Extracellular Glucose in Rat Ventromedial Hypothalamus During Acute and Recurrent Hypoglycemia

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The activity of neurons in the ventromedial hypothalamus (VMH) important for initiating compensatory responses to hypoglycemia is influenced by ambient glucose concentration. In the present study, we used in vivo microdialysis to evaluate interstitial glucose concentrations in rat VMH under various glycemic conditions. Using the zero-net-flux method, steady-state glucose concentration in the VMH was ~20% of blood glucose (~1.4 mmol/l) in fed rats but ~14% of blood glucose (~0.7 mmol/l) in overnight-fasted rats. During moderate hypoglycemia VMH glucose declined in parallel with blood glucose; however, VMH glucose decreased to a greater degree than blood glucose during a more severe hypoglycemic episode, falling to 10 ± 1.2% of blood levels ($P < 0.01$). To determine whether VMH glucose concentrations were influenced by recurrent episodes of hypoglycemia a second zero-net-flux study was conducted. Steady-state glucose concentrations in the VMH were ~20% lower after three episodes of recurrent hypoglycemia, a value 17.8 ± 0.8% of blood glucose, although the relative change in VMH glucose levels during the first and fourth hypoglycemic episodes were similar. From these results, we conclude that interstitial glucose concentrations in the VMH are not maintained at a constant level and are more dynamic than previously proposed. *Diabetes* 52:2767–2773, 2003

Hypoglycemia initiates compensatory responses, most notably the release of glucagon from the endocrine pancreas and sympathoadrenal activation resulting in secretion of epinephrine. There is strong evidence that the brain, in particular the hypothalamus, plays an important role in autonomic control of glucose balance (rev. in 1). Detection of local glucoprivation in the brain may be critical to the generation of appropriate compensatory responses (2). The ventromedial hypothalamus (VMH) has received attention because neurons in this area are responsive to both systemic hypoglycemia (3) and to changes in local extracellular glucose concentrations (4–6). Local glucoprivation by application of the glucose-analogue 2-deoxyglucose into this area replicated the increased neuroendocrine and sympathoadrenal output characteristic of systemic hypoglycemia (7). In addition, compensatory responses to systemic hypoglycemia were inhibited when the VMH was bilaterally perfused with glucose (8). In vitro studies, using tissue slices, demonstrated that ~30% of neurons throughout the VMH alter their firing rate to changes in glucose concentration in the bathing solution (4–6,9). It has been suggested that the cellular mechanisms by which hypothalamic neurons respond to glucose are similar to those of pancreatic β-cells, in part because they involve membrane-bound sulfonylurea receptors associated with ATP-sensitive potassium (K_{ATP}) channels (4,6,9). Neurons in the VMH of mice lacking Kir6.2, one of the subunits of K_{ATP} channels, are less responsive to changes in ambient glucose (10).

To be able to function as glucose sensors to initiate compensatory responses, neurons must be sensitive to physiological changes in ambient glucose levels. As a wide range of glucose values have been reported, it remains unclear what glucose levels are in the brains of intact animals. Glucose concentrations in brain interstitial fluid under euglycemic conditions have been examined with the zero-net-flux method using in vivo microdialysis, first described by Lönnroth et al. (11). They were found to be 0.3–0.5 mmol/l in striatum (12,13), 1 mmol/l in hippocampus (14), and ~3.3 mmol/l in the neocortex (15). Other reports, using a microelectrode coated with glucose-oxidase (16), indicated 0.35–1 mmol/l in striatum (17) and 2.4 mmol/l in cingulate cortex (18). To our knowledge, interstitial glucose levels in the hypothalamus have not yet been reported, nor are there reports of changes in hypothalamic glucose during systemic hypoglycemia. Bequet et al. (19) reported that during prolonged insulin-induced hypoglycemia, or after a 36-h fast, extracellular glucose in the cortex was closely coupled to glucose in the blood. Glucose is likely taken into the brain by facilitated diffusion; however, controversy remains as to what is the rate-limiting factor for the supply of glucose to the hypothalamus. Under normal conditions, glucose supply from the blood is believed to be in excess of metabolic requirements (20–22). On the other hand, increased neural activity may also influence the local extracellular glucose concentration (12,23).

Intensive insulin therapy is used in the treatment of type 1 diabetes to improve metabolic control and to reduce long-term diabetes complications. Unfortunately, frequent iatrogenic antecedent hypoglycemia diminishes warning

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AUC, area under the curve; K_{ATP}, ATP-sensitive potassium; PVP, polyvinylpyrrolidone; VMH, ventromedial hypothalamus.

