Metabolism-Independent Sugar Sensing in Central Orexin Neurons

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OBJECTIVE—Glucose sensing by specialized neurons of the hypothalamus is vital for normal energy balance. In many glucose-activated neurons, glucose metabolism is considered a critical step in glucose sensing, but whether glucose-inhibited neurons follow the same strategy is unclear. Orexin/hypocretin neurons of the lateral hypothalamus are widely projecting glucose-inhibited cells essential for normal cognitive arousal and feeding behavior. Here, we used different sugars, energy metabolites, and pharmacological tools to explore the glucose-sensing strategy of orexin cells.

RESEARCH DESIGN AND METHODS—We carried out patch-clamp recordings of the electrical activity of individual orexin neurons unambiguously identified by transgenic expression of green fluorescent protein in mouse brain slices.

RESULTS—We show that *1*) 2-deoxyglucose, a nonmetabolizable glucose analog, mimics the effects of glucose; *2*) increasing intracellular energy fuel production with lactate does not reproduce glucose responses; *3*) orexin cell glucose sensing is unaffected by glucokinase inhibitors alloxan, D-glucosamine, and N-acetyl-D-glucosamine; and *4*) orexin glucosensors detect mannose, D-glucose, and 2-deoxyglucose but not galactose, L-glucose, α-methyl-D-glucoside, or fructose.

CONCLUSIONS—Our new data suggest that behaviorally critical neurocircuits of the lateral hypothalamus contain glucose detectors that exhibit novel sugar selectivity and can operate independently of glucose metabolism. *Diabetes* **57:2569–2576, 2008**

Hypothalamic neurons maintain body energy balance by sensing energy status and initiating compensatory adjustments in food intake and energy expenditure (1,2). A key signal informing the brain of energy levels is the concentration of extracellular glucose (3,4). Specialized glucose-sensing (glucosensing) hypothalamic neurons respond to changes in ambient glucose levels with increases (glucose-excited neurons) or decreases (glucose-inhibited neurons) in their firing frequency (5,6). These responses are considered critical for orchestrating changes in hormone release, appetite, and food-seeking behavior that control body energy stores and blood glucose levels (4,7,8). Impaired hypothalamic glucosensing can lead to diabetes and obesity (9). It is thus important to understand how glucosensing neurons operate.

Until now, most studies examining the link between fluctuations in glucose levels and neuronal electrical activity have focused on glucose-excited neurons. There is now strong evidence that in most of these cells, extracellular glucose is carried into the cytosol by GLUT, phosphorylated by glucokinase, and subsequently metabolized to generate ATP, which closes membrane ATP-sensitive K^+ channels (K_{ATP} channels) and so electrically excites the cell (7,8,10). In contrast, glucose-inhibited neurons remain much less understood. Although the final effectors mediating glucose-induced inhibition have been recently defined as K^+ or Cl^- channels (11,12), the nature of glucosensing events upstream of these channels is unclear. There are some indirect indications that glucokinase is involved, for example, treatment of hypothalamic cell cultures with small interfering glucokinase mRNA accelerates the natural decline in their ability to exhibit glucose-induced inhibition (13). However, glucokinase appears to be found at low or undetectable levels in some glucosensing neurons (14), suggesting that other glucosensing modes are possible.

Clearly resolving the role of glucose metabolism in glucose-induced inhibition of behaviorally defined neurocircuits requires direct measurements of electrical responses in functionally defined neurons. Recently, it became clear that lateral hypothalamic neurons containing the neuropeptides orexins/hypocretins (orexin neurons) (15,16) are inhibited by glucose because of sugar-induced opening of postsynaptic K^+ channels (11,17). Orexin neurons are an excellent model for studying glucose-induced inhibition because they are readily identifiable in living brain slices by targeted expression of fluorescent proteins (17). Furthermore, changes in their electrical activity have well-defined physiological and behavioral implications. Through widespread projections to diverse brain areas expressing specific orexin receptors coupled to neuronal excitation, the firing of orexin neurons stimulates wakefulness, metabolic rate, and food intake (18,19). Orexin neurons are so critical for stable consciousness that their loss causes narcolepsy in a variety of mammalian species (20–22). Furthermore, key roles for orexins are emerging in reward-seeking and addiction (23,24). Understanding glucose responses of orexin cells would thus provide fundamental information about brain glucosensing in the context of vital adaptive behaviors.

To examine whether glucose metabolism is involved in glucosensing mediated by orexin cells, we directly measured their electrical responses to metabolizable and nonmetabolizable sugars, to stimulation of intracellular ATP production with lactate, and to glucokinase inhibitors. Our results suggest that sugar sensing in orexin neurons is independent of glucose metabolism or intra-

cellular ATP generation and displays a unique sugar-selectivity signature indicative of a novel glucose receptor.

RESEARCH DESIGN AND METHODS

Preparation of brain slices from transgenic mice. Animal procedures were performed according to the Animals (Scientific Procedures) Act, 1986 (U.K.). Transgenic "orexin-eGFP" mice were used in all experiments to identify orexin neurons, as in our previous studies (11,25). These mice expressed enhanced green fluorescent protein (eGFP) under the control of the *prepro-orexin* promoter, resulting in very specific targeting of eGFP only to orexin neurons (11). Mice were maintained on a 12-h light:dark cycle (lights on at 08:00 h) and had free access to food and water. Animals (14–21 days postnatal) were killed during the light phase. Coronal slices (250–300 μm thick) containing the lateral hypothalamus were cut with a Campden Vibroslice in ice-cold extracellular solution, incubated at 35°C in carbogen-gassed extracellular solution for 30 min, and then kept at room temperature until recording, as previously described (26). Typically, between 5 and 20 cells were evaluated from each animal used.

