Recurrent hypoglycemia reduces the glucose sensitivity of glucose-inhibited neurons in the ventromedial hypothalamus nucleus

Zhentao Song and Vanessa H. Routh

Department of Pharmacology and Physiology, New Jersey Medical School, Newark, New Jersey

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Song, Zhentao, and Vanessa H. Routh. Recurrent hypoglycemia reduces the glucose sensitivity of glucose-inhibited neurons in the ventromedial hypothalamus nucleus. Am J Physiol Regul Integr Comp Physiol 291: R1283–R1287, 2006. First published June 22, 2006; doi:10.1152/ajpregu.00148.2006.—Recurrent hypoglycemia blunts the brain’s ability to sense and respond to subsequent hypoglycemic episodes. Glucose-sensing neurons in the ventromedial hypothalamic nucleus (VMN) are well situated to play a role in hypoglycemia detection. VMN glucose-inhibited (GI) neurons, which decrease their firing rate as extracellular glucose increases, are extremely sensitive to decreased extracellular glucose. We hypothesize that recurrent hypoglycemia decreases the glucose sensitivity of VMN GI neurons. To test our hypothesis, 14- to 21-day-old Sprague-Dawley rats were subcutaneously injected with regular human insulin (4 U/kg) or saline (control) for three consecutive days. Blood glucose levels 1 h after insulin injection on day 3 were significantly lower than on day 1, reflecting an impaired ability to counteract hypoglycemia. On day 4, the glucose sensitivity of VMN GI neurons was measured using conventional whole cell current-clamp recording. After recurrent insulin-induced hypoglycemia, VMN GI neurons only responded to a glucose decrease from 2.5 to 0.1, but not 0.5, mM. Additionally, lactate supplementation also decreased glucose sensitivity of VMN GI neurons. Thus our findings suggest that decreases in glucose sensitivity of VMN GI neurons may contribute to the impairments in central glucose-sensing mechanisms after recurrent hypoglycemia.

P.O. Box 1709, Newark, NJ 07101–1709 (e-mail: routhvh@umdnj.edu).

Hypoglycemia is a major side effect of intensive insulin therapy in diabetic patients. Recurrent hypoglycemia impairs the sympathetic and neuroendocrine counterregulatory responses (CRR) to hypoglycemia that normally restore euglycemia. This results from a decreased ability of the brain to sense hypoglycemia (5). Severe hypoglycemia has potentially devastating effects on the brain, including impairment of memory and cognitive function (19). This is especially dangerous for the developing brain (13). However, the mechanisms by which the brain senses impending hypoglycemia and signals for the initiation of the CRR are unclear.

Local glycoproteins in the ventromedial hypothalamus (VMH), caused by the nonmetabolizable glucose analog, 2-deoxyglucose, triggers the release of the counterregulatory hormones, epinephrine, and glucagon (2). Conversely, VMH glucose infusion suppresses their release during systemic hypoglycemia (3). The ventromedial hypothalamic nucleus (VMN) within the VMH contains glucose-sensing neurons, which are exquisitely sensitive to decreases in extracellular glucose (16, 17). Thus VMN glucose-sensing neurons are well situated to play a key role in the detection of hypoglycemia and initiation of the CRR. Thus we hypothesize that impaired central glucose sensitivity after recurrent hypoglycemia is associated with reduced glucose sensitivity of VMN glucose sensing neurons.

Other fuel sources (e.g., lactate and ketone bodies) also support energy metabolism during systemic hypoglycemia (8, 18). For example, VMH lactate infusion suppresses the CRR during systemic hypoglycemia (4). Lactate regulates the activity of VMN glucose-sensing neurons (17). Furthermore, if VMN glucose-sensing neurons play a key role in hypoglycemia detection and initiation of the CRR, then their glucose sensitivity should be impaired in other situations in which the CRR to hypoglycemia is suppressed. Thus we also hypothesize that lactate supplementation will decrease the glucose sensitivity of VMN glucose-sensing neurons.