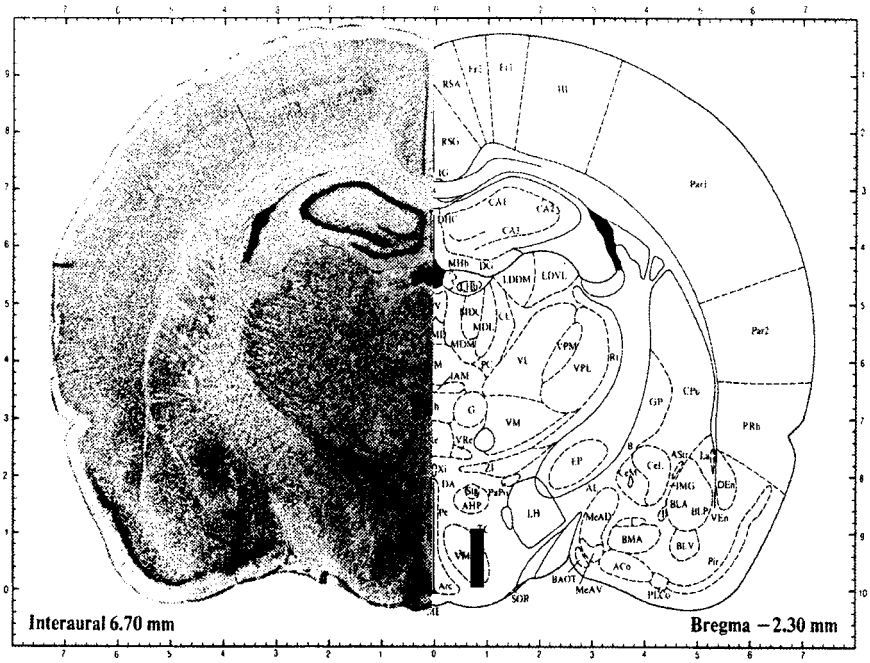

FIG. 1. Coronal view of cannula placement site in the lateral aspect of the VMH, 2.4 mm posterior from bregma using the stereotaxic atlas of Paxinos and Watson (32). Bar represents 0.25 × 1.0 mm tip of microdialysis probe.

symptoms and endogenous compensatory responses to subsequent hypoglycemia (24,25). This syndrome of "hypoglycemia unawareness" is responsible for considerable physical and psychological morbidity in diabetes (rev. in 26). It has been hypothesized that hypoglycemia unawareness is mediated by an increased efficiency of brain glucose uptake (27). While preserving the brain's glucose supply, detection of systemic hypoglycemia by putative brain glucose sensors may be impaired. There is some experimental evidence to support this theory. Lower symptom scores and blunted hormonal responses in type 1 diabetic patients with good glycemic control are associated with preserved brain glucose uptake and protected neural function during hypoglycemia (28). Similar findings have been reported in rats, where chronic hypoglycemia inhibited peripheral catecholamine secretion, increased glucose extraction by the brain (29,30), and even protected brainstem function (31). In the present study, the first objective was to determine, in vivo, the interstitial concentration of glucose in the VMH during systemic euglycemia and during an acute episode of hypoglycemia. We expected interstitial glucose concentrations in the VMH would decrease proportionally to a decrease in blood glucose. The second objective of this study was to evaluate the effect of recurrent hypoglycemia on VMH glucose and test the hypothesis that after repeated episodes of antecedent hypoglycemia there is less change in interstitial glucose concentrations during systemic hypoglycemia.

RESEARCH DESIGN AND METHODS

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

Surgical procedures. After a 1-week acclimation period, rats were fitted with a jugular vein catheter and microdialysis guide cannula as previously described (3). Briefly, rats were anesthetized with a mixture of Ketamine HCl, Xylazine HCl, and Acepromazine (30:6:1 mg/kg i.m.) and a 4-cm segment of Silastic tubing (0.64 mm i.d. × 0.94 mm o.d.) was inserted into the isolated right jugular vein. The catheter was exteriorized through an incision on top of the head, a piece of 21 gauge stainless steel tubing was inserted onto the end of the catheter, and the catheter was filled with a 40% polyvinylpyrrolidone (PVP) solution containing 500 units ml heparin and capped with a sealed piece of Tygon tubing to maintain patency. The rat was placed into a stereotaxic instrument (ASI instruments, Warren, MI), and a guide cannula was positioned 2 mm dorsal to the left VMH using the stereotaxic atlas of Paxinos and Watson (32). Coordinates for the tip of the guide cannula were AP = −2.3, L = 0.8, and D = 6.4 mm relative to Bregma. The tips of the microdialysis probes were designed to extend 2 mm beyond the end of the guide to direct the surface of the membrane into the lateral edge of the anterior portion of the VMH, immediately anterior to the perifornical area. Location of cannula placement is depicted in Fig. 1. The guide cannula and the end of the venous catheter were fixed in position with dental acrylic cement and anchored to the skull with four stainless-steel screws (Small Parts, Miami Lakes, FL). After surgery, rats were monitored until they had completely recovered from the anesthesia. Postsurgical analgesia was provided with Banamine (1.5 mg/kg s.c.). At the end of the study, cannula placement was verified histologically.