Chemicals and solutions. The extracellular solution was gassed with 95% O_2 and 5% CO_2 and contained 125 mmol/l NaCl, 2.5 mmol/l KCl, 2 mmol/l $MgCl_2$, 2 mmol/l $CaCl_2$, 1.2 mmol/l NaH_2PO_4, 21 mmol/l $NaHCO_3$, and 1 mmol/l glucose, except where indicated otherwise. We used a "K-chloride" intracellular (pipette) solution throughout to reduce junction potential errors in measurements of current-voltage relationships (27). This solution contained 130 mmol/l KCl, 0.1 mmol/l EGTA, 10 mmol/l HEPES, 5 mmol/l K_2ATP, 1 mmol/l NaCl, and 2 mmol/l $MgCl_2$, pH 7.25, with KOH. Where not indicated, the full chemical names of sugars and metabolites we used were D-(+)-mannose, L-lactate, D-(−)-fructose, D-(+)-galactose, D-(+)-glucose, L-(−)-glucose, and methyl-α-D-glucopyranoside. All chemicals were from Sigma (Poole, Dorset, U.K.). Chemicals were applied extracellularly by bath perfusion, unless indicated otherwise.

Data acquisition and analysis. Living orexin-eGFP neurons were visualized in brain slices using an Olympus BX50WI upright microscope equipped with epifluorescence as previously described (11). Whole-cell recordings, including switching to voltage-clamp mode (for recording membrane currents) and current-clamp mode (for recording membrane potential), were performed using an EPC 10 amplifier (Heka, Lambrecht, Germany) at 36°C (bath temperature controlled with Badcontroller V; Luigs & Neumann). Patch pipettes were pulled from borosilicate glass and had tip resistances of 3–6 mol/lΩ. Series resistances were in the range of 5–10 mol/lΩ and were not compensated. Data were sampled and filtered using Patchmaster software (Heka) and analyzed with custom-written Python software (http://www.python.org), Matplotlib (http://matplotlib.sourceforge.net), and Origin software (Microcal, Northampton, MA).

To monitor membrane resistance together with changes in membrane potential (Figs. 1*A* and 8*A*–*C*), cells were injected every 20–40 s with a fixed-amplitude (10–40 pA, 1-s duration) square pulse of hyperpolarizing current (26). The amplitude of the resulting downward deflections in membrane potential is proportional to membrane resistance (Ohm's law).

Current-voltage relationships (Figs. 2*A* and *B*, 3*B*, 4*B*, 5*B* and *C*, and 7*B* and *C*) were obtained by performing voltage-clamp ramps from 0 to −140 mV at a rate of 0.1 mV/ms ramp, which is sufficiently slow to allow leak-like K^+ currents to reach steady state at each potential (28). In Figs. 2*B* and 7*C*, the net current-voltage relationship was fitted with the Goldman-Hodgkin-Katz (GHK) current equation (29) in the following form:

$$I = P_K z^2 \frac{VF^2}{RT} \frac{[K^+]_i - [K^+]_0 \exp(-zFV/RT)}{1 - \exp(-zFV/RT)}$$

Here, I is current (in A), V is membrane potential (in V), z is the charge of a potassium ion (+1), $[K^+]_i$ is the pipette K^+ concentration (in mmol/l), $[K^+]_o$ is the extracellular K^+ concentration (in mmol/l), T is temperature (in Kelvins), and R and F have their usual meanings (29). P_K (a constant reflecting the K^+ permeability of the membrane) was the only free parameter during fits. The values of P_K used to obtain the fits shown in Figs. 2*B* and 7*C* were, respectively, 4.616×10^{-17} and 1.554×10^{-17} cm/s. Values are presented as means ± SE.

RESULTS

2-Deoxyglucose mimics the effects of glucose on orexin neurons.

We examined the effects of a variety of sugars and agents that affect energy metabolism on the membrane potential and currents of orexin neurons directly identified by selective expression of GFP using whole-cell patch-clamp recordings (11). We first tested 2-deoxyglucose,
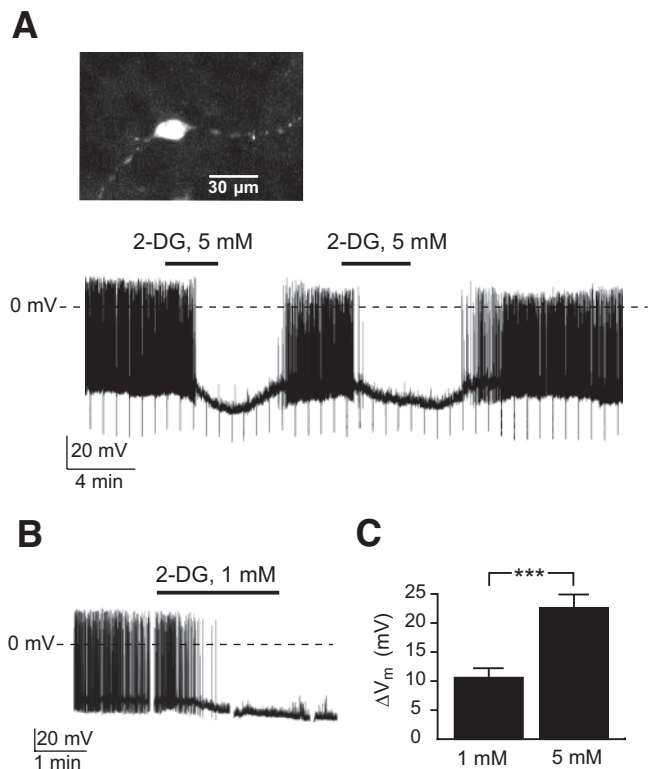

