Rate of fall in blood glucose and recurrent hypoglycemia affect glucose dynamics and noradrenergic activation in the ventromedial hypothalamus

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Barnes MB, Lawson MA, Beverly JL. Rate of fall in blood glucose and recurrent hypoglycemia affect glucose dynamics and noradrenergic activation in the ventromedial hypothalamus. Am J Physiol Regul Integr Comp Physiol 301: R1815–R1820, 2011. First published September 28, 2011; doi:10.1152/ajpregu.00171.2011.—Noradrenergic activity in the ventromedial hypothalamus (VMH) is increased and activates a sympathoadrenal response during hypoglycemia. How the rate at which hypoglycemia develops affects local glucose concentrations and norepinephrine (NE) release was evaluated by placing microdialysis probes into the VMH of male Sprague-Dawley rats receiving insulin (20 mU·kg⁻¹·min⁻¹) and variable glucose infusions. During a first episode of hypoglycemia, interstitial glucose concentrations in the VMH generally declined at the same rate as plasma glucose; however, the faster hypoglycemia developed, the greater the magnitude of the initial NE release in the VMH (r² = 0.72, P < 0.001). Following recurrent episodes of hypoglycemia, VMH glucose decreased at a slower rate than plasma glucose, and the initial NE release was attenuated at the same rates of blood glucose decline. The plasma glucose threshold for the initial NE release in VMH was similar for all groups (~3.23 mM); however, the VMH glucose threshold was stimulated and was lower when blood glucose declined more slowly (0.86 ± 0.06 vs. 1.06 ± 0.04 mmol/l, P < 0.01). The timing of the initial increase in NE release in VMH corresponded with an increase in plasma epinephrine during the first episode of hypoglycemia but not following recurrent hypoglycemia. Although a decrease in VMH glucose concentration is required for noradrenergic activation in VMH, there does not appear to be a set glucose threshold within the VMH for activation of this response.

recurrent hypoglycemia; glucose homeostasis; sympathoadrenal response; hypoglycemia-associated autonomic failure

Sensing and responding to decreased circulating glucose is essential to preserve brain function. Sensors in the brain and periphery detect hypoglycemia and coordinate neuroendocrine and autonomic responses to mobilize glucose. The ventromedial hypothalamus (VMH) plays a key role in glucose homeostasis through initiation of counterregulatory responses (CRR), increased plasma epinephrine, norepinephrine (NE), and glucagon to hypoglycemia. Within the VMH, the firing rates of specific neuron populations reflect ambient glucose concentrations and are responsible, in part, for sympathetic activation (29). The concentration of extracellular glucose in the VMH is 20–25% of plasma glucose under euglycemic conditions and generally declines in parallel with plasma glucose during hypoglycemia (8). When VMH glucose was maintained during systemic hypoglycemia, peripheral sympathoadrenal responses were attenuated (3, 10), demonstrating that the reduction of glucose within the VMH is both required and sufficient for activation of CRR. Activation of noradrenergic systems in the VMH increased sympathetic nervous system activity, a mediator of glucose mobilization (31), and there is an increase in NE release in the VMH during hypoglycemia (2) that occurs in response to the decrease in VMH glucose (10).

Hypoglycemia-associated autonomic failure, characterized by a blunted CRR during hypoglycemia, is clinically relevant in insulin-treated diabetes (15). Antecedent hypoglycemia alters both the CRR and the physiological state of the hypothalamus. A surprising observation was that basal glucose concentrations in the VMH were lower after recurrent hypoglycemia (8). In the same report, it was noted that basal VMH glucose concentrations after an overnight fast were at levels seen during an acute hypoglycemic episode (8); but there was no CRR in the fasted rats. A notable difference between the two observations is the interval over which VMH glucose concentrations decreased; the rate of decline during an overnight fast would be much slower than the decline induced in response to exogenous insulin. The present study was conducted to determine whether the rate of decline in blood glucose affected the rate of decline in VMH glucose concentrations influence NE release in the VMH and/or the development of hypoglycemia-associated autonomic failure.

MATERIALS AND METHODS

This study was approved by the University of Illinois Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (Charles River Laboratories), 250–300 g, were housed singly in Plexiglas cages (30 × 30 × 38 cm) in a light (12:12-h light-dark cycle; lights on at 0700 h)- and temperature (26 ± 2°C)-controlled room. Animals had free access to water and rodent diet (Harlan Teklabs, Madison, WI) at all times, except where stated otherwise.

