Differential Effects of Glucose and Lactate on Glucosensing Neurons in the Ventromedial Hypothalamic Nucleus

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Glucose directly alters the action potential frequency of glucosensing neurons in the ventromedial hypothalamic nucleus (VMN). Glucose-excited neurons increase, and glucose-inhibited neurons decrease, their action potential frequency as glucose increases from 0.1 to 2.5 mmol/l. Glucose-excited neurons utilize the ATP-sensitive K^+ channel (K_{ATP} channel) to sense glucose, whereas glucose opens a chloride channel in glucose-inhibited neurons. We tested the hypothesis that lactate, an alternate energy substrate, also regulates the action potential frequency of VMN glucose-excited and -inhibited but not nonglucosensing neurons. As expected, lactate reversed the inhibitory effects of decreased glucose on VMN glucose-excited neurons via closure of the K_{ATP} channel. Although increasing glucose from 2.5 to 5 mmol/l did not affect the activity of glucose-excited neurons, the addition of 0.5 mmol/l lactate or the K_{ATP} channel blocker tolbutamide increased their action potential frequency. In contrast to the glucose-excited neurons, lactate did not reverse the effects of decreased glucose on VMN glucose-inhibited neurons. In fact, it increased their action potential frequency in both low and 2.5 mmol/l glucose. This effect was mediated by both K_{ATP} and chloride channels. Nonglucosensing neurons were not affected by lactate. Thus, glucose and lactate have similar effects on VMN glucose-excited neurons, but they have opposing effects on VMN glucose-inhibited neurons. *Diabetes* 54:15–22, 2005

The ventromedial hypothalamic nucleus (VMN) plays an important role in the central regulation of glucose homeostasis (1). Electrical stimulation of the ventromedial hypothalamus (VMH), which contains the VMN, activates the sympathoadrenal system in a manner similar to that seen during initiation of the counterregulatory response to hypoglycemia (2). Moreover, local VMH glucopenia caused by delivery of the nonmetabolizable glucose analog 2-deoxyglucose into the VMH causes the release of counterregulatory hormones (3). In contrast, glucose infusion into the VMH suppresses their release during systemic hypoglycemia (4). We have described five subtypes of VMN glucosensing neurons that alter their action potential frequency in response to physiological changes in extracellular glucose from 2.5 to 0.1 or 5 mmol/l (5). Of these VMN glucosensing neurons, two subtypes are directly sensitive to decreases in extracellular glucose levels; glucose-excited neurons increase whereas glucose-inhibited neurons decrease their action potential frequency as extracellular glucose increases from 0.1 to 2.5 mmol/l (5). Like pancreatic β-cells, about half of both VMN glucose-excited and -inhibited neurons appear to utilize a special hexokinase known as glucokinase to sense glucose (6). The actual response to glucose is mediated by the ATP-sensitive K^+ (K_{ATP} channel) and a Cl^- channel for glucose-excited and -inhibited neurons, respectively (5).

Lactate may be an alternate energy source in the brain (7–9). Both neurons and astrocytes produce lactate (7). In vivo and in vitro studies suggest that lactate may substitute for glucose under conditions of energy deficit. Brain lactate utilization is elevated during hypoglycemia (10). Local lactate perfusion in the VMH suppressed the counterregulatory response to hypoglycemia (11). High concentrations of both lactate and glucose stimulated VMH glucose-excited neurons identified using extracellular recording (12). Finally, a recent study in humans showed that the brain can use circulating lactate to sustain metabolism during euglycemia (13).

We hypothesize that lactate may regulate the activity of VMN glucosensing neurons under conditions of energy deficit. That is, any energy source that raises the ATP-to-ADP ratio (e.g., lactate and ketones) will alter their activity in a similar fashion as glucose. To test this hypothesis, we investigated the effects of lactate on VMN glucose-excited and -inhibited neurons, in the presence of limiting and steady-state levels of extracellular glucose, using patch clamp recording techniques.

RESEARCH DESIGN AND METHODS

Preparation of brain slices. Male 14- to 21-day-old Sprague-Dawley rats were obtained from colonies at the Veterans Affairs Medical Center in East Orange, New Jersey. Animals were housed with their dams on a 12-h light/dark cycle at 22–23°C and fed a low-fat diet (Purina rat chow no. 5001) and water ad libitum. On the day of experiment, rats were anesthetized with ketamine/

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ACSF, artificial cerebrospinal fluid; CNS, central nervous system; K_{ATP} channel, ATP-sensitive K^+ channel; VMH, ventromedial hypothalamus; VMN, ventromedial hypothalamic nucleus.

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; containing [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 $CaCl_2$, 1.3 mmol/l $MgCl_2$) for the remainder of the day.