FIG. 1. Effects of 2-deoxyglucose on the membrane potential of orexin neurons. *A*: A GFP-expressing orexin neuron identified in brain slice by epifluorescence (full description and validation of the GFP-labeling method is given by Burdakov et al. [11]) (*top*). Effects of 5 mmol/l bath-applied 2-deoxyglucose (2-DG) on membrane potential and resistance (resistance monitored as voltage deflections in response to hyperpolarizing current injections, see RESEARCH DESIGN AND METHODS) (*bottom*). *B*: Effect of 1 mmol/l bath-applied 2-deoxyglucose on membrane potential. *C*: Magnitudes of membrane potential hyperpolarization (ΔV_m) induced by 1 and 5 mmol/l 2-deoxyglucose. ***$P < 0.005$, values are means ± SE, $n = 4$ cells for each condition.

a nonmetabolizable glucose analog that competes with glucose for binding to enzymes and transporters, thereby acting as an energy-depleting "antimetabolite." Thus, we expected this antimetabolite to have the opposite effects to glucose or no effects at all, because the cells that we recorded were provided with ATP from the pipette solution and hence presumably were energetically independent of extracellular glucose. However, 2-deoxyglucose mimicked the effects of glucose on the membrane potential and conductance of orexin cells, triggering dose-dependent and reversible membrane hyperpolarization and increase in membrane conductance (Fig. 1*A* and *B*, $n = 30$ cells). Analysis of membrane current responses to voltage-clamp ramps revealed that the changes in membrane potential were associated with activation of an ionic current that reversed at a membrane potential corresponding to E_K (Fig. 2*A*, $n = 7$ cells; predicted $E_K \sim -100$ mV with our solutions) and followed an outward current-voltage rectification well described by the GHK equation (Fig. 2*B*, $n = 4$ cells), as previously described for glucose (11). Like glucose, 2-deoxyglucose thus hyperpolarizes the orexin cell membrane by activating a leak-like K^+ current.

We also performed control experiments to ensure that the effects of 2-deoxyglucose on orexin neurons are a specific phenomenon rather than a general outcome of energy depletion caused by this antimetabolite). First, we applied 2-deoxyglucose to neurons that had similar membrane properties to orexin cells (depolarized potentials
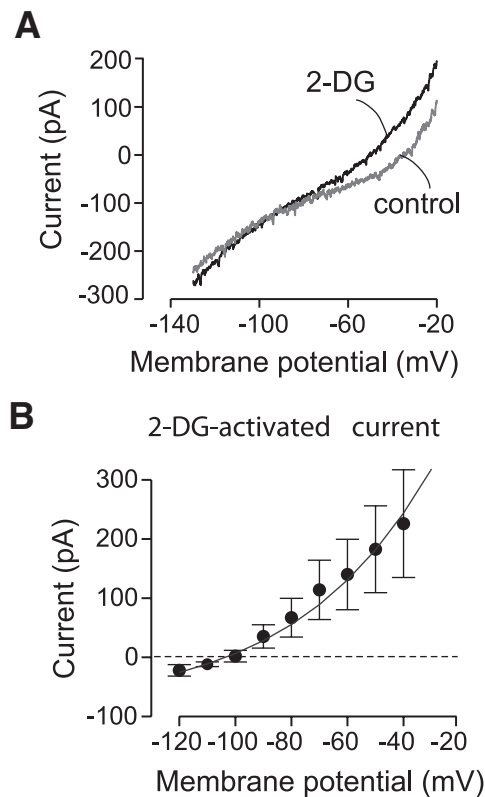
A

B

2-DG-activated current

FIG. 2. Effects of 2-deoxyglucose on the membrane currents of orexin neurons. Whole-cell voltage-clamp recordings. *A*: Effect of 2-deoxyglucose on membrane current-voltage relationships. *B*: Current-voltage relationship of the net current activated by 2-deoxyglucose. The line is a fit of the GHK equation to the data (see RESEARCH DESIGN AND METHODS). Values are means ± SE, *n* = 4 cells.
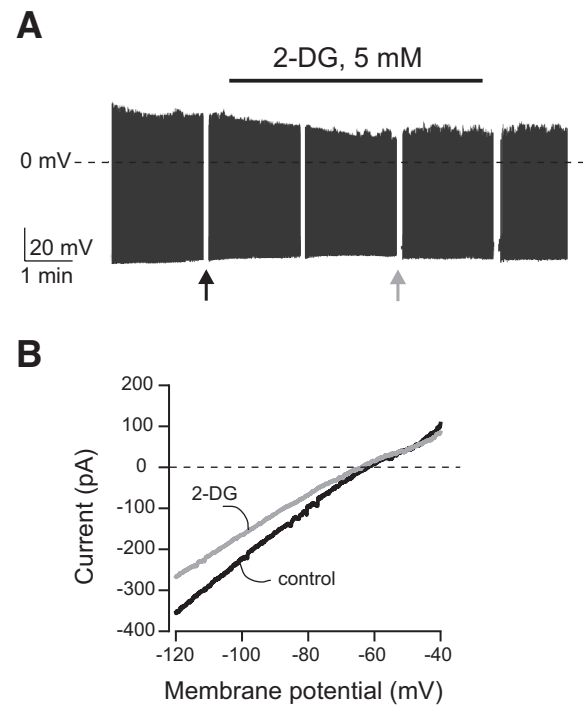
A