Surgical procedures. After a 1-wk acclimation period, rats were fitted with a jugular vein catheter, a femoral vein catheter, and a microdialysis guide cannula as previously described (2). Briefly, rats were anesthetized with a mixture of ketamine·HCl, xylazine·HCl, and acepromazine (30:6:1 mg/kg im) before placing Silastic catheters into the left femoral vein and right jugular vein. Catheters were exteriorized through an incision on top of the head and filled with a 40% polyvinylpyrrolidone solution containing 500 U/ml heparin to maintain patency. Rats were placed into a stereotaxic instrument (ASI Instruments, Warren, MI), and a guide cannula was positioned 2 mm dorsal to the lateral edge of the anterior portion of the VMH using the stereotaxic atlas of Paxinos and Watson (20). Coordinates for the tip of the guide cannula were 2.4 mm posterior of bregma, 0.8 mm lateral of midline, and 6.4 mm below the dura. The guide cannula and the ends of the catheters were fixed in position with dental acrylic cement and anchored to the skull with four stainless steel screws (Small Parts, Miami, FL). Following surgery, rats were monitored until they had recovered completely from the anesthesia, and postsurgical analgesia
was provided with Banamine (1.5 mg/kg sc). Cannula placements were verified histologically at the end of the study.

**Sample collection.** Animals (n = 18) were handled daily during the recovery period (5–7 days) and adapted to the experimental procedures. Experiments were conducted in the mid-light phase to minimize confounding diurnal-associated changes, and food was removed during the sampling period. Samples were collected from unrestrained animals in their home cages by using a liquid swivel (Instech, Plymouth Meeting, PA) connected to a weighted counterbalance lever. Microdialysis probes with a 0.20 x 1 mm cuprophan membrane (AKZO-Nobel, Wuppertal, Germany), connected to a 1-ml gas-tight syringe on a microinfusion pump (CMA Microdialysis, Sweden), were inserted 3 h prior to the sample collection and continuously perfused with Krebs Ringer buffer (CMA Microdialysis, Sweden).

Brain dialysate samples were inserted 3 h prior to the sample collection and continuously perfused with Krebs Ringer buffer (in mmol/l): 147 NaCl, 4 KCl, 2.4 CaCl2; pH 7.2. Dialysate was collected into chilled microtubes containing 0.5 μl 0.10 N perchloric acid and kept at −81°C until analyzed. Probe efficiencies were determined, in vitro, following their use.

Baseline samples were collected before starting a continuous infusion (10 μl/min of 0.6 U/ml insulin (Humulin, Eli Lilly, Indianapolis, IN) through the femoral vein (at time 0). With the use of a Y connector, a 30% dextrose solution was also infused into the femoral vein beginning at time 0 at a variable rate (8–15 μl/min) to control the fall of plasma glucose. Blood glucose levels were checked at 2-min intervals for the first 20 min and at 5-min intervals for the remainder of the study. Blood glucose (20 μl) was immediately analyzed using a handheld glucose monitor (Accu-Check Advantage; Roche Diagnostics, Indianapolis, IN), and the glucose infusion rate was adjusted accordingly to induce varying rates of decline. Blood samples (200 μl) for catecholamine analysis were collected and replaced with an equal volume of donor blood from littermates. Brain dialysate sample collection continued at 5- or 10-min intervals for the duration of the study. A second group of rats (n = 13) received a once-daily (1300 h) intravenous administration of insulin (2.0 U/kg) on three consecutive days. The rate of fall in blood glucose was not controlled during the recurrent hypoglycemia regimen. On day 4, rats were subjected to the controlled decline hypoglycemic clamp described above.

**Analytical methods.** Dialysate and plasma catecholamines were analyzed by reverse-phase HPLC and electrochemical detection. Samples were injected onto a 150 x 2 mm C18 (3 μm) Hypersil column (Keystone Scientific, Bellfonte, PA) fitted with a 2 mm C18 (3 μm) Hypersil javelin guard column (Keystone Scientific). The mobile phase was 75 mM NaH2PO4, 1.7 mM 1-octane sulfonic acid, 25 μM Na2 EDTA, 7% (vol/vol) acetonitrile, and 0.1% (vol/vol) triethylamine (pH = 3.0). An electrochemical detector (Decade; Antec Leyden, Leiden, The Netherlands), fitted with a VT-03 glassy carbon electrode (+0.75 V), and Dynamax MacIntegrator II and C module programs (Rainin Instruments, Woburn, MA), were used for peak integration and quantitation. Plasma catecholamines were analyzed following solid-phase extraction with aluminum oxide (BAS, West Lafayette, IN) and elution with 0.2 N perchloric acid. The interassay coefficient of variation was ±3%.