Electrophysiology. Viable neurons were visualized and studied under infrared differential-interference contrast microscopy using a Leica DMLFS microscope equipped with a 40× long-working-distance water-immersion objective, as described previously (5). Current clamp recordings (standard whole-cell recording configuration) from neurons in the VMN were performed using a MultiClamp 700A (Axon Instruments, Foster City, CA) and analyzed using pCLAMP 9 software. 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. The junction potential between the bath and the patch pipette was nulled before the formation of a GΩ seal. Membrane potential and action potential frequency were allowed to stabilize for 10–15 min after the formation of a GΩ seal. Neurons whose access resistance exceeded 20 MΩ after this time were rejected. Input resistance was calculated from the change in membrane potential measured during the last 5 ms of a small 500-ms hyperpolarizing pulse (−20 pA) given every 3 s. The membrane response was measured only after membrane potential and action potential frequency stabilized after each treatment. This value was then compared with controls measured immediately before treatment. Action potential frequency (in Hz) was calculated for the last 2 min of each treatment. The reversal potentials for changes in membrane conductance in response to glucose were derived from the voltage response to hyperpolarizing current steps varying from −20 to −120 pA by 20-pA increments. The time delay for the responses to glucose and lactate were defined subjectively as the time after solution change at which a progressive change in either action potential frequency or membrane potential was first observed. Extracellular glucose levels were altered and chemicals added to the ACSF as described in the figures. All chemicals were obtained from Sigma (St. Louis, MO).

Statistical analysis. All data were expressed as the means ± SE. Statistical analysis was performed using Student's t test. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of lactate on VMN glucose-excited neurons. VMN glucosensing neurons were characterized by their response to changes in extracellular glucose from 2.5 to 0.5 or 5 mmol/l, as described previously (5). This study focuses exclusively on glucose-excited and -inhibited and nonglucosensing neurons. VMN glucose-excited neurons reversibly hyperpolarize and decrease their action potential frequency when extracellular glucose levels were decreased from 2.5 to 0.1 mmol/l (Fig. 1*A*), with a concomitant decrease in input resistance as a result of a K_{ATP} channel opening (5). We show here that the glucose concentration-response relationship for input resistance in VMN glucose-excited neurons is well fit by an equation for a rectangular hyperbole, with the steepest slope <0.5 mmol/l and reaching a plateau at ~2.5 mmol/l ($r^2 = 0.99$) (Fig. 1*B*). Increasing glucose from 2.5 to 5 mmol/l did not increase action potential frequency (Fig. 2*A*), nor was input resistance altered ($n = 3$, $P = 0.75$) (Fig. 2*B*). As predicted, lactate (0.5 mmol/l L$^+$-lactic acid) reversed the effects of decreasing extracellular glucose on membrane potential, action potential frequency, and input resistance of VMN glucose-excited neurons ($n = 5$) (Fig. 1*A*). The reversal potential of the lactate response in 0.1 or 0.5 mmol/l glucose was −81 ± 7.0 mV ($n = 3$) (Fig. 1*C*), which is near the theoretical equilibrium potential for K^+ (E_K = −99 mV) in our solutions. Increased action potential frequency after the addition of lactate to 0.1 or 0.5 mmol/l glucose was observed after 1.63 ± 0.68 min ($n = 5$). This was significantly faster than that observed for the decrease in action potential frequency in response to decreased glucose (4.37 ± 0.33 min, $n = 7$, $P = 0.0026$). The increased action potential frequency in response to lactate also tended to occur faster than the increase when glucose levels were restored to 2.5 mmol/l (4.31 ± 1.18 min, $n = 7$); however, this did not reach statistical significance ($P = 0.11$). The K_{ATP} channel opener diazoxide (100 μmol/l) reversed the effects of lactate (Fig. 2*C*). Thus, lactate appears to close the K_{ATP} channel on VMN glucose-excited neurons. The effects of lactate on VMN glucose-excited neurons are presumed to be direct because it reversed the activation of a known postsynaptic K_{ATP} channel occurring in response to reduced glucose. Although VMN glucose-excited neurons did not respond to an increase in extracellular glucose from 2.5 to 5 mmol/l, the addition of 0.5 mmol/l lactate to 2.5 mmol/l glucose increased the action potential frequency (Fig. 2*A*) and input resistance ($n = 5$, $P = 0.04$) (Fig. 2*B*). Moreover, 100 μmol/l tolbutamide, a K_{ATP} channel blocker, also increased action potential frequency of VMN glucose-excited neurons in 2.5 mmol/l glucose ($n = 2$) (Fig. 2*A*).