METHODS

Induction of hypoglycemia. Male 14- to 21-day-old Sprague-Dawley rats were obtained from colonies at the New Jersey Medical School in Newark, NJ. Animals were housed with their dams on a 12:12-h light-dark cycle at 22–23°C and fed with low-fat diet (Purina rat chow #5001) and water ad libitum. At 9:00 AM, rats were subcutaneously injected with regular human insulin (4 U/kg, Eli Lilly, Indianapolis, IN) or saline (1 ml/kg) for 3 consecutive days. Pups were returned to their mothers after injection. Blood glucose was measured via tail blood with a blood glucose meter (OneTouch Ultra, LifeScan, Milpitas, CA) hourly, for 6 h postinjection in a group of animals not used for electrophysiological study.

Preparation of brain slices. On the fourth day, a separate group of rats were anesthetized with ketamine/xylazine and transcardially perfused with ice-cold oxygenated (95% O2/5% CO2) perfusion solution composed of the following (mM): 2.5 KCl, 1.25 NaH2PO4, 28 NaHCO3, 0.5 CaCl2, 7 glucose, 1 ascorbate, 3 pyruvate; osmolality 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, Camden, UK). The brain slices were maintained at 34°C in oxygenated artificial cerebrospinal fluid (aCSF; in mM: 126 NaCl, 1.9 KCl, 1.2 KH2PO4, 26 NaHCO3, 10 glucose, 1.3 MgCl2, and 2.4 CaCl2; osmolality was adjusted to ~300 mOsm with sucrose; pH 7.4) for 15 min and allowed to come to room temperature. They were then transferred to normal oxygenated aCSF (2.5 mM glucose) 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. Current clamp recordings (standard whole cell recording configuration) from neurons in the VMN were made using a MultiClamp 700A and analyzed using pCLAMP 9 software. During recording, brain slices were perfused at 10 ml/min.
Table 1. Blood glucose levels in 14- to 21-day-old Sprague-Dawley rats after subcutaneous injections of (4U/kg insulin) or saline injections

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Blood Glucose, mM</th>
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<tr>
<td></td>
<td>Day 1</td>
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<tr>
<td>Insulin</td>
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<tr>
<td>0</td>
<td>8.5±0.3</td>
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<tr>
<td>1</td>
<td>2.3±0.2</td>
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<tr>
<td>2</td>
<td>4.4±0.6</td>
</tr>
<tr>
<td>3</td>
<td>9.4±0.8</td>
</tr>
<tr>
<td>6</td>
<td>8.7±0.3</td>
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<tr>
<td>Saline</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.6±0.2</td>
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<tr>
<td>1</td>
<td>9.0±0.2</td>
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<td>2</td>
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Values are expressed as means ± SE; n = 10 for both groups. Glucose levels reached their nadir 1 h postsinsulin injection and recovered to control values by 3 h. On day 3, blood glucose levels at 1 h in the insulin-treated rats were significantly lower than on days 1 and 2 (P < 0.05). There was no significant effect of saline injections on blood glucose levels.

with normal oxygenated aCSF. 1- to 3-MΩ electrodes were filled with an intracellular solution containing (in mM): 128 K-gluconate, 10 KCl, 4 KOH, 10 HEPES, 4 MgCl₂, 0.5 CaCl₂, 5 EGTA, and 2 Na₂ATP; pH 7.2. Osmolarity was adjusted to 290–300 mOsm with sucrose. Input resistance (IR) was calculated from the change in membrane potential measured during the last 1 min of a small 500-ms hyperpolarizing pulse (−20 pA) given every 3 s. Extracellular glucose levels were altered as described in the figures. Each figure shows data from consecutive current-clamp recordings from VMN neurons in brain slices using conventional whole cell patch clamp.

Changes in glucose sensitivity were quantitated using the percent change in IR. This is because action potential frequency (APF) can vary between brain slices due to variation in presynaptic inputs, which remain intact as a result of the exact location of the slice. However, we have found that changes in IR in response to glucose are extremely consistent between slices, as well as animals (17, 21).

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

RESULTS

Fourteen- to twenty-one-day-old Sprague-Dawley rats were subcutaneously injected with regular human insulin (4 U/kg) or saline (control) for 3 consecutive days. As shown in Table 1, blood glucose levels 1 h after insulin injection on day 3 were significantly lower than on day 1 (1.4±0.1 vs. 2.3±0.2 mM, respectively, n = 10 rats/group; P < 0.05), reflecting an impaired ability to counteract hypoglycemia. Blood glucose levels gradually recovered to normal levels 3 h after insulin injection. Saline injection had no effect on blood glucose levels at any of the times measured. These data indicate that the ability to counteract hypoglycemia was selectively impaired in the insulin-treated animals and was not a result of injection stress.