B

FIG. 3. Effects of 2-deoxyglucose on cortical neurons. *A*: Effect of 2-deoxyglucose on the membrane potential. Breaks in the trace correspond to where the recording was interrupted to expose the cell to voltage-clamp ramps (see RESEARCH DESIGN AND METHODS). Arrows show where the voltage-clamp ramps shown in *B* were taken. *B*: Effect of 2-deoxyglucose on membrane current-voltage relationship of the cell shown in *A*. No activation of K^+ current is observed, but 2-deoxyglucose inhibits a current with a reversal potential of about −50 mV.

and spontaneous firing) but were located in the cerebral cortex, a nonglucosensing brain region (6). Unlike in orexin cells, in cortical neurons, 2-deoxyglucose induced neither hyperpolarization (Fig. 3*A*, *n* = 7 cells) nor activation of K^+ currents (Fig. 3*B*, *n* = 7 cells). Second, we filled orexin neurons with 350 μmol/l tolbutamide and 5 mmol/l ATP by including these chemicals in the pipette solution used for whole-cell recording. This procedure is expected to block their K_{ATP} channels by ~100% (30), thereby disrupting the ubiquitous coupling between intracellular ATP metabolism and membrane potential in neurons (31). Under these conditions, orexin cells still exhibited full-blown responses to 2-deoxyglucose (Fig. 4*A* and *B*, *n* = 6 cells) and glucose (*n* = 3; data not shown). In the presence of tolbutamide, hyperpolarization elicited by 2-deoxyglucose (17.0 ± 2.5 mV, *n* = 6) was not different from that in control cells (19.9 ± 2.5 mV, *n* = 9, *P* = 0.437). This implies that orexin cell responses to 2-deoxyglucose are a specific sensing phenomenon of orexin cells rather than a general neuronal response related to energy metabolism.

Lactate does not mimic the effects of glucose despite being metabolized. To further examine the effects of energy metabolism on the membrane potential of orexin cells, we stimulated them with lactate. Lactate is used as fuel for mitochondrial ATP production and is considered to be the preferred energy fuel for central neurons (32). Lactate (5 mmol/l) neither hyperpolarized orexin neurons nor activated their K^+ currents (Fig. 5*A* and *B*). Instead, it triggered a slight membrane depolarization and an increase in action potential firing frequency, both of which

were reversible upon removal of lactate (Fig. 5*A*, *n* = 10 cells). To examine membrane currents underlying these excitatory effects, we looked at membrane current-voltage relationships before and after lactate action, which revealed that lactate inhibited a membrane current with a reversal potential close to that of K^+ (Fig. 5*B*, *n* = 4 cells). This observation is consistent with lactate being converted to ATP and the latter blocking the K_{ATP} channels. We observed that the net lactate-inhibited current (Fig. 5*C*, *n* = 4 cells) exhibited an inward current-voltage rectification typical of K_{ATP} channels (30) but not of glucose-activated K^+ channels (11). These data imply that in orexin neurons, lactate-induced stimulation of mitochondrial ATP production causes membrane depolarization through closure of K_{ATP} channels and does not mimic the activating effects of glucose on leak-like K^+ channels.

Glucokinase inhibitors do not block glucose responses. Our experiments with lactate imply that mitochondrial ATP generation does not induce glucosensing responses in orexin cells. However, it is possible that the exact site of ATP production is critical for glucose-sensing, for example, in glucose-excited β-cells; pyruvate does not trigger glucosensing responses despite being metabolized (33,34). Therefore, we next tested whether glucosensing depends on glucose metabolism in the glycolytic pathway. To block glycolytic metabolism of glucose, we pharmacologically inhibited glucokinase with high concentrations of three selective inhibitors, alloxan, D-glucosamine, and *N*-acetyl-D-glucosamine (35). However, in orexin neurons, glucokinase inhibition did not change the effects of glucose on the membrane potential (Fig. 6*A–C*, *n* = 14 cells) or K^+ currents (*n* > 3 cells for each inhibitor; data not shown). In the presence of alloxan, D-glu-