Effects of lactate on VMN glucose-inhibited neurons. VMN glucose-inhibited neurons, which are generally quiescent in 2.5 mmol/l glucose, reversibly depolarize, increase input resistance, and show spontaneous action potentials when extracellular glucose levels decrease to 0.1 mmol/l (Fig. 3*A*). We have shown previously that the increased input resistance in response to decreased glucose results from closure of a Cl^- channel (5). The glucose concentration-response relationship for input resistance in VMN glucose-inhibited neurons decreases exponentially between 0.1 and 1 mmol/l, with an apparent plateau at 2.5 mmol/l glucose ($r^2 = 0.90$) (Fig. 3*B*). In contrast to our findings in glucose-excited neurons, 0.5 mmol/l lactate did not reverse the excitatory effects of decreased glucose in VMN glucose-inhibited neurons. In fact, 0.5 mmol/l lactate further increased action potential frequency in both 0.1 and 0.5 mmol/l ($n = 13$, $P = 0.016$) (Figs. 3*A* and 6) as well as initiating action potentials in 2.5 mmol/l glucose ($n = 5$) (Fig. 4). Lactate did not reverse the increased input resistance associated with decreasing glucose to 0.1 or 0.5 mmol/l, and, in fact, it significantly increased input resistance in the presence of 2.5 mmol/l glucose ($n = 5$, $P = 0.02$). There was no significant difference between the percent increase in action potential frequency or input resistance in response to 0.5 ($n = 13$) or 5 ($n = 3$) mmol/l lactate in the presence of low (0.1 or 0.5 mmol/l) glucose ($P = 0.44$ and 0.53, respectively). Action potential frequency increased after the addition of lactate to 2.5 mmol/l glucose after 1.89 ± 0.27 min ($n = 11$). This was significantly faster than the increase in action potential frequency observed when glucose levels were reduced to 0.1 or 0.5 mmol/l (3.54 ± 0.27 min, $n = 13$, $P = 0.0002$). The excitatory effects of lactate persisted in high-Mg^{2+} (3.1 mmol/l) and low-Ca^{2+} (0.3 mmol/l) ACSF, which blocks

