences in the maintenance of synaptic inputs in the individual slice preparations.

Finally, tolbutamide altered the action potential frequency of several different types of VMN neurons. As expected, tolbutamide (200 μmol/l) depolarized and in-

creased the action potential frequency of GE neurons as well as the two PIR neurons that were also inhibited by 0.1 mmol/l glucose ($n = 6$). GI and PED neurons were depolarized and their action potential frequencies increased in the presence of tolbutamide ($n = 2$). In contrast, tolbutamide hyperpolarized and decreased the action potential frequencies of VMN PIR neurons that were not inhibited as glucose was decreased to 0.1 mmol/l ($n = 2$). Surprisingly, tolbutamide also depolarized and increased the action potential frequency of three VMN neurons that had no apparent response to changes in extracellular glucose.

Responses of VMN neurons in DIO-prone rats. In contrast to the DR rats, VMN glucosensing neurons in DIO-prone rats were both fewer in number and showed abnormal responses to glucose (Table 1). Only 6% (3 of 53) of the VMN neurons in the DIO-prone rats were inhibited by 0.1 mmol/l glucose (Fig. 5*A* and *B*, top trace). These VMN GE neurons were hyperpolarized to a similar extent as those in DR rats (6 ± 1.7 mV). However, two of three neurons were abnormal, as illustrated by their slow and blunted response (Fig. 5*A*) and incomplete recovery (Fig. 5*B*). Furthermore, only 1 of the 36 (2.7%) VMN neurons in DIO-prone rats was inhibited by increasing extracellular glucose to 10 mmol/l. In this case, the action potential frequency decreased slightly, and the neuron was hyperpolarized by only 2 mV (Fig. 5*B*, middle trace). This neuron was stimulated by tolbutamide in the presence of 10 mmol/l glucose (Fig. 5*B*, lower trace).

Only 6% (3 of 53) of the VMN neurons in the DIO-prone rats depolarized (1 ± 0.4 mV; $n = 3$) and increased their action potential frequency when extracellular glucose levels were decreased from 2.5 to 0.1 mmol/l. For two of these three neurons, the response was barely detectable because of a high steady-state action potential frequency (Fig. 6*A*). The subtype of these neurons could not be determined because this high action potential frequency precluded investigation of presynaptic effects. Finally, only 2.7% (1 of 36) of the VMN neurons in DIO-prone rats depolarized (3 mV) and increased their action potential frequency when extracellular glucose was increased to >5 mmol/l (Fig. 6*B*, bottom trace). This neuron was not excited by decreasing extracellular glucose levels (Fig. 6*B*, top trace).