Dialysate glucose was measured with a TD-700 fluorometer (Turner Designs, Sunnyvale, CA) equipped with a minicell, by using a double-enzyme method described by de Vries et al. (8). Excitation wavelengths were between 300 and 400 nm, and emission wavelengths were between 410 and 610 nm. Inter- and intra-assay variations were between 2 and 5%.

**Data analysis.** Linear regression with analysis of variance was used to determine the relationship between rate of fall and the magnitude of the first increase in NE release > 95% confidence interval of baseline interstitial NE. Breakpoint analysis was performed using segmented linear regression analysis. Changes in VMH vs. plasma glucose over the first 60 min were analyzed by linear regression and comparison of slopes. All other variables were compared by one-way ANOVA and Scheffé’s post hoc multiple comparison test (Prism 5.0; GraphPad, San Diego, CA). Values are reported as means ± SE. Data from two of the recurrent animals with rates less than 0.9 mg/dl were not used, although they were statistically not different from the mean of rats with rates greater than 0.9 mg/dl. In addition, we were unable to obtain a complete set of plasma catecholamine results from five animals (3 slow and 2 recurrent), and they were removed from all analyses except that in Fig. 1. Samples from three animals were not analyzed because we could not confirm probe placement in the VMH.

**Supplies.** Ketamine, acepromazine, and Banamine were obtained from Aveco (Fort Dodge, IA). Xylazine was obtained from Vedco (St. Joseph, MO). All other reagents were purchased from Sigma (St. Louis, MO).

### RESULTS

The rate at which hypoglycemia developed significantly affected the magnitude of noradrenergic activation in the VMH (Fig. 1). NE activation was defined a priori as an increase in NE levels above the 95% confidence interval of the baseline concentration. The magnitude of NE release in VMH increased as the rate of hypoglycemia induction increased (r² = 0.72, P < 0.001) during the initial hypoglycemic episode but was attenuated (r² = 0.35, P = 0.04) in animals having undergone recurrent hypoglycemia. Breakpoint analysis of the data indicated a differential response above and below rates of 0.9 mg·dl⁻¹·min⁻¹ during the initial hypoglycemic episode. The magnitude of the NE release was greater (P < 0.001) when blood glucose declined greater than 0.9 mg·dl⁻¹·min⁻¹ (78.8 ± 6.6% increase) than rates less than 0.9 mg·dl⁻¹·min⁻¹ (38.2 ± 4.7% increase). The magnitude of NE release in VMH at the higher rate was similar to that produced by bolus insulin administration that produced a 3–5 mg·dl⁻¹·min⁻¹ decrease in blood glucose (2, 10). This relationship did not hold for the recurrent group, in which there was no effect of the rate of decline on NE release in the VMH (increases of 30.4 ± 4.2% vs. 23.8 ± 3.1%).

The relative change in VMH glucose to change in blood glucose (Fig. 2) was affected by the rate at which hypoglycemia developed and by antecedent hypoglycemia. VMH glucose...
In the initial hypoglycemic episode induced at a slower rate or following recurrent episodes of insulin-induced hypoglycemia (5.9 ± 0.3 vs. 9.4 ± 1.1 nmol/l, P = 0.003).

In the initial hypoglycemia groups, the increase (P < 0.001) in plasma epinephrine (Fig. 4) occurred concomitant with the increase in VMH NE levels. In the recurrent hypoglycemia group, the initial increase in VMH NE occurred during the 20–30 min sample period, yet a significant increase in plasma epinephrine was not apparent until 60–70 min. Because onset of sympathoadrenal activation in the slower-fall and recurrent groups occurred late in the sampling period, we did not obtain recurrent group. As blood glucose remained below the activation threshold, there was continued release of NE in the VMH. At the slower rates, NE eventually reached a maximal concentration 186 ± 8% of baseline by the end of the infusion period that was similar to the maximal increase at the faster rate (217 ± 15% of baseline). The maximal increase in NE in recurrent animals reached 152 ± 4% of baseline, lower (P < 0.05) than during the initial episode. Similar to our previous report (9), baseline NE values were higher following recurrent hypoglycemia (5.9 ± 0.3 vs. 9.4 ± 1.1 nmol/l, P = 0.003).