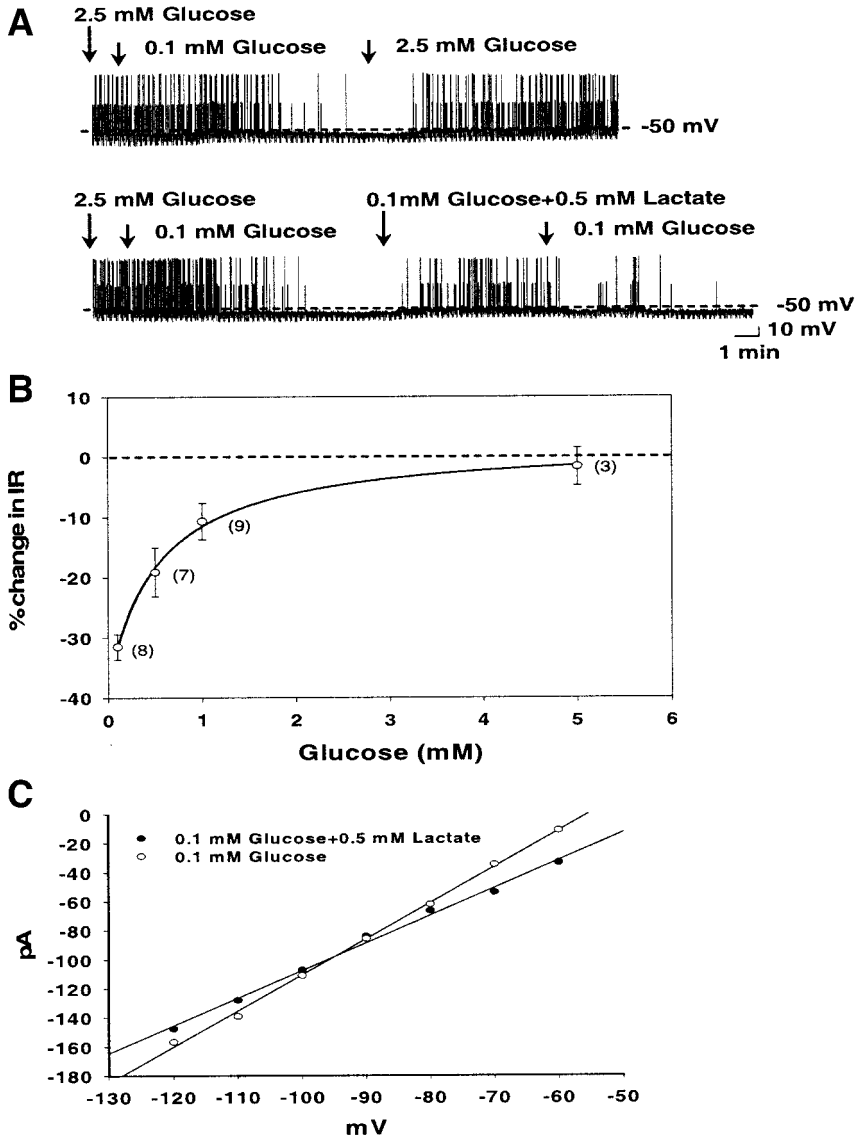

FIG. 1. ***A*: Consecutive whole-cell current clamp recordings of spontaneous electrical activity in a VMN glucose-excited neuron. Resting membrane potential is noted to the right of each trace in this and subsequent figures. Input resistance (IR) was calculated from the downward deflections (as described in the RESEARCH DESIGN AND METHODS). Upward deflections represent action potentials. Membrane potential, action potential frequency, and input resistance reversibly decreased when glucose levels decreased from 2.5 to 0.1 mmol/l (*upper trace*). Lactate (0.5 mmol/l) reversed the effects of decreased glucose ($n = 3$, *bottom trace*). *B*: Glucose concentration-response relationship for VMN glucose-excited neurons. Values are expressed as percent change in input resistance in each concentration of glucose vs. 2.5 mmol/l glucose. Data points represent the means ± SE; n values are in parentheses. The dashed line represents 0% change from 2.5 mmol/l glucose. The data fit the equation for a rectangular hyperbole: percent change = $\{[E_{max} \times (\text{glucose})]/[EC_{50} + (\text{glucose})]\} + i$, where i is a constant that does not force the rectangular hyperbole to pass through the origin (30). $E_{max} = 40.6$, $i = -38.2$, and $EC_{50} = 0.52$ ($r^2 = 0.99$). *C*: Voltage-current relations indicate that the lactate sensitive conductance in 0.1 mmol/l glucose reverses at −95 mV for this glucose-excited neuron.**

presynaptic transmission ($n = 3$) (Fig. 4*B*). Interestingly, the reversal potential for the response to lactate was significantly different in 0.1 or 0.5 mmol/l vs. 2.5 mmol/l glucose (−72.4 ± 7.2 mV, $n = 5$, and −97.0 ± 2.0 mV, $n = 3$, respectively; $P = 0.04$) (Fig. 5). The former is between E_{Cl} (−55 mV) and E_K, whereas the latter is very near E_K. Finally, the lactate-induced increase in action potential frequency in the presence of 0.5 mmol/l glucose was significantly reduced by 100 μmol/l diazoxide, a K_{ATP} channel opener ($n = 6$, $P = 0.031$) (Fig. 6).

Effects of lactate on VMN nonglucosensing neurons. VMN nonglucosensing neurons did not change membrane potential, action potential frequency, or input resistance in response to changes in extracellular glucose from 2.5 to 0.1 or 0.5 mmol/l (Fig. 7), nor did they respond to lactate in either low (0.1 or 0.5 mmol/l, $n = 6$, $P = 0.64$ [action potential frequency]) or steady-state glucose (2.5 mmol/l, $n = 7$, $P = 0.43$ [action potential frequency]) (Fig. 7).

DISCUSSION

Nutrient utilization in the central nervous system (CNS) is more complex than originally believed. The traditional view supported the primacy of glucose as the preferred neuronal fuel under normal conditions, whereas ketones, produced by fatty acid oxidation, substitute for glucose under pathological conditions (e.g., hypoglycemia) (14). This view has been challenged recently, and lactate has emerged as an alternative CNS energy source during times of energy deficit. For example, lactate is an obligatory energy substrate for recovery of neuronal function after hypoxia-ischemia (15). Insulin-induced hypoglycemia increases lactate utilization in the brain (10), and local perfusion of lactate into the VMN suppresses the counterregulatory response to hypoglycemia (11). In fact, the astrocyte-neuron lactate shuttle hypothesis suggests that neurons may actually prefer glial-derived lactate to glucose as a fuel for neuronal activity (7,16). According to the hypothesis, neuronal activity increases extracellular glutamate, which stimulates glial anaerobic glycolysis and converts glucose to lactate. Lactate is transported out of glia by monocarboxylate transporter 1 and into neurons by monocarboxylate transporter 2, where it is metabolized to pyruvate via lactate dehydrogenase-1. This pyruvate then enters the neuronal tricarboxylic acid cycle to generate