Dialysate sample collection. Rats were allowed 5–7 days of recovery after surgery and only animals with body weights greater than on the day of surgery were used. During this time, the animals were handled daily and were adapted to the experimental procedures. Blood and dialysate samples were collected from unrestrained animals in their home cages using sampling lines through a liquid swivel (Instech, Plymouth Meeting, PA) connected to a weighted counterbalance lever. Experiments were conducted during the mid-light phase to minimize possible confounding diurnal-associated changes. Microdialysis probes of 0.20 × 1 mm were constructed with cuprophan membrane (AKZO-Nobel, Wuppertal, Germany). Probe efficiencies were determined, in vitro, after their use. To minimize the effect of tissue disruption at the sampling site, probes were inserted into the brains 3 h before samples were collected. The probes were connected through a liquid swivel to a 1-ml gas-tight syringe on a microinfusion pump (Bioanalytical Systems, West Lafayette, IN) and continuously perfused with Kreb's Ringer buffer (in mmol/l: 147 NaCl, 4 KCl, and 2.4 $CaCl_2$; pH 6.4) at 1.5 μl/min. Dialysate was collected into chilled microtubes and kept at −84°C until assayed for glucose. Thirty minutes before the start of each experiment, the PVP-heparin solution was flushed from the catheters and replaced by sterile 0.9% saline.

Experiment 1: Zero-net-flux experiment to determine extracellular glucose concentration in the VMH under baseline conditions, both in fed and overnight-fasted rats. Rats were placed into one of two groups and had either free access to food (n = 11) or food was removed at the beginning of the dark period before the day of the experiment (n = 10). The zero-net-flux method with regression analysis, adapted from Lönnroth et al. (11), was used to calculate the glucose concentration in the interstitial space. In each animal, the probe was perfused with a series of Kreb's Ringer buffers containing 0, 0.5, 1, or 1.5 mmol/l glucose in order of increasing concentration. At each level, the

glucose flux across the probe's membrane was allowed to equilibrate for 20 min. A dialysate sample was then collected for 10 min. The net glucose flux (out − in) was measured at every perfusate concentration and regression analysis used to calculate the actual concentration of interstitial glucose in the VMH. Blood samples were collected at the midpoint of each 10-min sample period from the jugular vein catheter.

Experiment 2: Effect of different degrees of acute hypoglycemia on extracellular glucose in the VMH of fed rats. Glucose concentrations in blood and extracellular fluid of the VMH were evaluated to a single intravenous administration of insulin (0.5 or 5.0 units kg^{-1}ml; Humulin, Eli Lilly, Indianapolis, IN) or an equal volume of saline. Insulin or saline was injected via the jugular vein catheter at $t = 0$ min. Blood glucose was measured at $t = -10$, 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 90 min, as described above. Dialysate samples for evaluation of changes in extracellular glucose were collected at 10-min intervals beginning 30 min before inducing systemic hypoglycemia.

Experiment 3: Two studies to evaluate the effect of recurrent hypoglycemia on interstitial glucose in the VMH. In the first study, rats received a once-daily intravenous administration of saline ($n = 6$) or insulin (1.0 units kg^{-1}ml, $n = 9$) for 3 consecutive days. The injections were given in the middle of the light period. At the end of the light period on day 3, food was removed and all animals were fasted overnight. On day 4, blood and interstitial glucose were evaluated before and after an intravenous injection of 0.5 units/kg insulin. All rats received insulin. Timing and sample collection were as described in experiment 2.

The zero-net-flux procedure was used in a second study to confirm the changes in basal glucose levels observed in the first study. To remove the possible confounding effect of fasting, rats in this study were allowed ad libitum access to food at all times, except during the dialysis procedure. Steady-state interstitial glucose concentrations were measured after 3 consecutive days of saline (i.e., before either the first episode of hypoglycemia; $n = 10$) or 3 consecutive days of 1.0 units kg^{-1}ml insulin (e.g., before the fourth episode of hypoglycemia; $n = 10$).