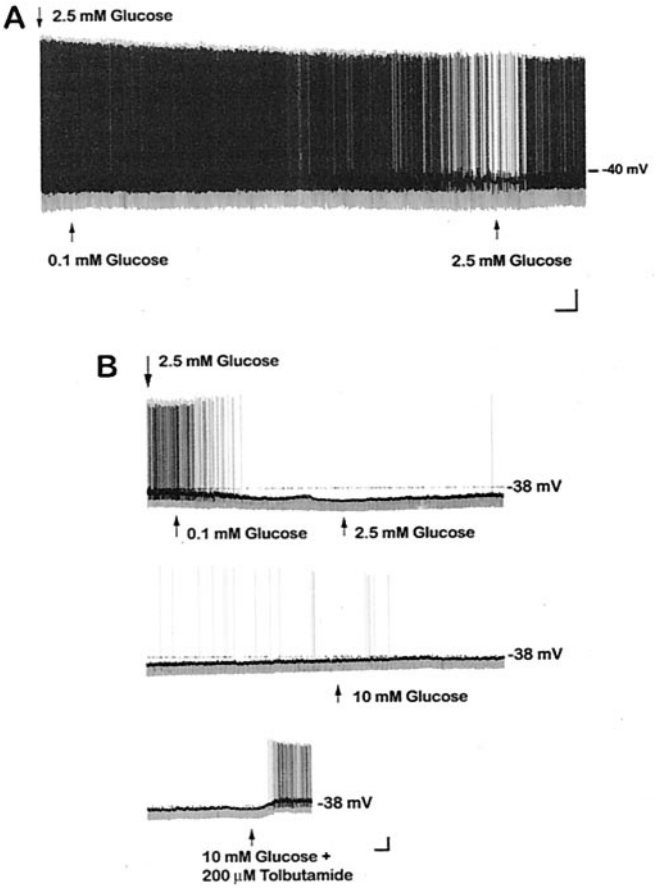

FIG. 5. Electrophysiological characterization of VMN GE and PIR neurons in DIO-prone rats. Shown are standard whole-cell current-clamp recordings of spontaneous electrical activity in VMN neurons in brain slices. RMP is indicated by the dotted lines and/or noted to the right of each trace. Each panel shows consecutive traces from the same neuron. The downward deflections represent the membrane voltage response to a constant current pulse. *A*: The inhibitory response to decreasing extracellular glucose from 2.5 to 0.1 mmol/l is delayed and blunted in this GE neuron from a DIO-prone rat. *B*: The inhibitory response to increasing extracellular glucose was extremely blunted (middle and bottom traces) in this VMN PIR neuron. Moreover, the inhibitory response to decreasing extracellular glucose to 0.1 mmol/l did not recover (top trace). Tolbutamide caused this neuron to depolarize and increase its action potential frequency (bottom trace). The vertical scale is 10 mV, and the horizontal scale is 1 min.

DISCUSSION

The present studies demonstrate that glucosensing by VMN neurons involves a complex convergence of pre- and postsynaptic mechanisms. These mechanisms are summarized in Fig. 7. Under physiological conditions, there are two types of neurons with inherent glucosensing properties in the VMN (Table 1). The first, which we have named GE, is analogous to the classic GR neuron (1,2), in which the inhibitory effect of decreasing extracellular glucose from 2.5 to 0.1 mmol/l is mediated by a somatic (or dendritic) K_{ATP} channel. Interestingly, VMN GE neurons were also inhibited when extracellular glucose decreased from 2.5 to 0.5 mmol/l but not from 2.5 to 1 mmol/l ($n = 4$). This suggests that GE neurons only respond to the large decreases in extracellular glucose that accompany profound systemic hypoglycemia, rather than the relatively small changes expected with the 10–15% dips in plasma glucose levels that precede some meals (16). Thus, VMN GE neurons may be more likely to play a role in the counterregulatory response to hypoglycemia (17) and glucoprivic feeding (18) than in the physiological control of meal initiation. This is consistent with recent studies of mice having deletions of the Kir6.2 pore-forming unit of the K_{ATP} channel ($Kir6.2^{-/-}$). These mice had no functional VMN GR neurons, and their counterregulatory response and stimulation of feeding induced by glucoprivation were attenuated. On the other hand, they maintained normal ingestive responses to the physiological regulators of ingestion, leptin, and neuropeptide Y (4). However, it is important to note that there are no data concerning actual meal-to-meal variations in extracellular brain glucose levels. Moreover, the data of Silver and Erecinska (12) show that the relation between plasma glucose levels and extracellular brain glucose levels is not linear at <2.5 mmol/l. Thus, further studies are needed before a final role in glucose homeostasis is assigned to the GE neuron.