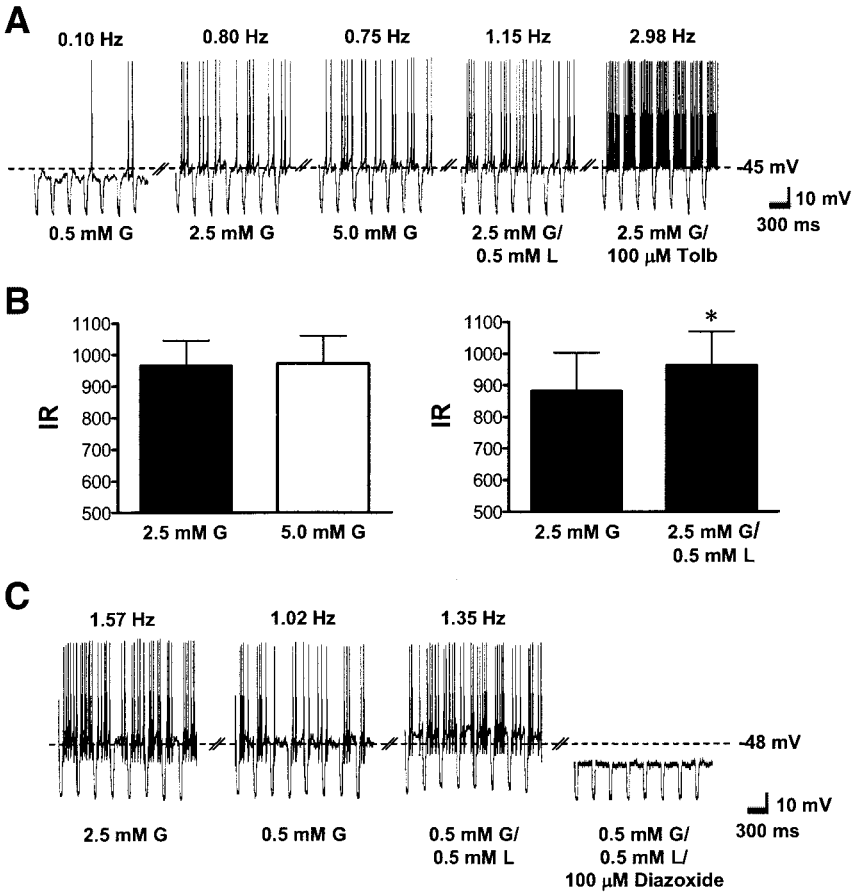

FIG. 2. *A* and *C*: Whole-cell current clamp recordings in VMN glucose-excited neurons. *A*: Increasing glucose (G) from 2.5 to 5 mmol/l did not alter the action potential frequency. Lactate (L) and tolbutamide (Tolb; 100 μmol/l) in 2.5 mmol/l glucose increased action potential frequency. *B*: Input resistance (IR) did not differ in 2.5 and 5 mmol/l glucose. Lactate in 2.5 mmol/l glucose significantly increased input resistance (*right*). *C*: Diazoxide (100 μmol/l) reversed the lactate-induced increase in membrane potential, action potential frequency, and input resistance in 0.5 mmol/l glucose.

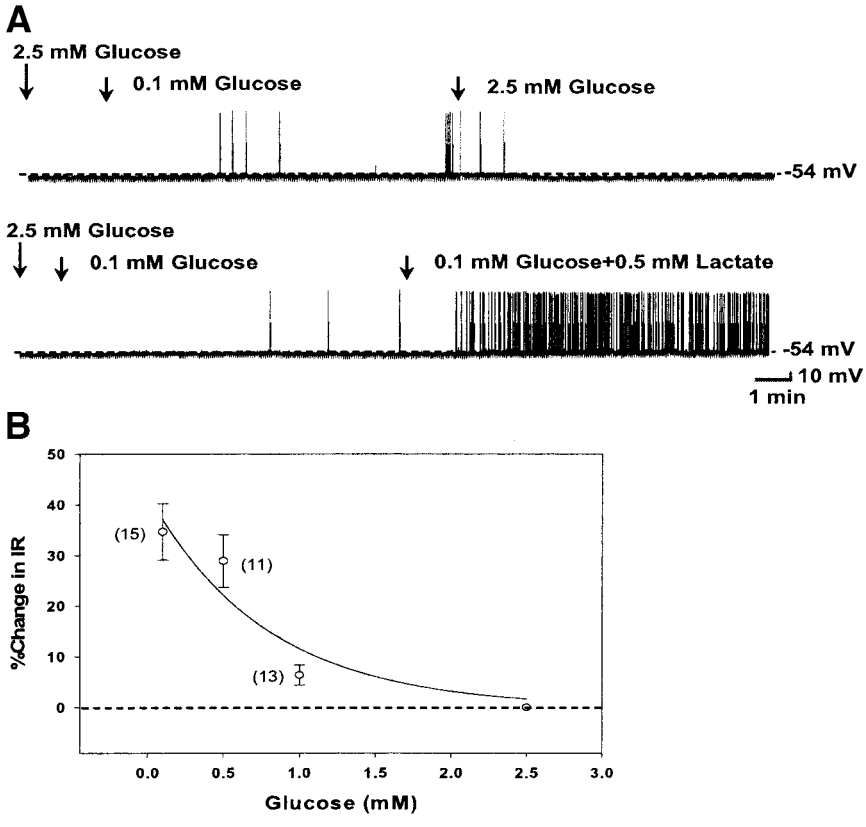

FIG. 3. *A*: Consecutive whole-cell current clamp recordings in a VMN glucose-inhibited neuron. Membrane potential, action potential frequency, and input resistance (IR) reversibly increased when glucose levels decreased from 2.5 to 0.1 mmol/l. (*upper trace*). Lactate further increased membrane potential, action potential frequency, and input resistance in the presence of 0.5 mmol/l glucose (*bottom trace*). *B*: Glucose concentration-response relationship for VMN glucose-inhibited neurons. The data were initially transformed by adding an integer to give all positive values, as described by Winer (38). The data fit the equation for exponential decay: $A_c = A_0 \times e^{-k[\text{glucose}]}$ with $A_0 = 42.1$ and $k = 1.29$, where A_c = IR at any concentration of glucose, A_o = IR, e = exponential, and k = decay constant.