Analytical methods. Glucose in dialysate was measured with a Turner TD-700 fluorometer (Turner Designs, Sunnyvale, CA) equipped with a minicell, using a method similar to McNay et al. (14). Briefly, glucose is converted into 6-phosphate-gluconolactone in enzymatic reactions catalyzed by hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49). Simultaneous conversion of $NADP^+$ to NADPH was detected fluorometrically. Dialysate samples (10 μl) were mixed with 65 μl reagent mix (in Tris buffer with pH = 8.1; 1 mmol/l $MgCl_2$, 3 mmol/l ATP, 3 mmol/l $NADP^+$, 3 mmol/l Dithiothreitol, 0.4 units/ml glucose-6-phosphate dehydrogenase, and 4 units/ml hexokinase) and assayed for glucose after a 60-min incubation. Filters restricted excitation wavelengths to between 300 and 400 nm and emission wavelengths to between 410 and 610 nm. The procedure was modified to provide sensitivity (threshold detection was 50 pmol glucose) to detect small changes in glucose concentration. A standard curve was linear between 0 and 250 μmol/l, and samples were adjusted to fall into this range by dilution with Krebs-Ringer bicarbonate. Inter- and intra-assay variation was between 2 and 5%. Blood glucose measurements were obtained using an Accu-Chek Instant (Roche Diagnostics, Indianapolis, IN) for experiment 2 and the first part of experiment 3. A Beckman Glucose Analyzer 2 (Beckman Instruments) was used in the zero-net-flux studies after separation of plasma by centrifugation.

Data analysis. Results are presented as means ± SE. The sum change in glucose concentrations was determined using the trapezoid rule to calculate the area under the curve (AUC). Differences in baseline values between fed and fasted rats and in recurrent hypoglycemic episodes were analyzed by Student's t test. Differences among treatment groups in responses of blood glucose, extracellular glucose, and brain/blood ratios over time were analyzed by repeated-measures ANOVA, and AUC was evaluated by one-way ANOVA. When ANOVAs were significant, a Scheffé's multiple-comparison test was used to determine differences among treatment groups. Changes from baseline were analyzed by repeated-measures ANOVA followed by paired t tests. In vitro recoveries, determined using an unstirred 1 mmol/l glucose solution, were 4.6 ± 0.8%.

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

RESULTS

Experiment 1. Interstitial glucose concentrations in the VMH were 1.42 ± 0.08 mmol/l, ~19% of blood glucose concentration (Table 1). After an overnight fast, glucose concentrations fell to a greater extent in the VMH than in the blood; the VMH-to-blood ratio decreased to ~14%. Baseline glucose concentrations determined by dividing dialysate glucose concentration by probe efficiency were similar to those determined by the zero-net-flux method; VMH glucose was calculated to be 1.41 0.10 mmol/l in fed rats and 0.84 0.10 mmol/l in fasted rats. The difference between fed and fasted states using probe efficiency was also statistically significant ($t_{18} = 3.55$, $P < 0.01$)

TABLE 1
Glucose concentrations in blood and VMH under baseline conditions in fed and overnight-fasted rats

	Fed	Fasted	t	P
n	11	10		
Blood glucose (mmol/l)	7.7 ± 0.2	5.8 ± 0.2	3.21	0.007
VMH glucose (mmol/l)	1.42 ± 0.08	0.73 ± 0.05	5.76	0.0002
VMH/blood (%)	18.7 ± 1.4	13.8 ± 0.8	3.21	0.007

Data are means ± SE. Extracellular glucose in VMH determined with zero-net-flux method.

Experiment 2. Baseline blood glucose concentrations were similar among treatment groups and decreased ($F_{24,192} = 7.62$, $P < 0.001$) rapidly following the administration of insulin (Fig. 2, *top*). The measured glycemic nadir of 3.4 ± 0.4 mmol/l was reached 10 min after injection of 0.5 units/kg insulin. This decrease was followed by a steady increase back to euglycemia by 60 min postinjection. The higher dose of insulin (5.0 units/kg) reduced blood glucose to a similar nadir, but glucose values remained low throughout the sampling period resulting in a greater total decrease in blood glucose, as determined by AUC ($F_{2,16} = 74.2$, $P < 0.001$).

