Hypoglycemia-induced noradrenergic activation in the VMH is a result of decreased ambient glucose

Martin G. de Vries,1,2 Marcus A. Lawson,1 and J. Lee Beverly1,2
1Department of Animal Sciences and 2Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana Illinois

Submitted 7 June 2005; accepted in final form 23 June 2005

The objective of the present study was to examine whether increased NE release in the VMH during hypoglycemia is mediated by changes in local glucose concentration in this brain area is known to play an important role in integrated hormonal and behavioral responses to systemic hypoglycemia. Selective glucoprivation restricted to the VMH is both necessary and sufficient to initiate secretion of counterregulatory hormones. The present study was designed to investigate whether increased release of NE in the VMH depends on detection of glucoprivation localized in this area. In awake, chronically catheterized male Sprague-Dawley rats, extracellular NE in the VMH was monitored using 1-mm microdialysis probes perfused with Krebs Ringer buffer (KRB) or KRB + 100 mM D-glucose (D-Glc). During insulin-induced hypoglycemia (glycemic nadir ~2.4 mM) extracellular NE was increased to >160% of baseline (P < 0.01) only in the KRB + insulin group. There was no increase in NE from baseline when glucose was added to the perfusate to maintain euglycemia at the periprobe environment. The sympathoadrenergic response to hypoglycemia, present in the KRB + insulin group, was attenuated in the D-Glc + insulin group. The present results confirm that noradrenergic activation in the VMH during systemic hypoglycemia depends on detection of glucoprivation locally in this area. These data provide additional support for the importance of increased noradrenergic activity in the VMH in the counterregulatory hormonal responses to hypoglycemia.

in vivo microdialysis; extracellular fluids; freely moving rats; glucosensing neurons

COMPENSATORY PHYSIOLOGICAL mechanisms that defend against systemic hypoglycemia, including both neural and hormonal efferent pathways, have been well studied (reviewed in Refs. 5 and 17). However, it is still not clear how and where glucoprivation is detected or what integrative neural circuitry is responsible for directing the appropriate compensatory output. The central nervous system seems to be the dominant center responsible for detection and integration of glucoprivic signals. Infusion of glucose into the carotid and vertebral arteries inhibited compensatory hormonal responses to systemic hypoglycemia (13). In addition, these hormonal responses could be inhibited by delivery of glucose specifically and bilaterally to the VMH in the face of glucoprivation everywhere else (7).

We have recently demonstrated that under euglycemic conditions the concentration of extracellular glucose in the VMH is ~20% that in the blood and that this ratio is largely maintained during mild hypoglycemia (10). There is wide support for the view that neurons in the VMH have the capability to detect glucoprivation in their local environment. During systemic hypoglycemia, detection of glucoprivation by neurons in the VMH seems necessary to trigger secretion of compensatory hormones because they were inhibited by concurrent bilateral perfusion of the VMH with glucose (7). Importantly, 2DG-induced glucoprivation limited to the VMH was also sufficient to initiate these hormonal responses and hence induce hyperglycemia (8). Because hypothalamic neurons express ATP-sensitive potassium channels, it has been suggested that glucose is sensed in the hypothalamus through a cellular mechanism similar to the one found in pancreatic β-cells (17). However, neurons in the VMH that alter their firing rate to changes in glucose concentration have been documented primarily in vitro (15, 31).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
area. For this purpose, a permanent catheter into the jugular vein for delivery of insulin was combined with unilateral VMH microdialysis in Sprague-Dawley rats. Glucose was delivered directly to the VMH of awake, unrestrained animals by adding glucose to the microdialysis perfusate. This way, systemic hypoglycemia effected glucoprivation in all peripheral tissues and in all brain areas except for the ipsilateral VMH. Confounding factors, such as anesthesia and the spreading of glucose to other brain areas, could thus be avoided. Here we test the hypothesis that a decrease in local glucose concentration in the VMH is necessary for increased noradrenergic activity in this area to occur during systemic hypoglycemia. If true, detection of glucoprivation in the VMH is essential for increased neuroendocrine and sympathoadrenal output to hypoglycemia by acting upon hypothalamic noradrenergic neurons.

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) weighing 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)- and temperature (25 ± 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.

After a 1-wk acclimation period, rats were fitted with a jugular vein catheter and a microdialysis guide cannula as previously described (10, 11). Briefly, rats were anesthetized with a mixture of ketamine·HCl, xylazine·HCl, and acepromazine (30:6:1 mg/kg im) and a 4-cm segment of Silastic tubing (0.64 mm ID; 0.94 mm OD) was inserted into the isolated right jugular vein, exteriorized through an incision on top of the head, and kept patent by filling the catheter with a 40% polyvinylpyrrolidone solution containing 500 U/ml heparin. A microdialysis guide cannula was stereotaxically positioned so that the tip of the guide was 2.4 mm posterior of bregma, 0.8 mm lateral of midline, and 6.4 mm below the dura (21). Tips of the microdialysis probes were designed to extend 2 mm beyond the end of the guide to place the 1-mm dialysis membrane into the lateral edge of the anterior portion of the VMH (immediately anterior to the perifornical region). 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 Beach, FL). After surgery, rats were monitored until completely recovered from the anesthesia and provided an analgesic (Banamine, 1.5 mg/kg sc). Cannula placements were verified histologically at the end of the study.