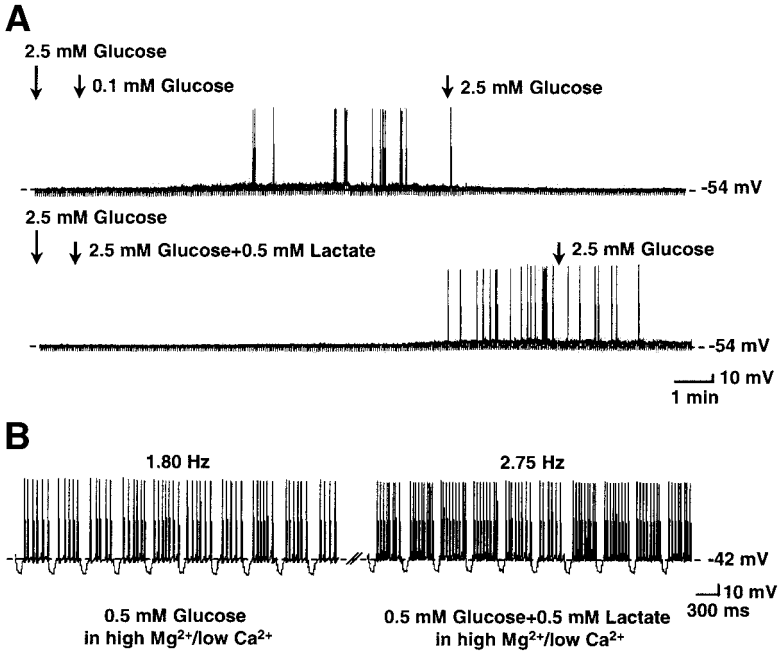

FIG. 4. Consecutive whole-cell current clamp recordings in a VMN glucose-inhibited neuron. *A*: Lactate significantly increased membrane potential, action potential frequency, and input resistance in 2.5 mmol/l glucose (*bottom trace*). *B*: The response to lactate persisted in high Mg^{2+}/low Ca^{2+} ACSF, which abolishes presynaptic transmission.

ATP (17). In support of this hypothesis, a recent study showed that the human brain preferentially uses circulating lactate to sustain metabolism even at a normal glucose concentration (13). Finally, there may also be a role for fatty acids as neuronal fuels (18).

Thus, the brain, like the periphery, utilizes a wide variety of fuels. However, unlike the periphery, the brain uses these fuels not only to sustain cellular function, but also as signals of central and peripheral energy balance. By sensing and integrating these signals, the brain is able to maintain whole-body energy homeostasis through regulation of the autonomic nervous system. For this reason there must be a link between CNS nutrient status and neuronal activity. The existence of neurons that change their action potential frequency as extracellular glucose levels change has been known for many years (19). We have described five subtypes of these glucosensing neurons that respond to physiologically relevant changes in extracellular glucose. Two of these, glucose-excited and -inhibited neurons, directly change their action potential frequency as extracellular glucose levels increase from 0.1 to 2.5 mmol/l. The remaining three are presynaptically modulated by changes in extracellular glucose (5). If VMN glucosensing neurons play a role in sensing overall CNS fuel status and maintaining whole-body energy homeostasis, then it is logical to hypothesize that glucose is not the only CNS fuel that regulates their activity. To test this hypothesis, we investigated the effects of lactate on VMN glucose-excited and -inhibited neurons. Our expectations were that lactate would reverse the effects of decreased glucose. Furthermore, we expected that lactate (or any CNS fuel that increases the ATP-to-ADP ratio) would have an effect similar to that of increased glucose on the regulation of VMN glucosensing neurons.

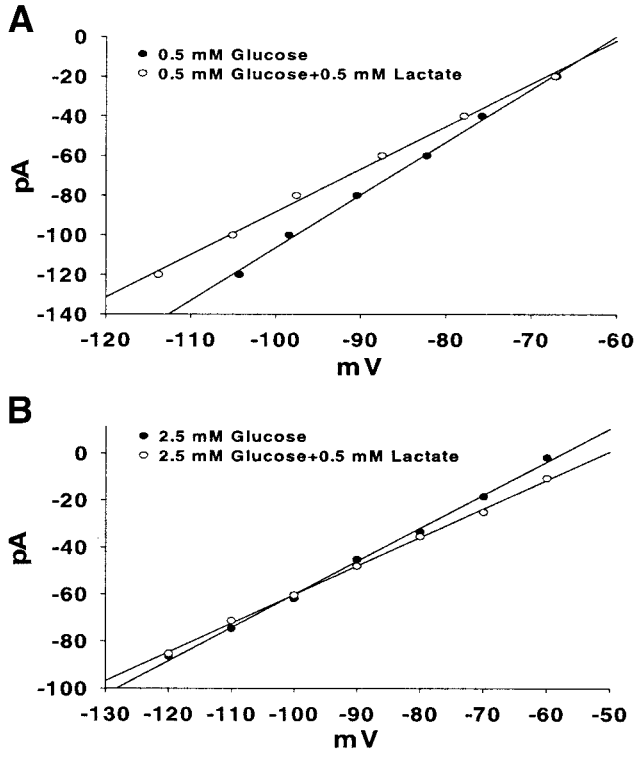

FIG. 5. *A*: Voltage-current relations indicate that the lactate-sensitive conductance in 0.5 mmol/l glucose for this VMN glucose-inhibited neuron reverses at −65 mV. *B*: The lactate-sensitive conductance in 2.5 mmol/l glucose for this VMN glucose-inhibited neuron reverses at −100 mV.