Baseline concentrations of interstitial glucose in the VMH were 1.64 ± 0.38, 1.53 ± 0.21, and 1.68 ± 0.61 for rats in the saline, 0.5 units insulin, and 5.0 units insulin groups, respectively. VMH glucose was reduced ($F_{22,154} = 14.01$, $P < 0.001$) after insulin administration (Fig. 2, *middle*) with the decline mirroring the level of blood glucose. Total decrease in VMH glucose during the sampling period, determined by analysis of AUC, was different among all three groups ($F_{2,16} = 5.7$, $P = 0.01$). The VMH–to–blood glucose ratios (Fig. 2, *bottom*), expressed as percentage of blood glucose, were ~23% during the baseline period. The ratios increased during the 0- to 10-min interval in response to either insulin treatment but were not significantly different among treatment groups; however, there was a significant time-by-dose interaction ($F_{18,135} = 4.75$, $P < 0.001$), and the decrease from baseline was different from that of controls (as determined by AUC) only after administration of the high dose of insulin ($F_{2,16} = 7.58$, $P = 0.005$). After the higher insulin dose, VMH glucose tended to continue to decline throughout the data collection period, being significantly lower than other groups during the last 30 min.

Experiment 3. There was no difference in the degree or pattern of hypoglycemia induced after the first or fourth dose of insulin (Fig. 3, *top*). Similar to the results in the second experiment, the decline ($F_{9,90} = 27.37$, $P < 0.0001$) in interstitial glucose concentrations in the VMH paralleled the changes in blood glucose (Fig. 3, *middle*). The lowest levels were reached during the 20- to 30-min interval, 0.22 ± 0.05 and 0.08 ± 0.02 mmol/l in the first and fourth hypoglycemic episodes, respectively. Throughout the