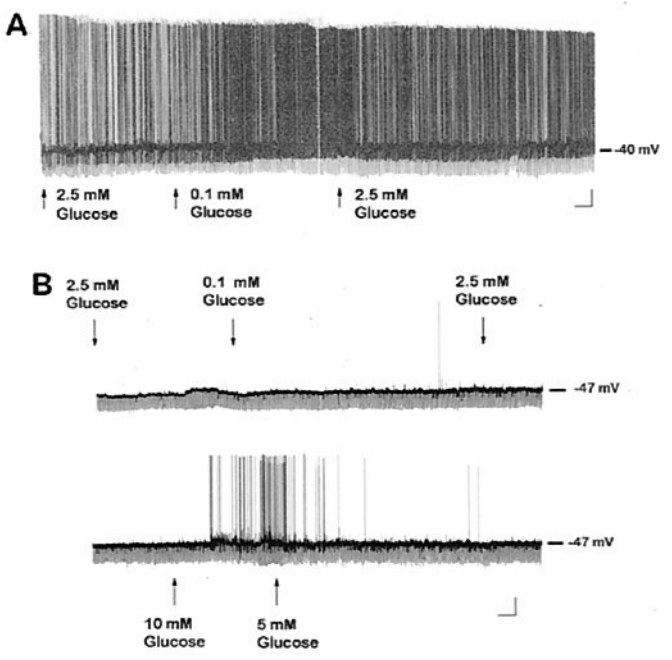

FIG. 6. Electrophysiological characterization of those VMN neurons in DIO-prone rats that are excited by changes in extracellular glucose. Shown are standard whole-cell current-clamp recordings of spontaneous electrical activity in VMN neurons in brain slices. The RMP is noted to the right of each trace. Each panel shows consecutive traces from the same neuron. The downward deflections represent the membrane voltage response to a constant current pulse. *A*: The excitatory response to decreasing extracellular glucose levels is difficult to detect in this neuron because of high steady-state action potential frequency. This high steady state activity precluded subtype characterization of this VMN glucosensing neuron. *B*: This VMN PER neuron depolarized and increased its action potential frequency only in response to extracellular glucose levels over 5 mmol/l. The vertical scale is 10 mV, and the horizontal scale is 1 min.

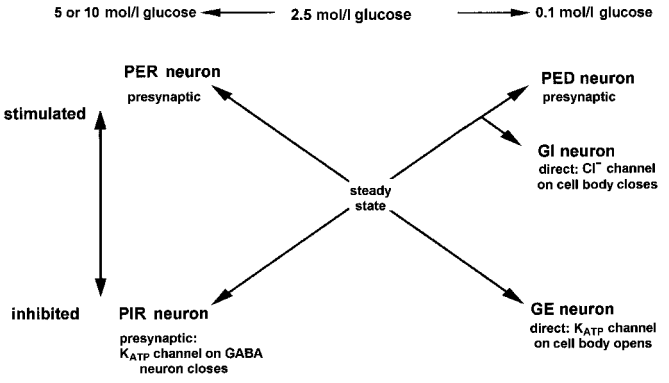

FIG. 7. Schematic overview of the subtypes of VMN glucosensing neurons and the mechanisms by which they sense glucose. Glucose concentration increases from right to left, and neuronal activity increases from bottom to top in the diagram. The mechanism is listed below each neuronal subtype. Thus, PED and GI neurons are shown to the upper right corner, indicating that they are excited (presynaptically or via closure of a Cl^- channel, respectively) as extracellular glucose levels decrease. GE neurons are shown on the lower right corner, indicating that they are inhibited by decreased extracellular glucose directly via opening of a K_{ATP} channel. PIR neurons are shown on the lower left corner, indicating that they are inhibited as extracellular glucose levels are raised (presynaptically via closure of a K_{ATP} channel on a GABA neuron), and PER neurons are shown on the upper left, indicating that they are (presynaptically) excited as extracellular glucose is raised.

A less common type of VMN neuron with inherent glucosensing properties is analogous to the GS neuron, which is found in low abundance in the VMN (1). We refer to these as GI neurons because they are inhibited by glucose. GI neurons were excited when extracellular glucose was decreased from 2.5 to 0.1 mmol/l. Further dose-response curves with glucose will be required to determine the functional response range of these VMN GI neurons. Regardless of the range of glucose needed to alter their firing, the present data lead us to hypothesize that their direct response to decreased glucose levels is mediated by the inactivation of a Cl^- channel. Because decreasing extracellular glucose levels should lower intraneuronal ATP levels, such a Cl^- channel should be responsive to changes in the ATP-to-ADP ratio. The cystic fibrosis transmembrane regulator (CFTR) is one such Cl^- conductance, and it is activated by ATP and blocked by sulfonylureas (19). Moreover, CFTR mRNA and protein are expressed in human and rat hypothalamus (20,21). There is also an ATP-activated Cl^- conductance in pancreatic islet cells. Decreased levels of ATP or high levels of the sulfonylurea gliburide inactivate this channel and depolarize the islet cells (22). Given these facts, it is interesting that the sulfonylurea tolbutamide stimulated GI neurons in our study. Although this may reflect the ubiquitous nature of the K_{ATP} channel (23), it may also indicate a lack of specificity of the sulfonylureas for the K_{ATP} channel. The SUR and CFTR belong to the same family of ATP-binding cassette transporters and have significant homology (6,24). Thus, tolbutamide may depolarize GI neurons by blocking the same Cl^- conductance that is reduced when extracellular glucose (or ATP) levels are decreased. These data also suggest that caution is warranted when using sulfonylureas as the sole indicator of the presence of GE neurons and/or K_{ATP} channels. For this reason, GE neurons in this study were characterized by a response to glucose that was not only blocked by sulfonylureas but also reversed at the K^+ equilibrium potential. Finally, although the GI neurons we observed were similar, we cannot be certain that they were identical to the GS neurons previously described by Oomura (1). This is because the GI neurons in our study also received presynaptic input from neurons that were excited when extracellular glucose levels were increased to >2.5 mmol/l. Thus, it is uncertain whether these GI neurons would change their action potential frequency in response to a linear change in glucose between 0 and 10 mmol/l.