A

2-DG, 5 mM

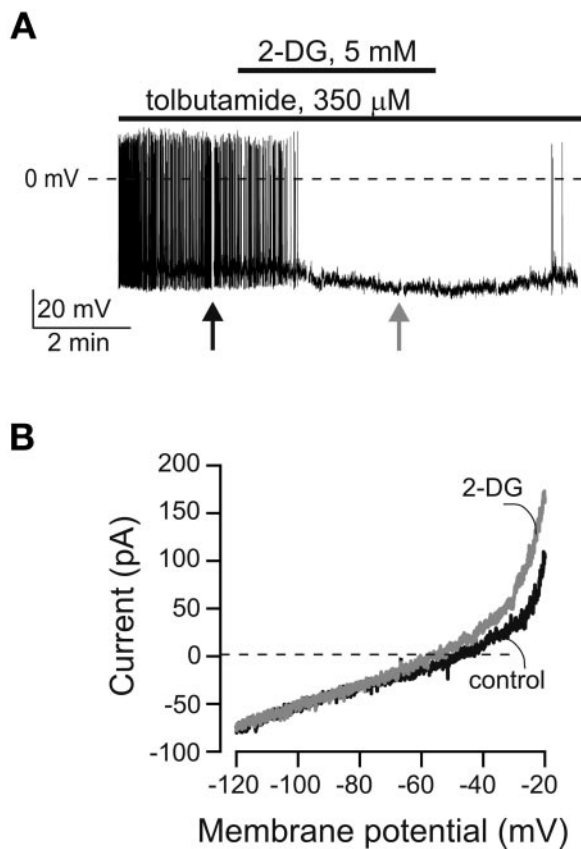tolbutamide, 350 μM

0 mV – – – – – –

20 mV
2 min

B

FIG. 4. Effects of 2-deoxyglucose on orexin neurons in the presence of tolbutamide. *A*: Effect of 2-deoxyglucose on the membrane potential in the presence of intracellular (pipette) tolbutamide. Arrows show where the voltage-clamp ramps shown in *B* were recorded. *B*: Effect of 2-deoxyglucose on membrane current-voltage relationships of the cell shown in *A*.
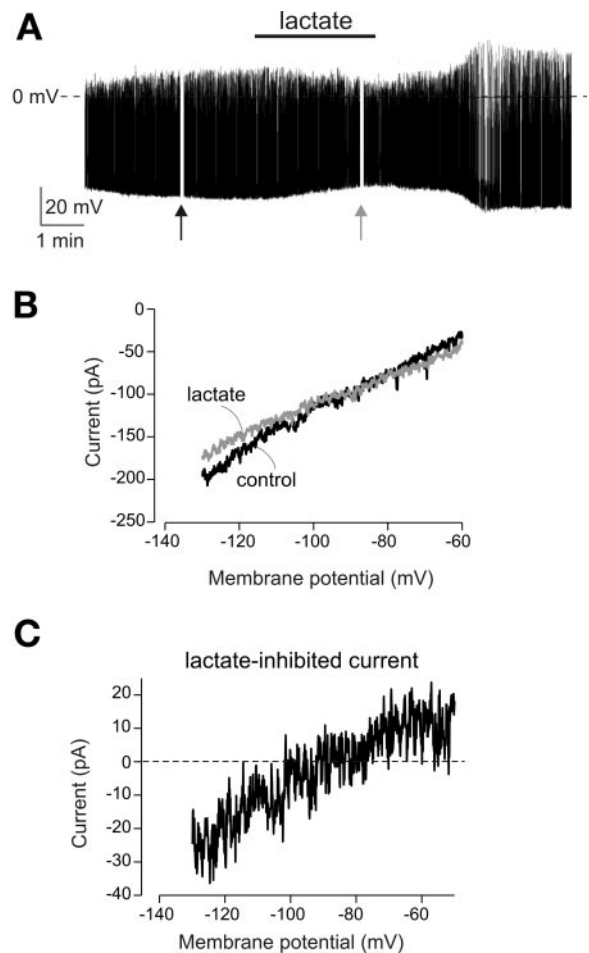
A

lactate

0 mV – –

20 mV
1 min

B

C

FIG. 5. Effects of lactate on orexin neurons. *A*: Effect of lactate on the membrane potential. Arrows show where voltage-clamp ramps shown in *B* were taken. *B*: Effect of lactate on the membrane current-voltage relationship of the cell shown in *A*. *C*: Current-voltage relationship of the net current inhibited by lactate.

cosamine, and *N*-acetyl-D-glucosamine, respectively, a switch from 1 to 5 mmol/l glucose caused hyperpolarization of 21.7 ± 3.7 ($n = 5$), 22.5 ± 3.1 ($n = 5$), and 28.0 ± 2.4 mV ($n = 4$), which is indistinguishable from control value (25.1 ± 3.7 mV, $n = 6$; $P = 0.533$, 0.616, and 0.582, respectively).

These results indicate that preventing intracellular glucose from being converted to its metabolizable form by glucokinase-catalyzed phosphorylation does not affect the ability of orexin cells to sense glucose. In fact, intracellular glucose in general is unlikely to play a key role in orexin cell glucosensing because artificial infusion of glucose into orexin cells does not trigger their inhibitory glucose responses (11). Here, we repeated the latter experiment with higher concentrations of intracellular glucose and lower concentrations of extracellular glucose than in this original study (Fig. 6*D*). We found that internally dialyzing orexin cells with 10 mmol/l glucose does not evoke glucosensing responses but, subsequently, that switching from 1 to 2.5 mmol/l glucose outside the same cell elicits normal responses (Fig. 6*D*, $n = 4$ cells). Thus, it is unlikely that glucose-derived energy metabolites or intracellular glucose plays a role in glucose responses of orexin neurons.

Effects of other sugars on orexin neurons. Despite the importance of orexin neurons for behavioral coordination, and the potent inhibitory effects of glucose on these cells, their sensitivity to other sugars has not been examined. Determining the sugar selectivity of glucose effects on orexin neurons can provide valuable information about

the nature of the glucose-detection device in these cells, because sugar binding sites on known glucose-binding proteins exhibit specific sugar-selectivity signatures (see DISCUSSION). In addition to 2-deoxyglucose (Fig. 1), therefore, we examined responses to five other sugars, mannose, L-glucose, α-methyl-D-glucoside, fructose, and galactose. Of these, only mannose mimicked the effects of glucose on the membrane potential of orexin cells (Fig. 7*A*, $n = 8$ cells). The responses of orexin cells to mannose were also identical to glucose responses in terms of activation of membrane currents: Like glucose, mannose triggered a K-selective current (Fig. 7*B*, $n = 6$ cells) whose current-voltage relationship was well approximated by the GHK current equation (Fig. 7*C*). In contrast, at 5 mmol/l, none of the other sugars that we tried affected the membrane potential (Fig. 8, $n > 4$ cells for each sugar tested) or membrane currents ($n > 3$ cells for each sugar; data not shown) of orexin cells. To validate glucose-sensing viability of each cell we examined, we stimulated it with 5 mmol/l glucose at the end of the experiment, which invariably triggered a full-blown hyperpolarizing response (Fig. 8).