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<th>Blood and VMH glucose concentrations of rats during an initial episode of hypoglycemia at a slower (&lt;0.9 mg·dl⁻¹·min⁻¹) or faster (&gt;0.9 mg·dl⁻¹·min⁻¹) rate or following recurrent episodes of insulin-induced hypoglycemia</th>
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<td>Blood glucose, mmol/l</td>
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<td>Basal</td>
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<td>5.70 ± 0.15</td>
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<td>NE activation</td>
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<td>VMH glucose, mmol/l</td>
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<td>NE activation</td>
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Data are means ± SE; n = 6–8/group. VMH, ventromedial hypothalamic; NE, norepinephrine. Values within a row having different superscripts differ by at least P < 0.05.

decreased in parallel with blood glucose at rates of < 0.9 mg·dl⁻¹·min⁻¹ (Fig. 2, top), but the decline in VMH glucose was delayed when blood glucose declined at the faster rate (Fig. 2, middle). The slopes for change in VMH and plasma glucose were similar for both groups. In the recurrent hypoglycemic group, the relative decrease in VMH glucose was less (P = 0.03) than the change in blood glucose throughout the hypoglycemic period (bottom). The relative change in VMH glucose (as a % of baseline), however, was similar during the initial hypoglycemic episode induced at the faster rate and in the recurrent rats.

The increase in NE release above baseline (Fig. 3) occurred earlier at the faster rates during an initial episode of insulin-induced hypoglycemia (Table 1) and at the same time in the initial hypoglycemia groups, the increase (P < 0.001) in plasma epinephrine (Fig. 4) occurred concomitant with the increase in VMH NE levels. In the recurrent hypoglycemia group, the initial increase in VMH NE occurred during the 20–30 min sample period, yet a significant increase in plasma epinephrine was not apparent until 60–70 min. Because onset of sympathoadrenal activation in the slower-fall and recurrent groups occurred late in the sampling period, we did not obtain recurrent group. As blood glucose remained below the activation threshold, there was continued release of NE in the VMH. At the slower rates, NE eventually reached a maximal concentration 186 ± 8% of baseline by the end of the infusion period that was similar to the maximal increase at the faster rate (217 ± 15% of baseline). The maximal increase in NE in recurrent animals reached 152 ± 4% of baseline, lower (P < 0.05) than during the initial episode. Similar to our previous report (9), baseline NE values were higher following recurrent hypoglycemia (5.9 ± 0.3 vs. 9.4 ± 1.1 nmol/l, P = 0.003).
samples to appropriately measure the full sympathoadrenal response.

The concentration of plasma glucose when NE activation first occurred in the VMH (−3.3 mmol/l) was independent of the rate at which hypoglycemia developed and was similar in both the initial and recurrent hypoglycemic groups. However, VMH glucose concentration at the time when VMH NE was activated was affected by the rate of decline in blood glucose (Table 1). At NE activation in the VMH, local tissue glucose concentrations were lower (P < 0.005) when blood glucose declined at a slower rate. Glucose concentration in the VMH of recurrent hypoglycemic rats was lower (P = 0.005) prior to onset of hypoglycemia, both in absolute levels and as a percent of blood glucose, similar to our previous report (8). However, recurrent hypoglycemia did not affect the local glucose concentration at which NE activation in the VMH occurred (1.04 ± 0.06 mmol/l).