Although the present studies support our hypothesis that VMN glucose-excited and -inhibited neurons respond to other CNS fuels, the results were not always as expected. For VMN glucose-excited neurons, the addition of 0.5 mmol/l lactate did indeed reverse the inhibitory effects of decreased glucose. This level of lactate was chosen based on microdialysis measurements indicating that the brain lactate is ~0.3–1.1 mmol/l in the rat (20–22). A value at the low end of this range was used because VMN neurons in brain slice preparations are likely being provided with some glial-derived lactate. The excitatory response of glucose-excited neurons to lactate in the presence of low glucose reversed at E_K, and it was

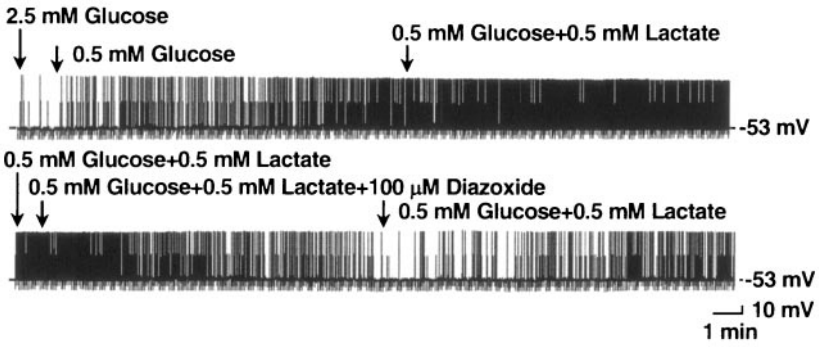

FIG. 6. Consecutive whole-cell current clamp recordings in a VMN glucose-inhibited neuron. Diazoxide reversed the effect of lactate in 0.5 mmol/l glucose.

reversed by the K_{ATP} channel opener diazoxide. Thus, lactate reverses the inhibition of VMN glucose-excited neurons induced by the reduction of glucose, and it does so through reversal of the resulting K_{ATP} channel activation.

The observation that lactate caused a further excitation of VMN glucose-excited neurons in the presence of 2.5 mmol/l glucose was surprising. The concentration-response relation for VMN glucose-excited neurons shows that they are not sensitive to changes in extracellular glucose >2.5 mmol/l (Fig. 1*B*). This is in contrast to our recent data in glucose-excited neurons in the hypothalamic arcuate nucleus (23). In these glucose-excited neurons, whereas the response to increased glucose plateaus at >2.5 mmol/l, there is still a significant increase in action potential frequency and input resistance with a corresponding decrease in K_{ATP} channel currents as glucose levels are increased to 10 mmol/l. There are several possible explanations for this discrepancy between glucose-excited neurons located in the VMN and arcuate nucleus. First, the proximity of the arcuate nucleus to the median eminence, where the blood-brain barrier is leaky, may result in arcuate nucleus glucose-excited neurons being exposed to higher levels of glucose than those that occur in other brain regions (24). Thus, they may have evolved mechanisms for sensing higher glucose levels. Furthermore, current evidence indicates that the arcuate nucleus is associated with the regulation of food intake and energy balance (25–27). In contrast, the VMN appears to be associated with the generation of the counterregulatory response to hypoglycemia (4). Thus, arcuate nucleus and VMN glucose-excited neurons may play different roles in the regulation of glucose homeostasis. Our current data showing that VMN glucose-excited neurons are exquisitely sensitive to decreases in glucose between 0.1 and 0.5 mmol/l (analogous to brain glucose levels seen during the initiation of the counterregulatory response to hypoglycemia) (14,28), whereas the sensitivity of arcuate nucleus glucose-excited neurons is shifted to the right, are consistent with this hypothesis.

Interestingly, lactate appears to be a more potent stimulus of VMN glucose-excited neurons than glucose itself. Tolbutamide, in the presence of 2.5 mmol/l glucose, increases action potential frequency and input resistance, suggesting that the K_{ATP} channel is not completely closed in 2.5 mmol/l glucose. The addition of lactate to 2.5 mmol/l glucose also increases action potential frequency and input resistance, though not as strongly as tolbutamide. This suggests that further closure of the K_{ATP} channel resulting in increased action potential frequency in VMN glucose-excited neurons requires simultaneous elevations in both glucose and lactate. Alternatively, more than one subtype of K_{ATP} channel may exist on glucose-excited neurons that have preferential sensitivity for the metabolic byproducts of glucose or lactate. Thus, there is an additive interaction between the effects of glucose and lactate on the K_{ATP} channel, and therefore the action potential frequency, of VMN glucose-excited neurons.