DISCUSSION

In this study, we document several previously unknown features of sugar-induced block of orexin neurons, cells
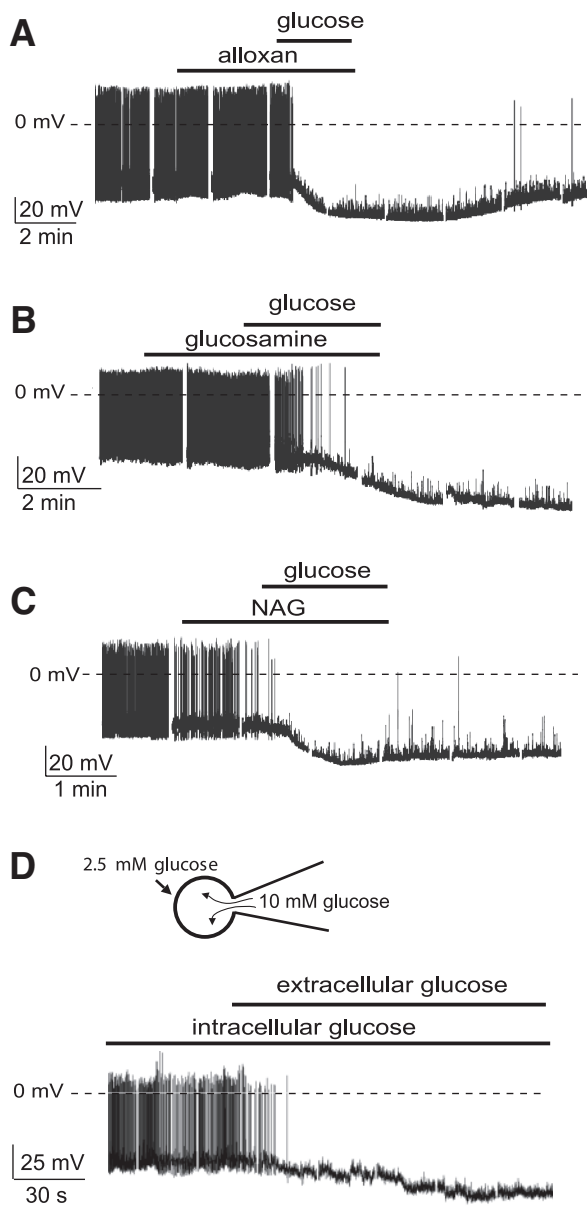
FIG. 6. Effects of glucokinase inhibitors and intracellular glucose on the glucose response of orexin neurons. *A*: Effect of 5 mmol/l glucose on the membrane potential in the presence of 10 mmol/l bath alloxan. *B*: Effect of 5 mmol/l glucose on the membrane potential in the presence of 10 mmol/l bath D-glucosamine. *C*: Effect of 5 mmol/l glucose on the membrane potential in the presence of 4 mmol/l bath *N*-acetyl-D-glucosamine (NAG). *D*: Cartoon showing experimental protocol (*top*). Effect on the membrane potential of 10 mmol/l interacellular glucose and 2.5 mmol/l extracellular glucose (*bottom*).
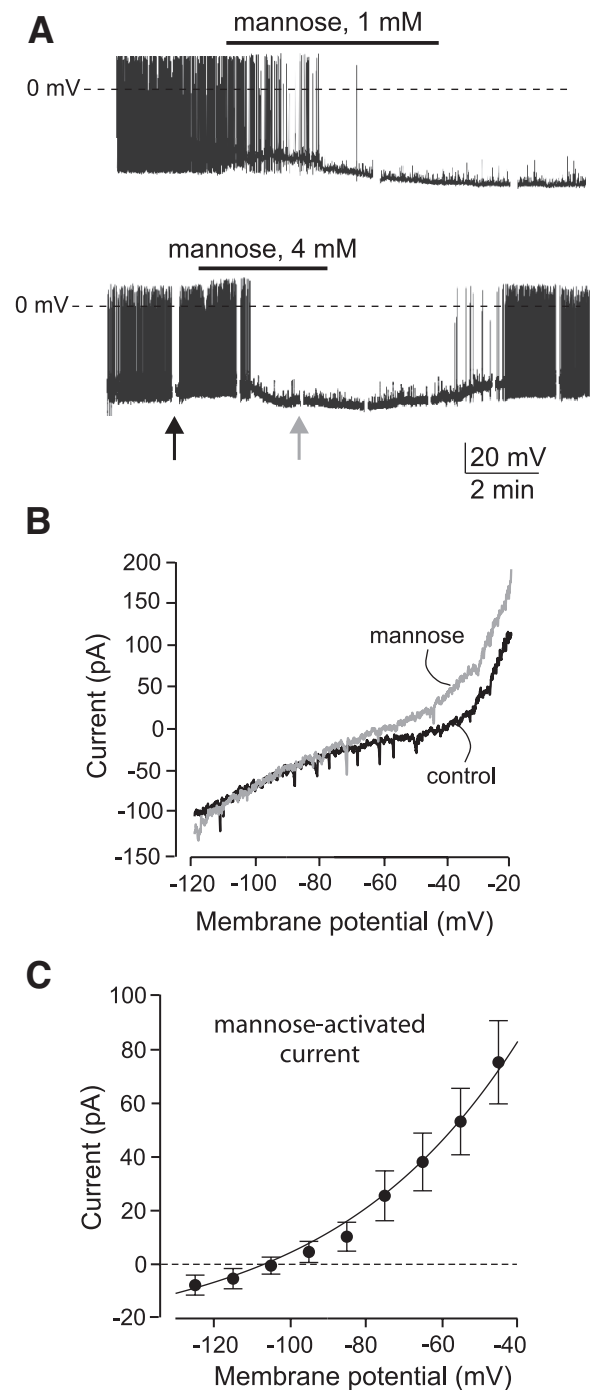