DISCUSSION

The rate at which hypoglycemia developed affected both local glucose concentrations and the degree of noradrenergic activity within the VMH. It has been known for some time that noradrenergic activity increases in the VMH during hypoglycemia (28), an increase due to the reduction in local glucose concentrations (10). Activation of adrenoceptors in the VMH is necessary for the sympathoadrenal response to exercise (23), glucoprivation (27), and hypoglycemia (10). In the present study, the initial increase in VMH NE release coincided with increased plasma epinephrine regardless of the rate at which hypoglycemia developed during an initial episode of hypoglycemia. The apparent local glucose threshold for VMH NE activation, however, was affected by the rate at which hypoglycemia developed. The slower the decline in blood glucose, the lower the glucose concentration in the VMH fell before NE activation occurred. The magnitude of the increase in VMH NE also did not correlate with VMH glucose concentrations at the time of activation, suggesting that other inputs influence NE activity in the VMH during hypoglycemia. Similarly, an increase in NE activation in VMH may not be sufficient to induce the sympathoadrenal response to hypoglycemia under some conditions. In recurrent animals, NE release in VMH was significantly elevated by 30 min, but there was no increase in plasma epinephrine until the 50- to 60-min sample period. This disconnect between VMH NE activity and the sympathoadrenal response following recurrent hypoglycemia was observed when hypoglycemia was induced much more rapidly (e.g., 3–5 mg·dl⁻¹·min⁻¹) by bolus insulin administration (9). The elevated basal NE tone in the VMH of recurrent animals and normal plasma catecholamine concentrations, provides further support that increased VMH NE levels do not independently activate a sympathoadrenal response. We are unaware of any report of hypoglycemia or recurrent hypoglycemia inducing changes in noradrenergic receptor number or affinity in the VMH. Decreased sensitivity or downregulation of VMH adrenoceptors by the elevated NE tone would be consistent with the apparent discordance between increased VMH NE and sympathoadrenal activation. Alternatively, the impaired sympathoadrenal response following recurrent hypoglycemia may reflect the absence of some feature normally present or the presence of an inhibiting condition in the VMH or downstream circuitry.

Plasma glucose concentrations were similar for all groups at first activation of VMH NE activity (−3.2 mmol/l), supporting a more stable plasma glucose threshold. This value is similar to the plasma glucose threshold for activating the sympathoadrenal response reported by Amiel et al. (1) and Donovan et al. (12). Glucose sensors associated with the hepatic portal are responsive to changes in portal glucose concentrations and influence the activity of neurons in the hypothalamus (25). Glucose infusions into the portal vein during systemic hypoglycemia (11) and denervation of afferents from the portal vein (16) blunted the plasma catecholamine response during hypoglycemia. However, preventing a decline in glucose in only the VMH was sufficient to prevent both the increased NE release in the VMH and the plasma catecholamine response during a hypoglycemic episode (10). Findings by Saberi et al. (22) suggest that the locus for detection of hypoglycemia shifts between the periphery and the brain depending on the rate of fall in blood glucose. Using portal and superior mesenteric vein denervation, they demonstrated that when hypoglycemia developed slowly, peripheral input was required for sympathoadrenal responses. However, as hypoglycemia developed, faster peripheral denervation had no significant impact on CRR, as measured by increased plasma epinephrine and NE. Based on these observations, we suggest integration of the stimulus generated by peripheral glucose sensors with local glucose concentrations in the VMH to influence the magnitude of NE activation in the VMH. The largest increase in NE release (initial-fast) occurred when blood glucose changed proportionally more than VMH glucose, while the smallest increase occurred in those animals in which blood and VMH glucose changed to the same degree.

The rate at which hypoglycemia developed had no effect on the magnitude of hormonal responses, cognitive function, or the hierarchy of CRR to hypoglycemia (1, 19). Because we were interested in glucose concentrations at activation, we failed to follow plasma catecholamine responses sufficiently to determine the full degree of sympathoadrenal activation in our slow fall group and did not document whether the overall responses to slow and fast fall were similar or different. However, the patterns of change in plasma epinephrine and
plasma glucose threshold in our study were similar to those reported by Amiel et al. (1). In the Amiel study, there was no substantive differences in the counterregulatory response activated by the rate of fall in blood glucose.

There does not appear to be an absolute glucose threshold in the VMH for noradrenergic activation, nor did antecedent hypoglycemia alter either plasma glucose or VMH glucose thresholds for sympathoadrenal activation. There are glucose-responsive neurons in the VMH whose firing rates are affected by changes in ambient glucose concentrations (reviewed in Refs. 17 and 21) and a decrease in local glucose is required for the increased NE release in the VMH during hypoglycemia (10). Using ibotenic acid to selectively lesion cells with the VMH, effectively prevented the increase in NE during hypoglycemia and attenuated plasma catecholamine and glucagon levels (4, 14). Yet when hypoglycemia developed at a slower rate, VMH glucose concentrations were 20% lower before noradrenergic activation in the VMH first occurred. Additionally, VMH glucose concentrations fell at a slower rate and only half as much following recurrent hypoglycemia. Recurrent hypoglycemia was reported to reduce the sensitivity of some glucose sensory neurons in the VMH (30). If VMH glucose-sensing neurons do mediate NE release in the VMH, the present results are consistent with their responsiveness being affected by the relative rate of change in ambient glucose concentration.