On day 4, brain slices (350 μM) through the VMN from 38 saline-treated and 19 insulin-treated rats were perfused with aCSF and evaluated for glucose sensitivity. Using conventional...
whole cell current-clamp recording, we have previously established that VMN GI neurons increase their APF and IR in response to physiological decreases in extracellular glucose levels (17). Thus GI neurons were initially defined in saline- and insulin-treated rats by an increase in APF and IR as glucose decreased from 2.5 to 0.1 mM. To be certain that this initial exposure of the brain slice to 0.1 mM glucose did not alter subsequent glucose sensitivity, a subset of neurons in saline-treated rats was exposed to a second decrease in glucose from 2.5 to 0.1 mM. There was no significant difference in the percent increase in IR between the two 0.1 mM glucose challenges (1st exposure: 53 ± 11%; 2nd exposure: 52 ± 9%; n = 8; P = 0.79, Students paired t-test).

Similar percentages of GI neurons were observed in the saline- (15 out of 62 recorded neurons, 23%) vs. insulin- (6 out of 26 recorded neurons, 24%) treated animals. There were no significant differences between membrane potential (MP) and IR in 2.5 mM glucose for the saline (MP: −54 ± 2 mV; IR: 556 ± 39 nA; n = 15) and insulin-treated rats (MP: −47 ± 4 mV; IR: 557 ± 121 nA; n = 6). VMN GI neurons from saline-injected rats reversibly increased APF, IR, and MP as extracellular glucose decreased (Fig. 1A). IR and MP increased significantly more as glucose levels decreased from 2.5 to 0.1 mM (55.6 ± 6.2%, n = 15, 17.0 ± 1.7%, n = 15, respectively) than from 2.5 to 0.5 mM (24.1 ± 8.0%, n = 9, P < 0.05; 7.9 ± 2.4%, respectively, n = 9, P < 0.05). In contrast, after recurrent hypoglycemia VMN GI neurons failed to respond to a decrease in extracellular glucose from 2.5 to 0.5 mM (%change in IR: −0.7 ± 1.7%, n = 6 vs. saline: 24.1 ± 7.9%, n = 9; P < 0.001; Figs. 1B and 2A). However, IR increased to a similar degree in insulin-treated and control rats, as glucose decreased to 0.1 mM (49.4 ± 20.5%, n = 6; vs. 55.6 ± 6.3%, respectively, n = 9; P = 0.7; Fig. 2B). Lactate addition also decreased the glucose sensitivity of VMN GI neurons. APF increased as glucose decreased from 2.5 to 0.1 but not to 0.5 mM (Fig. 3). IR of VMN GI neurons only increased by 8.5 ± 4.7% (n = 5) in the presence vs. 36.9 ± 10% (n = 5) in the absence of added lactate (P < 0.05; Fig. 4). Thus recurrent hypoglycemia and lactate addition significantly reduced the glucose sensitivity of VMN GI neurons.

DISCUSSION

In vivo human and animal studies in adults clearly demonstrate that recurrent hypoglycemia blunts the CRR (6, 14). This results from an increased brain glucose threshold for the detection of hypoglycemia. Thus brain glucose levels are allowed to fall to dangerous or even lethal levels before the CRR is initiated (1, 5). We are the first to show that recurrent hypoglycemia also impairs the ability to counteract hypoglycemia in young and most importantly, suckling rats, as evidenced by lower blood glucose levels after recurrent hypoglycemia. In vivo studies also show that the CRR is blunted when lactate is infused into the VMH during systemic hypoglycemia (4). We have previously shown that VMN glucose-sensing neurons have the potential to sense impending hypoglycemia (17). Here, we found that recurrent hypoglycemia and lactate supplementation significantly reduced the glucose sensitivity of VMN GI neurons in suckling rats whose ability to counteract hypoglycemia was impaired. These data provide strong support for our hypothesis that VMN GI neurons play a key role in hypoglycemia detection and initiation of the CRR.

Because these studies were carried out in juvenile animals, caution must be exercised in extrapolating these data to the adult human. However, we feel this animal model has important implications. First, the developing brain may be more sensitive to the damaging effects of hypoglycemia. Children under 10 years of age with type 1 diabetes mellitus show a broad degree of neurocognitive dysfunction, involving perceptual, motor, memory and attention tasks (13). Recurrent hypoglycemia impairs hippocampal long-term potentiation in young rats (22). Furthermore, infant (nursing) humans exhibit an impaired CRR to hypoglycemia (11). Finally, the CRR is initiated at lower glucose levels in young children (10).