FIG. 7. Effects of mannose on orexin neurons. *A*: Effect of 1 mmol/l mannose on the membrane potential (*top*). Effect of 4 mmol/l mannose on the membrane potential (*bottom*). Arrows show where membrane ramps shown in *B* were taken. *B*: Effect of mannose on membrane current-voltage relationships of the cell shown in A (*bottom*). *C*: Current-voltage relationship of the net current activated by mannose. The line is a fit of the GHK equation to the data (see RESEARCH DESIGN AND METHODS). Values are means ± SE, *n* = 6 cells.

critical for normal cognitive arousal and feeding behavior. First, 2-deoxyglucose, a nonmetabolizable glucose analog causing energy depletion, selectively mimics the inhibitory effects of glucose (Figs. 1–3). Second, stimulating ATP generation with lactate does not reproduce the effects of glucose (Fig. 5). Third, orexin cell glucosensing is not affected by glucokinase inhibitors, which block glucose entry into glycolysis (Fig. 6). Fourth, glucose-induced inhibition of orexin neurons exhibits a unique sugar-selectivity signature: It is caused by D-glucose, mannose (Fig. 7), and 2-deoxyglucose (Fig. 1) but not by L-glucose, galactose, α-methyl-D-glucoside, or fructose (Fig. 8). Together, these data indicate that behaviorally vital neurocircuits of the lateral hypothalamus contain

pharmacologically novel glucose detectors that can operate independently of glucose metabolism.

Metabolic and nonmetabolic modes of brain glucose sensing. To the best of our knowledge, this study is the first direct demonstration of metabolism-independent glucose sensing in glucose-inhibited neurons. Our evidence for this comes from direct electrophysiological measurements of glucosensing in identified orexin cells and is based on three complementary pharmacological observa-
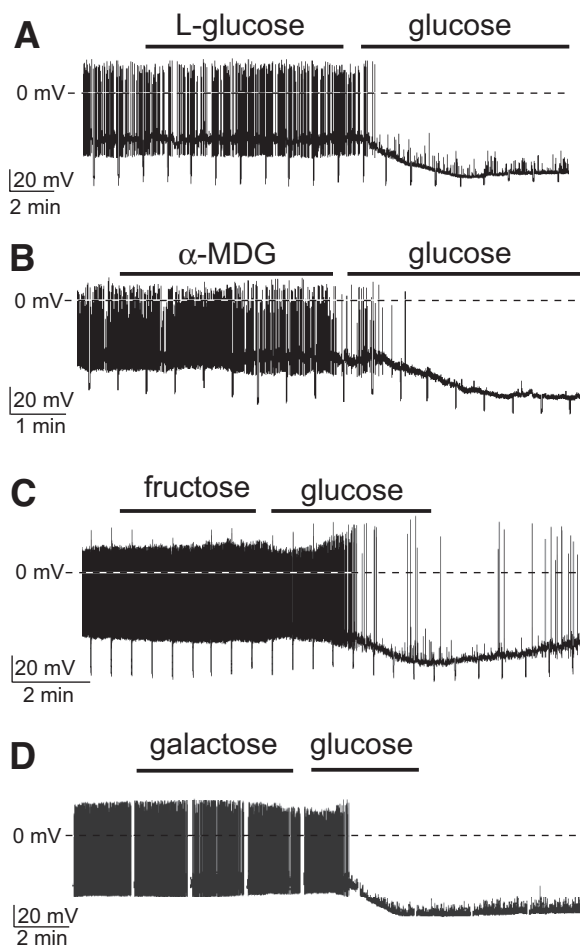
FIG. 8. Sugars that were not effective in hyperpolarizing orexin neurons. *A*: Lack of effect of 5 mmol/l L-glucose on the membrane potential and resistance, followed by normal response to 5 mmol/l glucose. *B*: Lack of effect of 5 mmol/l α-methyl-D-glucoside (α-MDG) on the membrane potential and resistance, followed by normal response to 5 mmol/l glucose. *C*: Lack of effect of 5 mmol/l fructose on the membrane potential and resistance, followed by normal response to 5 mmol/l glucose. *D*: Lack of effect of 5 mmol/l galactose on the membrane potential, followed by normal response to 5 mmol/l glucose.

tions: *1*) specific and reversible stimulation of gluco-sensing responses by a nonmetabolizable glucose analog 2-deoxyglucose (Figs. 1–3); *2*) lack of stimulation of glucosensing responses by neuronal energy fuel lactate (Fig. 5); and *3*) Insensitivity of glucosensing responses to suppression of intracellular glucose metabolism to ATP with glucokinase blockers (Fig. 6). The latter experiment is an important functional complement to previous work that suggested that orexin neurons do not express gluco-kinase mRNA (35), because molecular determination of glucokinase expression is notoriously difficult.