The slower fall in VMH glucose following recurrent hypoglycemia may reflect a change in the availability of glucose in this area or the use of alternative energy substrates. Increased uptake of glucose across the blood-brain barrier after recurrent hypoglycemia was proposed as a possible mechanism to provide more efficient uptake of glucose and buffer changes in brain glucose (5). This suggestion received support when an increase in GLUT1 following prolonged hypoglycemia was reported (26). However, brain glucose concentrations were not increased following antecedent hypoglycemia in humans (7) nor were cerebral glucose transport, metabolism, or blood flow (24). Brain glycogen has also been proposed to contribute to the maintenance of interstitial glucose concentrations (6, 13), and following a single episode of hypoglycemia, brain glycogen levels rebounded to three times the prehypoglycemic levels (6). Thus, mobilization of glycogen stores from astrocytes could contribute to the slower fall in interstitial glucose during recurrent hypoglycemia. The slower rate and attenuate decline in VMH glucose following recurrent hypoglycemia was in contrast to the hippocampus, where glucose was reported to decrease faster and to a greater degree during hypoglycemia in recurrent animals (18).

In summary, extracellular glucose concentration in the medial hypothalamus is surprisingly dynamic. Both the rate at which blood glucose decreases and prior episodes of hypoglycemia alter the magnitude of the NE response in the VMH, without changing the apparent plasma glucose threshold for NE activation. Decrease in VMH glucose likely alters the activity of neurons within the VMH involved in glucose homeostasis and is required for the hypoglycemia-induced increase in noradrenergic activity required for sympathoadrenal counterregulatory activation. It remains to be determined the importance of relative vs. absolute changes in VMH glucose concentration in glucose homeostasis and how these alterations in glucose contribute to hypoglycemic unawareness.

**Perspectives and Significance**

The VMH is a critical brain area for monitoring and responding to hypoglycemia. However, local glucose dynamics during hypoglycemia and its impact on neural circuits in the VMH activating CRR to hypoglycemia are unknown. Hypoglycemia-associated autonomic failure, a blunting of the CRR, likely involves hypoglycemia-induced changes in the VMH. These changes may contribute to the slower decline in VMH glucose than plasma glucose following recurrent hypoglycemia. Whether the slower decline reflects a greater efficiency of uptake from blood, mobilization of local glucose resources, or a shift in glucose utilization, requires further study. The initial increase in NE release in the VMH coincided with an increase in plasma epinephrine during a first episode of hypoglycemia but not subsequent episodes, indicating that an increase in VMH NE by itself is insufficient to activate a sympathoadrenal response. A decrease in sensitivity of glucose sensing elements in the VMH following recurrent hypoglycemia has been proposed (30). The reduced magnitude of NE release following recurrent hypoglycemia is consistent with a decreased activation of local glucose sensors. However, glucose concentrations in the VMH when NE activity increased were the same during the first and subsequent episodes of insulin-induced hypoglycemia. A difference in sensitivity across populations of glucose sensors so that fewer are activated at the same glucose concentration would explain this apparently contradictory result. During a first episode of hypoglycemia, VMH glucose concentrations at the first significant increase in NE release were significantly different depending on the rate of change in blood glucose; VMH glucose was ~ 20% lower when glucose fell more slowly. This result does not support a simple glucose threshold for glucose sensors in the VMH. The fact that plasma glucose concentrations were similar when the initial increase in VMH NE release occurred supports a strong role for peripheral glucose sensors in the response. Because maintaining VMH glucose concentrations during systemic hypoglycemia prevented the increases in VMH NE and plasma catecholamines (10), we suggest integration of peripheral and local glucose sensors in determining the magnitude of NE activation in the VMH and sympathoadrenal activation.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

M.B.B. and J.L.B. conception and design of research; M.B.B. and M.A.L. performed experiments; M.B.B., M.A.L., and J.L.B. analyzed data; M.B.B., M.A.L., and J.L.B. interpreted results of experiments; M.B.B. and J.L.B. prepared figures; M.B.B. drafted manuscript; M.B.B. and J.L.B. edited and revised manuscript; M.B.B., M.A.L., and J.L.B. approved final version of manuscript.

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