On the other hand, young suckling animals exhibit higher levels of monocarboxylic acid transporters, suggesting that their brains may be using more lactate and ketone bodies than mature animals (20). This may reduce the effectiveness of our
model of recurrent hypoglycemia. Furthermore, the pups may not all suckle equally when returned to their dams. This potentially increases the variability in this study. Therefore, this may not be the most robust model in which to study impaired glucose-sensing mechanisms after recurrent hypoglycemia. However, despite these limitations, the data in Table 1 clearly demonstrate that even though the pups were returned to their dams to suckle after the insulin injection, they experienced a hypoglycemic challenge sufficient to impair glucose recovery during subsequent insulin-induced hypoglycemia. Because recurrent hypoglycemia (and VMH lactate perfusion) similarly impairs glucose recovery in adults, we believe that these results are germane to the deleterious effects of recurrent hypoglycemia observed clinically. It is possible that the injections elicited a stress response, independent of hypoglycemia. However, this does not seem likely since the saline-injected pups did not exhibit stress-induced hyperglycemia.

A final benefit of using young rats relates to the paucity of GI neurons in the VMN (12, 16). These neurons cannot be identified before electrophysiological recording. Additionally, at least 5 to 10 min are required to observe glucose effects (17). Because of variations in neuronal activity, we are never absolutely confident of a glucose response unless we observe reversal upon washout. Therefore, we must maintain a giga-ohm seal in a healthy neuron for an hour to measure the response to at least two glucose concentrations. It is much easier to obtain healthy cells that can withstand extended recordings in younger animals. Moreover, we have published detailed glucose concentration-response relations for glucose-sensing neurons in animals of this age, using the whole cell patch-clamp configuration (17, 21). Thus we did not have to reestablish a half-maximal concentration of glucose, which would most likely reveal a difference in glucose sensitivity between saline and insulin-treated animals. Overall, the use of young, suckling animals facilitates the study of the mechanisms underlying the impaired CRR after recurrent hypoglycemia in a model that is extremely relevant to pediatric medicine.

The mechanism by which lactate reduces glucose sensitivity is not clear. One possibility is that it is being used simply as a fuel to support neuronal activity in both glucose-sensing and nonglucose-sensing neurons. In support of this, inhibition of lactate transport during hypoglycemia increases neuronal damage, which suggests that lactate fuels neurons during hypoglycemia (9). Increased lactate could supply ATP and replace glucose in the regulation of GI neurons. However, this is not consistent with our previous studies showing that lactate regulated the activity of VMN glucose-sensing neurons in a manner distinct from that of glucose. For example, although GI neurons were inhibited by glucose, they were excited by lactate in both high and low glucose concentrations (17). Another possibility is that lactate replaces glucose as a fuel source only for nonglucose-sensing neurons, while also serving as a signal that regulates neuronal activity in glucose-sensing neurons.

In either case, we speculate that hypoglycemia induces local neuroprotective mechanisms that impair the glucose sensitivity of VMN GI neurons. In support of this, hypoglycemia increases neuronal and blood-brain barrier glucose and lactate transporter expression (7, 9, 15). This would protect the brain during an acute hypoglycemic event. However, recurrent hypoglycemia may result in sustained upregulation of nutrient availability/utilization to protect the brain. As a result, glucose-sensing neurons would not perceive the intensity of the glucose deficit and signal for an appropriate CRR. Finally, these data indicate the importance of approximating the physiological milieu surrounding glucose sensing neurons to properly study
them. Clearly, lactate, which is a component of the extracellular fluid in the brain, has a significant effect on the function of glucose-sensing neurons. Thus it is very important that studies, especially of isolated neurons, include lactate in the perfusion solution. This is less of a concern for studies of brain slices that contain glial cells, as these cells presumably provide a certain level of lactate to adjacent neurons.

In conclusion, our findings indicate that decreased glucose sensitivity of VMN GI neurons in young suckling rats may contribute to their impaired glucose restoration following recurrent hypoglycemia. These data support a role for VMN GI neurons in hypoglycemia detection and initiation of the CRR.

GRANTS

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REFERENCES