An important finding of these studies is that there are many neurons in the VMN that have no inherent glucosensing capacity of their own. Instead, their firing rate is regulated by presynaptic inputs from other glucosensing neurons that are presumably outside of the VMN. One subtype of these neurons (PED) is excited by decreasing extracellular glucose from 2.5 to 0.1 mmol/l. Such PED neurons differ from GI neurons in several ways. First, the glucose modulation of their action potential frequency and membrane properties is presynaptic. Second, conductance is increased rather than decreased. Finally, because the current-voltage relations were parallel between −60 and −120 mV, this conductance increase appears unrelated to opening of K^+ or Cl^- channels. If the current-voltage relations remain parallel at more positive potentials, it might suggest the involvement of an ATP-dependent pump such as the Na^+/K^+-ATPase. This has been previously suggested for GS neurons (1). Two additional subtypes of

VMN neurons are presynaptically modulated by raising extracellular glucose levels. The first of these (PIR) was inhibited at extracellular glucose levels to >2.5 mmol/l. Because bicuculline reversed and tolbutamide mimicked the effect of increased extracellular glucose, we hypothesize that this response may be mediated by inactivation of a K_{ATP} channel on a presynaptic GABA cell body or nerve terminal. A comparable finding has been described for GABAergic inputs to the substantia nigra (25–27). Although the VMN itself contains GABA neurons (28), we found no VMN neurons with inherent glucosensing responses at levels >2.5 mmol/l glucose. Thus, it is likely that the presynaptic inputs to PIR neurons were from GABA neurons whose cell bodies lay outside the VMN. A likely candidate is the population of neuropeptide Y neurons in the adjacent arcuate nucleus that co-express GABA (29). These neurons are regulated by metabolic perturbations and have molecular properties that make them likely to be GR neurons (30). Also, neurons in the arcuate nucleus might be exposed directly to plasma glucose levels that exceed extracellular brain glucose levels because the arcuate nucleus is adjacent to the median eminence, which has a defective blood brain barrier (31).

Increasing extracellular glucose from 2.5 mmol/l to either 5 or 10 mmol/l presynaptically excited another subtype of VMN neuron (PER). An increase in extracellular glucose concentration to 5 mmol/l would be seen in the brain after a meal (12). In addition, these neurons were also excited when glucose levels were decreased. Thus, these PER neurons may be part of a regulatory system that becomes active when extracellular glucose levels increase above a steady-state level of ~2.5 mmol/l. This could include the hypothalamo-pituitary and autonomic systems, which participate in the assimilation, expenditure, and storage of ingested calories.

Thus, our data support that of others suggesting that VMN glucosensing neurons are involved in the responses to both pathological and physiological stimuli. The DIO rat exhibits several abnormalities of physiological function related to defects in central glucose sensing (9–11). Here, we show that these defects may be related to abnormalities in VMN glucosensing neurons. Not only were VMN glucosensing neurons of all subtypes fewer in number in DIO-prone rats, but their glucose responses were also abnormal. The K_{ATP} channel appears to play a role in the glucose sensing of both GE and PIR neurons. Thus, abnormalities in GE and PIR neurons are consistent with the reduced low-affinity sulfonylurea binding in the VMN and arcuate of DIO-prone rats (11). These data suggest that defective VMN glucosensing neurons may play a role in the altered central glucose sensing in DIO-prone rats.

In summary, we have shown that GE and GI neurons in the VMN respond directly to physiological changes in extracellular glucose, but only when glucose falls to <2.5 mmol/l. GE neurons use the K_{ATP} channel to sense glucose, whereas GI neurons may use a Cl^- conductance. Importantly, other VMN neurons alter their firing rates in response to a variety of extracellular glucose concentrations, but none of these neurons are inherently glucosensing. Instead, the observed effects are caused by presynaptic inputs from other glucosensing neurons, whose cell bodies may reside outside the VMN. Finally, DIO is associated with defective glucosensing neurons.

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