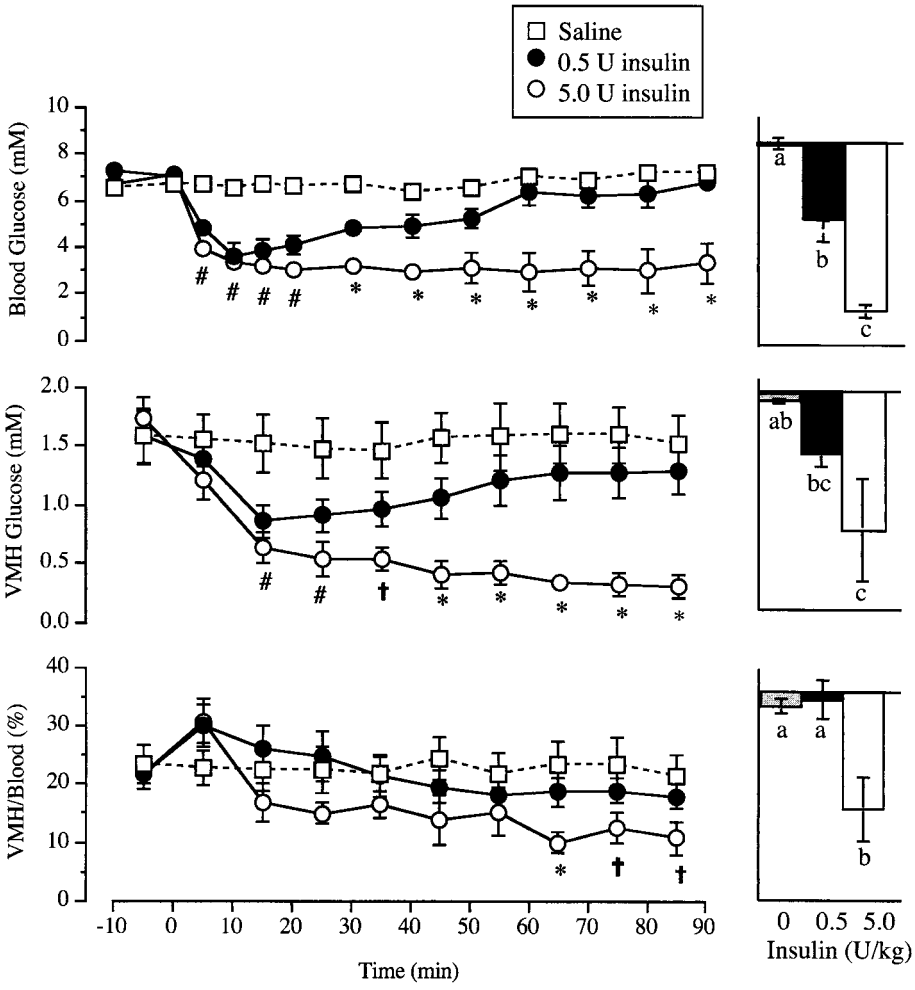

FIG. 2. Effect of different amounts of insulin on glucose concentration in blood (*top*), VMH interstitial fluid (*middle*), and on VMH-to-blood ratios (*bottom*). Insulin (0.5 or 5.0 units/kg regular insulin, ●, $n = 8$; and ○, $n = 6$, respectively) or an equal volume of saline (□) ($n = 6$) was administered into the jugular vein at time 0. Total change in glucose concentration from baseline (AUC) to the right of each panel. Data are means ± SE and were analyzed by repeated-measures ANOVA and Scheffé's posthoc test. #Both insulin doses differ from saline but not from each other. *Saline and 0.5 units/kg dose of insulin similar and different from 5.0 units/kg dose of insulin. †5.0 units/kg dose of insulin different from saline only. Bars with different superscripts are different ($P < 0.05$).

fourth hypoglycemic episode, VMH glucose values remained lower than during the first hypoglycemic episode with total decrease in VMH glucose, as determined by AUC; this was the same for both groups ($P = 0.89$). Surprisingly, baseline glucose concentrations in the VMH were lower after 3 days of insulin treatment (0.79 ± 0.10 vs. 0.60 ± 0.10). The baseline glucose values before the first episode were similar to those of fasted rats in the first zero-net-flux study. This decrease was confirmed in the second zero-net-flux study in which rats were not fasted overnight before testing (Table 2).

The ratio of VMH to blood glucose during the baseline period was reduced in both studies (Fig. 3, *bottom*; and Table 2) and was independent of whether the animals were fed or fasted overnight. During the first episode of insulin treatment, changes in VMH interstitial glucose paralleled the changes in blood glucose. With the exception of the first 10-min sample period, when blood glucose values appeared to decline faster than VMH glucose values, interstitial glucose concentrations remained at ~15% of blood glucose levels. However, during the fourth episode of insulin-induced hypoglycemia there was a greater decline in VMH glucose concentrations than occurred in the blood ($F_{9,72} = 2.116$, $P < 0.05$), and the AUC was greater during the fourth episode ($F_{1,12} = 5.47$, $P = 0.04$). There was no significant difference (F1,12 = 3.29, $P = 0.09$) between treatments in the change from baseline ratio throughout the sample period. The greatest difference was apparent between 20 and 50 min when VMH glucose levels were only 6–9% of blood levels. The ratio returned to near baseline (12.5 ± 2.5%) by the end of the experiment. The Accu-Chek monitor was less accurate when blood glucose values were <2.2 mmol/l. Because not all blood glucose values were verified on the Beckman Glucose Analyzer, it is possible that during the 30-min period when hypoglycemia was <2.2 mmol/l, blood glucose values were slighter higher and the VMH-to-blood ratio slightly lower than reported.

DISCUSSION

The objective of this study was to determine the dynamics of interstitial glucose in the VMH when blood glucose levels were stable and during a hypoglycemic episode. Accurate determination of interstitial glucose in the VMH, in vivo, is necessary to better understand glucose sensing mechanisms that may reside in this area of the brain. At normal blood glucose levels, VMH interstitial glucose was ~1.6 mmol/l and was consistent across experiments. This value is lower than that reported by Silver and Erecinska (18) using glucose microelectrodes in the VMH of anesthetized rats. In the current study, samples were collected from conscious free-moving rats and, in general, reports of interstitial glucose levels in hippocampus, striatum, and