Glucose-inhibited orexin neurons thus appear to oper-ate very differently from classical glucose-excited neurons. In the latter, glucosensing responses are not triggered by 2-deoxyglucose, mimicked by lactate, and abolished by glucokinase inhibitors (36). This clearly indicates that glucose-excited neurons need glucose metabolism for their sugar-sensory activity, and it has been recently theorized that glucose-inhibited neurons follow a similar strategy (13,35). However, for glucose-inhibited neurons, such theories have so far been examined by measuring the effects of reducing glucokinase mRNA expression on cytosolic Ca^{2+} signals in neuronal cultures (13) and by looking at effects of pharmacological glucokinase inhibi-tors and activators on spontaneous Ca^{2+} signals (13,35). How these data relate to the coupling between extracellu-lar glucose and membrane currents in situ is difficult to interpret. On the other hand, direct patch-clamp measure-ments from glucose-inhibited neurons of the ventromedial hypothalamus suggested that these cells share at least some similarities with the orexin neurons analyzed here. For example, like orexin cells (Fig. 5), the ventromedial glucose-inhibited neurons are not inhibited by lactate (37). A comprehensive evaluation of whether energy-independent glucosensing is found widely in glucose-inhibited cells requires the development of specific markers for identifying glucose-inhibited neurons in other brain regions, combined with direct measure-ments of their electrical activity. Currently, our study indicates that, in glucose-inhibited orexin neurons at least, conventional glucose metabolism is not required for sugar sensing.

Substrate selectivity signatures of sugar sensors. Although the physiological importance of orexin neurons and their ability to sense glucose have been recognized for several years, the nature of the glucose detector in orexin cells remains elusive. Our study provides the first phar-macological characterization of this sugar-sensing de-vice by examining the effects of other sugars on orexin cells. For the orexin cell glucosensor, we found activa-tion by D-glucose, 2-deoxyglucose (Fig. 1), and mannose (Fig. 7), but not by fructose, D-galactose, α-methyl-D-glucoside, or L-glucose (Fig. 8). Thus, the glucosensing device in glucose-inhibited orexin cells appears pharma-cologically distinct from that in glucose-excited neu-rons, because the latter is activated by galactose but not by 2-deoxyglucose (36).

The sugar selectivity profile defined here provides im-portant information about the orexin cell glucose detector because known glucose-binding proteins exhibit charac-teristic sugar-selectivity signatures. For example, the glu-cose transporter protein hSGLT3, which acts as an extracellular glucose sensor not requiring transport or metabolism of the sugar for its sensing role, is activated by glucose and α-methyl-D-glucoside but not by galactose, fructose, or mannitol (38). In contrast, both glucose and galactose are active substrates of its relative, SGLT1. Another family of glucose transporters, GLUTs, which can also operate as glucose receptors independently of their ability to transport or metabolize sugars (39), exhibit yet different sugar selectivity. For example, GLUT2, unlike SGLT3, is activated by fructose. The sugar selectivity of the orexin cell glucosensor thus appears distinct from the glucose-binding proteins mentioned above: Unlike hSGLT3, it is not activated by α-methyl-D-glucoside; unlike SGLT1, it is not activated by galactose; and unlike GLUT2, it is not activated by fructose. We are not aware of any glucose-binding proteins that have similar sugar selectivity to the glucose detector in orexin neurons. This provides a valuable criterion for future screening of different glucose-sensing molecules as candidate molec-ular correlates for the orexin cell glucosensor.

Physiological implications. Here, we studied neuro-chemically defined orexin neurons, whose physiological importance in the control of feeding and wakefulness is well established (19) and whose firing can lead to behav-ioral alterations if it changes by as little as 5 Hz (18). Understanding the mechanisms regulating the electrical activity of orexin can thus provide direct insights into

cellular bases of vital behaviors. In higher animals, complex brains produce survival advantages by predicting and then avoiding danger before it becomes disruptive to bodily functions. The dissociation between sensing and use of metabolic fuels may allow the brain to sense glucose levels and to carry out compensatory adjustments before cellular metabolism is disrupted. It is tempting to speculate that the energy-independent glucose sensing described here can occur outside the glucose concentration range where extracellular glucose affects intracellular ATP levels. This would enable metabolism-independent glucose-inhibited neurons to act as "predictive" sensors, and metabolism-dependent glucose-excited neurons to act "emergency alarms" signaling dangerously low glucose levels. Comparisons of glucose sensitivity of lateral hypothalamic neurons suggest that glucose-inhibited neurons may sense glucose in a higher concentration range than glucose-excited neurons (26). Further elucidation of relative roles of glucose-excited and glucose-inhibited neurons are a key subject for future research. In this context, our finding that glucose-inhibited orexin neurons do not sense galactose (Fig. 8D) offers a potential new way to discriminate between physiological effects of activation of glucose-inhibited versus glucose-excited neurons, because the latter do sense galactose (36). For example, a suppression of feeding by glucose but not galactose (e.g., as reported by Kurata et al. [40]) may suggest an involvement of glucose-inhibited neurons. Future in vivo studies guided by the cellular information presented here should provide new insights into physiological and pathological impact of different glucosensing neurocircuits.

ACKNOWLEDGMENTS

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