Lactate also regulates the activity of VMN glucose-inhibited neurons. These results were even more surprising than those for the glucose-excited neurons. That is, whereas glucose inhibits VMN glucose-inhibited neurons, lactate increases their action potential frequency in both low and steady-state glucose. This effect appears to be mediated by both K^+ and Cl^- channels. In low glucose the response to lactate reverses between E_K and E_{Cl}. In contrast, in 2.5 mmol/l glucose, the lactate response reverses at E_K. Because the effect of lactate on glucose-inhibited neurons is reversed by diazoxide, we speculate that lactate is regulating a K_{ATP} channel on both glucose-excited and glucose-inhibited neurons. One hypothesis explaining these results is presented as follows based on the assumption that the K_{ATP} channel subtype on glucose-

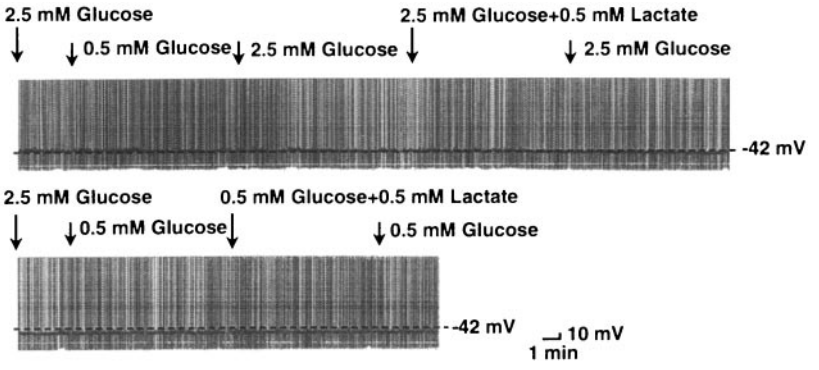

FIG. 7. Consecutive whole cell current clamp recordings in a VMN nonglucosensing neuron. Neither lactate nor glucose altered membrane potentiation, action potential frequency, or input resistance in nonglucosensing neurons.

inhibited neurons is responsive to lactate metabolism only, while the Cl^- channel is modulated by the metabolic byproducts of both lactate and glucose. In low glucose, the Cl^- channel will be mostly closed, whereas the K_{ATP} channel will be open. The addition of lactate in low glucose will close the K_{ATP} channel and open the Cl^- channel, hence the mixed reversal potential. However, in 2.5 mmol/l glucose this subtype of K_{ATP} channel is still open, as is the Cl^- channel. The Cl^- channel cannot be opened further by the addition of lactate; however, the K_{ATP} channel will close. Thus, the reversal potential will be at E_K. These data indicate that there is an inverse interaction between glucose and lactate on VMN glucose-inhibited neurons. Interestingly, the excitatory effect of lactate on glucose-inhibited neurons did not vary with lactate concentration. This may be because glucose-inhibited neurons are maximally stimulated by 0.5 mmol/l lactate. Alternatively, the lactate concentration–response relationship may be bell shaped. Thus, detailed concentration-response relationships for lactate on VMN glucose-excited and -inhibited neurons will be the focus of future studies.

The observation that glucose and lactate regulate VMN glucosensing neurons via distinct mechanisms suggests that the end points and/or byproducts of their metabolism mean very different things in terms of intracellular signaling. One explanation may involve the anaplerotic entry of pyruvate into the tricarboxylic acid cycle. That is, because of the high affinity of lactate dehydrogenase-1 for lactate (29), an increase in extracellular lactate concentration will drive the lactate dehydrogenase reaction from lactate to pyruvate. This would presumably result in a higher concentration of intracellular pyruvate than that produced by the lower-capacity glycolytic enzymes (30). Our observation that VMN glucosensing neurons appear to respond more quickly to lactate than to glucose is consistent with this hypothesis. Pyruvate can be converted to acetyl CoA by pyruvate dehydrogenase, which results in ATP production via oxidative phosphorylation. It can also be converted to oxaloacetate by pyruvate carboxylase (anaplerotic pathway), and ultimately back to pyruvate, via the pyruvate-malate or -citrate cycles (31,32). NADPH is a byproduct of either pyruvate cycle (33), and it may be involved in K_{ATP} channel–independent glucose-stimulated insulin secretion in pancreatic β-cells (34). Malonyl CoA is an important byproduct of the pyruvate-citrate cycle because it inhibits fatty acid oxidation (35). Thus, it is a potential site of interaction between glucose, lactate, and fatty acid signaling in neurons. Finally, the increased production of NADH when lactate is converted to pyruvate increases the cytosolic NADH-to-NAD^+ ratio, which has a variety of intracellular consequences, including alteration of intracellular pH and inhibition of glycolysis (36,37).

In conclusion, the activity of VMN glucose-excited and -inhibited neurons, but not nonglucosensing neurons, was altered by the addition of lactate to either low or steady-state glucose. This supports our hypothesis that VMN glucosensing neurons are regulated by CNS fuels other than glucose. Furthermore, glucose and lactate exert similar effects on glucose-excited neurons but opposite effects on glucose-inhibited neurons. These data lead us to hypothesize that the activity of VMN glucosensing neurons varies as a function of the overall status of intracellular metabolism. We further hypothesize that their sensitivity to peripheral signals of energy homeostasis, as well as to feeding-relevant peptides, is dependent on their metabolic state at any given time. Thus, it is necessary to fully characterize the effects of CNS fuels including glucose, lactate, and fatty acids on glucosensing neurons, singly and in combination, to determine the role of glucosensing neurons in the maintenance of glucose and energy homeostasis.

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