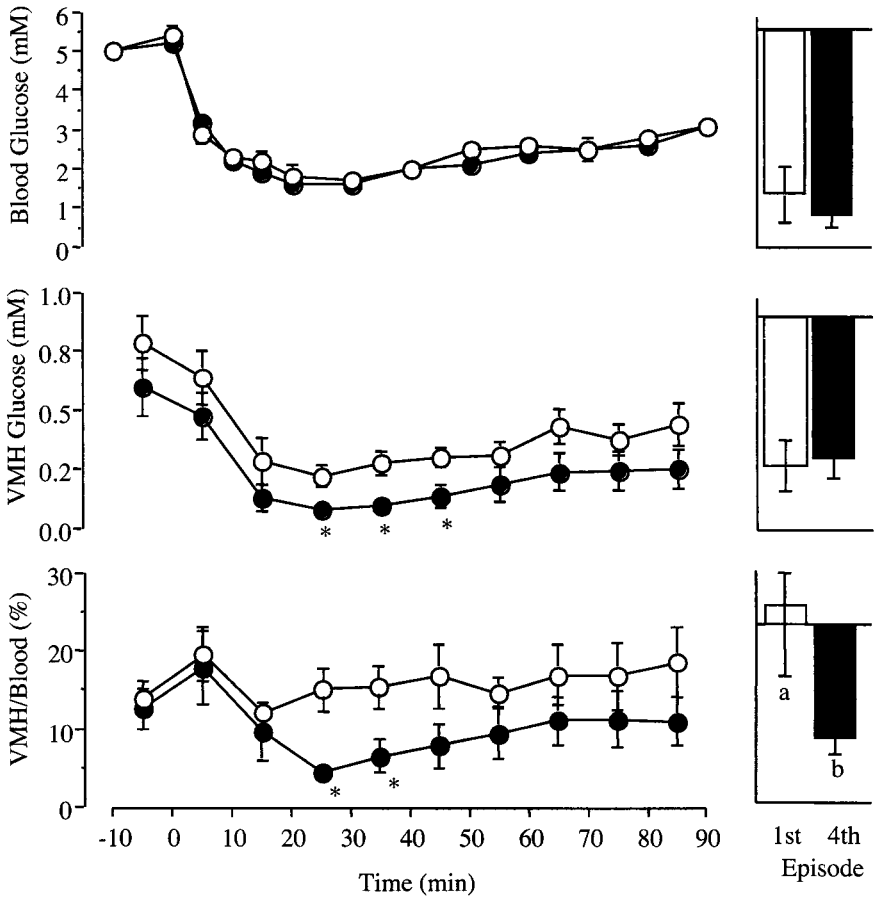

FIG. 3. Effect of recurrent hypoglycemia on changes in glucose concentration in blood (*top*), VMH interstitial fluid (*middle*), and on VMH-to-blood ratios (*bottom*) during the first (○; $n = 6$) or fourth (●; $n = 8$) hypoglycemic episode. The rats were fasted overnight before insulin (0.5 units/kg regular insulin) was infused into the jugular vein (time 0). Total change in glucose concentration from baseline (AUC) to the right of each panel. Data are means ± SE and were analyzed by repeated-measures ANOVA and Scheffé's post hoc test. *Fourth episode different from first episodes ($P < 0.05$).

cortex of anesthetized rats are higher than when free-moving rats were used (15,16,18). Compared with other reports using microdialysis (some using the zero-net-flux method), the level of glucose we report for the VMH is similar to the hippocampus (14) and higher than the striatum (12,13,33). Whether the differences reflect variation among brain areas or differences in technique remains to be determined. Baseline VMH glucose concentrations were similar across experiments in the present study, and there was good agreement between values determined by dividing dialysate glucose concentrations by probe efficiencies and those obtained by the zero-net flux method: 1.42 vs. 1.41 (fed), 0.73 vs. 0.84 (fasted), 1.71 vs. 1.62 (first episode), and 1.42 vs. 1.51 (fourth episode).

A relatively stable relationship between interstitial glucose concentration in the VMH and blood glucose apparent in control rats was only observed during a moderate degree of insulin-induced hypoglycemia. In these situations VMH glucose changed proportionally with the decline in blood glucose, remaining at ~20% of blood values, consistent with previous reports of extracellular glucose changing in parallel with changes in blood glucose (18,19). The stable VMH-to-blood ratio during moderate hypoglycemia is suggestive of a constant, predictable relationship between the methods for determining glucose concentrations in the different fluids. The higher VMH-to-blood ratio during the first 10-min sample period is consistent with blood glucose levels falling faster than the fall in interstitial glucose and may reflect a limited buffering capacity. Any buffering capacity was quickly overwhelmed when a more severe degree of systemic hypoglycemia was induced. VMH glucose concentrations declined to a greater degree than blood glucose, to ~10% of blood glucose levels. The steady decline in this ratio would be expected if glucose transport into the VMH was reduced, perhaps by a decrease in glucose uptake, a decrease in blood flow to the VMH, or if glucose metabolism in VMH was increased to a greater degree than glucose uptake from the blood. We are unaware of any reports documenting reduced blood-to-brain glucose transport or reduced blood flow in the hypothalamus during hypoglycemia. To the contrary, both cerebral blood flow (27,34) and glucose extraction (29,35) were reported to be increased during hypoglycemia. A decline in interstitial glucose may be related to increased metabolic and neural activity in the VMH, as ambient interstitial glucose was reduced when neural activity was induced (12,18,36). During hypoglycemia

TABLE 2
Baseline glucose concentrations in blood and VMH to recurrent hypoglycemia in fed rats

	Prior to 1st episode	Prior to 4th episode	t	P
n	10	10		
Blood glucose (mmol/l)	7.8 ± 0.1	7.5 ± 0.4	0.62	0.55
VMH Glucose (mmol/l)	1.71 ± 0.05	1.43 ± 0.07	4.09	0.0006
VMH/blood (%)	22.3 ± 0.6	17.8 ± 0.8	4.85	0.0001

Data are means ± SE. Extracellular glucose in VMH determined with zero-net-flux method.

there is an increase in noradrenergic and GABAergic activity in the VMH (3).