After a 5- to 7-day recovery period during which animals were handled daily and adapted to the experimental procedures, the rats were randomly assigned to a treatment group in the two-by-two factorial design. Blood and dialysate samples were collected from conscious unrestrained animals in their home cages during the midlight phase to minimize possible confounding by diurnal-associated changes. Food was removed during the sample collection period. Microdialysis probes with a 0.20 × 1 mm cuprophan membrane (AKZO-Nobel, Wuppertal, Germany) were placed into the VMH 3 h before sample collection at 1000. The probes were connected through a liquid swivel (Instech, Plymouth Meeting, PA) on a counterbalanced lever to a 1-ml gas-tight syringe on a microinfusion pump (CMA, North Chelmsford, MA). Probes were continuously perfused (flow rate, 1.5 μl/min) with Krebs-Ringer buffer (KRB). Twelve minutes before starting baseline collections, probes of half the animals were switched to a KRB containing 100 mM D-glucose (D-Glc). The probes contained in (mM) 147 NaCl, 4 KCl, and 2.4 CaCl2 (pH 6.4). Starting at 1300, dialysate was collected at 10-min intervals into chilled microtubes containing 3 μl 0.01 N perchloric acid and kept at −82°C until assayed. After collecting three baseline samples, rats received a bolus intravenous infusion (1 ml/kg) of sterile 0.9% saline or 2 U/kg regular insulin (Humulin, Eli Lilly, Indianapolis, IN). Blood samples (250 μl) were taken at t = −15, 0, 5, 15, 30, 45, 60, and 90 min. Blood glucose (50 μl) was immediately analyzed using a handheld glucose analyzer (Roche Diagnostics, Indianapolis, IN). The remaining 200 μl was transferred to chilled centrifuge tubes containing heparin, EDTA, and aprotinin. From these samples, plasma was stored...
at ~82°C until assayed for catecholamines. Donor blood from litters was infused after each sample to avoid diminution of blood volume. Probe efficiencies were determined in vitro in a standard NE solution and a flow rate of 1.5 μl/min.

Catecholamines were analyzed by reverse-phase HPLC and electrochemical detection (ESA, Chelmsford, MA) using a C18 (3 μm) Hypersil column (Keystone Scientific, Belfonte, PA) fitted with a 2-mm C18 (3 μm) Hypersil javalin guard column (Keystone Scientific) and mobile phase containing 75 mM NaH2PO4, 1.7 mM 1-octanesulfonic acid, 25 μM Na2EDTA, 7% (vol/vol) acetonitrile, and 0.1% (vol/vol) tetrahydrofuran (pH 3.0). The interassay coefficient of variation was ~3%. Plasma catecholamines were analyzed following solid-phase extraction with aluminum oxide (Bioanalytical Systems, West Lafayette, IN) and elution with 0.2 N perchloric acid. Dihydroxybenzylamine was used as internal standard to determine extracellular NE concentration.

Probe efficiencies were determined in vitro in a standard NE solution and a flow rate of 1.5 μl/min.

Results are presented as means ± SE. Blood glucose and catecholamines were analyzed by two-way repeated-measures ANOVA followed by Scheffé’s post hoc test. Main effects were buffer (KRB vs. d-Glc) and hypoglycemia (saline vs. insulin). The final number of rats in each group was 15. Increase in noradrenergic activity was apparent in the KRB control given insulin (Fig. 4). Extracellular NE increased to 170% of baseline during the first 10 min after insulin was administered to the KRB group [F(8,136) = 2.498, P < 0.01]. However, there was no change in extracellular concentration NE in the VMH of d-Glc rats administered insulin. Baseline NE concentration was 11% higher in the d-Glc group (7.35 ± 0.69 vs. 6.61 ± 0.50 mM, P = 0.52).

DISCUSSION

The main finding we report here is the increase in noradrenergic activity in the VMH during insulin-induced hypoglycemia, which is in response to a decrease in local glucose concentrations within the VMH. Maintaining glucose concentrations in the VMH in the face of systemic hypoglycemia prevented the increase in extracellular NE. Care was taken not to deliver too much glucose to the targeted site because local hyperglycemia is known to stimulate hypothalamic neurons and to cause increased sympathetic output to the periphery (26). To avoid these confounding responses, timing of the delivery of glucose and the concentration of glucose in the dialysate were adjusted to minimize any increase in glucose in the periprobe environment. Under similar conditions as this

plasma NE concentration was lower, there was no significant effect of adding glucose to the KRB [F(7,84) = 1.18, P = 0.32].

Increase in noradrenergic activity was apparent in the KRB control given insulin (Fig. 4). Extracellular NE increased to 170% of baseline during the first 10 min after insulin was administered to the KRB group [F(8,136) = 2.498, P < 0.01]. However, there was no change in extracellular concentration NE in the VMH of d-Glc rats administered insulin. Baseline NE concentration was 11% higher in the d-Glc group (7.35 ± 0.69 vs. 6.61 ± 0.50 mM, P = 0.52).