The lower steady-state interstitial glucose concentrations in the VMH following repeated episodes of hypoglycemia are difficult to reconcile with reports of an increase (29,35) or of no change (37) in brain glucose uptake and the suggestion that hypoglycemia unawareness is the result of enhanced brain glucose uptake (38). Receptor density of GLUT1 (localized on capillary endothelium of the blood-brain barrier) was increased after 1 week of chronic hypoglycemia (39–42). We are unaware of any studies documenting changes in GLUT1 expression after a single episode or multiple separate episodes of hypoglycemia. An increase in GLUT1 expression would support glucose uptake as being increased and, if true, an increase in cellular (i.e., neuronal and glial) glucose uptake and metabolism would be expected to account for the lower interstitial glucose. An increase in GLUT3, the primary isoform-associated glucose uptake into neurons, would be consistent with increased cellular uptake of glucose. However, neither an increase in GLUT3 expression nor an increase in cerebral glucose metabolism has been consistently measured during hypoglycemia (39,40). If glucose transport into the brain is increased, the lower steady-state glucose concentrations may reflect an increase in glycogen. Brain glycogen levels reduced during hypoglycemia rebounded to almost threefold the concentration before hypoglycemia (43). Fillenz et al. (36) proposed a role of glial glycogen in maintaining interstitial glucose concentrations, and the slight delay in the decline in VMH glucose could reflect mobilization of glycogen reserves from astrocytes, which contain glucose-6-phosphatase (44). A neuroprotective role for brain glycogen, which may also contribute to hypoglycemic unawareness, proposed by Fillenz et al. (36) and Choi et al. (43), is not supported by the present results. If greater glycogen was present in the VMH, the VMH–to–blood glucose ratio should have been improved during the fourth consecutive episode of hypoglycemia. In the present study, the fall in VMH glucose concentration was greater during the fourth hypoglycemic episode than during the first.

Surprisingly, a stable relationship between interstitial and blood glucose concentrations was not in evidence after a moderate chronic decrease in blood glucose (e.g., an overnight fast). Steady-state basal levels of glucose were reduced to a greater extent in the VMH than in blood, as evidenced by the reduced brain-to-blood ratio. This result contrasts that of Bequet et al. (19) who measured a similar decline (~32%) in both blood and cortical glucose following a 36-h fast. Steady-state glucose levels were also lower after recurrent episodes of moderate hypoglycemia and were further reduced by fasting. This result indicates that the mechanisms responsible for the lower steady-state VMH glucose after an overnight fast or recurrent hypoglycemia may be independent. During moderate episodes of hypoglycemia, the interstitial glucose declined to a similar degree (75–80% of baseline) during induced hypoglycemia regardless of steady-state levels before inducing hypoglycemia.

Ambient glucose concentrations in the VMH influence the activity of glucosensory neurons that likely initiate compensatory responses during hypoglycemia (4). Lesions of VMH suppress counterregulatory response (45), and maintaining a local glucose concentration in the VMH during systemic hypoglycemia reduced the increase in both plasma catecholamines and glucagon (8). The neurochemical phenotype of glucosensory neurons is uncertain; however, noradrenergic activity in the VMH was increased during an episode of induced hypoglycemia (3) and noradrenergic circuits in the VMH affect circulating concentrations of glucose and glucose-mobilizing hormones (46). Stimulation of VMH increased sympathetic efferent activity in the liver, hepatic glucose output (47,48), metabolic rate (49), and adrenal nerve activity (50). It is unclear from the present results whether activation of compensatory responses occurs in response to a relative change in interstitial glucose or an absolute change, such as reaching a threshold level. After an overnight fast, steady-state glucose levels in the VMH were lower than the nadir reached during an induced hypoglycemic episode; however, compensatory mechanisms are fully activated only in the latter situation. Most human studies evaluating physiological responses to hypoglycemia tend to fast overnight, while rodent studies do not. The rate of decline in ambient glucose concentrations may be an important factor in altering neural activity in the VMH. Most studies induce rapid declines in available glucose that would differ from the expected gradual decline expected during an overnight fast.

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