RESULTS

There was an immediate decrease [F(1,120) = 264, P < 0.001] in blood glucose following insulin administration (Fig. 1), which stabilized at ~2.4 mM during the dialysate collection period. There was no difference between the KRB and d-Glc groups. Increase [F(3,12) = 11.90, P < 0.001] in plasma epinephrine (Fig. 2) following insulin administration was significantly reduced by adding glucose to KRB [F(7,84) = 2.39, P < 0.05]. Epinephrine concentration at 90 min was different from baseline in the d-Glc group [t(1,11) = 9.93, P < 0.01]. Plasma NE (Fig. 3) was also increased [F(3,12) = 6.65, P < 0.01] by insulin-induced hypoglycemia; however, whereas
study, VMH glucose concentrations fell from ~1.7 to ~0.4 mM (10). The 100 mM glucose in dialysate and probe efficiency of 2.52 ± 0.20% for the ß-Glc groups should have buffered the fall in extracellular glucose. In a pilot study, using 50 mM glucose in the KRB was not sufficient to suppress noradrenergic activation in the VMH (M. G. de Vries, unpublished data). We do not know how broad an area of the VMH was maintained by diffusion of glucose from the probe site. However, absence of an increase in extracellular NE supports that diffusion was sufficient to reach those glucose-responsive elements in the VMH regulating local NE release.

Hypoglycemia-induced sympahtoadrenal activation was also attenuated when VMH glucose concentrations were maintained during systemic hypoglycemia. This result is consistent with the report of Borg et al. (7), who perfused the VMH bilaterally with glucose. These authors noted that the sympathetic response to systemic hypoglycemia was absent when 100 mM, but not 50 mM, ß-Glc was added to the dialysate. In the present study, there was an attenuation of the plasma epinephrine released during hypoglycemia, especially compared with the fourfold greater increase by the KRB + insulin group. Plasma epinephrine concentrations of the ß-Glc + insulin group did increase to nearly twice the two control groups (565 ± 85 vs. 290 ± 91 and 275 ± 33 pg/ml) by 90 min. This partial sympahtoadrenal response is likely a result of glucose being maintained only unilaterally; the concentration of extracellular glucose in the VMH contralateral to the perfused area was allowed to fall as it normally would (10). Restricting neuroglucopenia to the VMH was effective in evoking a counterregulatory hormonal response to elevate plasma glucose only when administered bilaterally (6, 8).

Noradrenergic systems in the hypothalamus play an important role in the regulation of compensatory hormonal responses to acute glucoprivation. Administration of NE into the hypothalamus increased the concentrations of blood glucose and circulating glucose-raising hormones (28, 32), whereas noradrenergic receptor antagonists in the medial hypothalamus blocked autonomic and neuroendocrine responses to 2DG (28, 29). With the use of microdialysis, endogenous release of NE and GABA in the hypothalamic extracellular space was carefully monitored during glucoprivation with 2DG (2, 3), as well as during systemic hypoglycemia induced by insulin (4, 11).

In the VMH, ~30% of neurons respond to changes in ambient glucose (19); almost two-thirds of these increase their firing rate when the glucose concentration is increased (glucose-excited) and the remaining one-third decrease their firing rate to an increase in glucose (glucose-inhibited) (31). The opposite effect on firing rate occurs when ambient glucose falls. The relationship of these glucosensory neurons to the noradrenergic inputs is unclear. It is unlikely that NE is released directly by glucosensing neurons. The VMH contains axon terminals of brainstem monoamine neurons, especially in the lateral fiber shell from which the current dialysate measurements were taken (12). We therefore suggest that detection of glucoprivation takes place presynaptically from NE release, probably by modulating the activity of neural projections from the brainstem. NE cell groups A1, A2, and A6, as well as epinephrine cell groups C1-C3, respond to glucoprivation (24, 25) and project to the medial hypothalumus (20, 22). It has been suggested that the brainstem is the primary site for generating most of the counterregulatory responses to glucoprivation, whereas the forebrain serves to regulate food intake and to modulate and integrate the various metabolism-related signals (17, 23). In the present study, we expect that these hindbrains were subjected to normal hypoglycemic changes in glucose, yet the blunted sympathoadrenal response when glucose was maintained in the VMH supports an important role of the VMH in counterregulatory responses to hypoglycemia.

Detection of hypoglycemia may occur when glucose-excited neurons are inhibited and/or glucose-inhibited neurons excited. To reconcile our current finding of increased NE release by neural projections in the VMH during hypoglycemia with one of the two types of presynaptic glucosensing neurons, the latter would have to be either glucose-inhibited while providing stimulatory input (e.g., release glutamate) or be of the glucose-excited type and thereby relieve inhibitory input (e.g., disinhibition from GABA). We demonstrated earlier that NE mediates an increase in GABAergic activity in the VMH during systemic glucoprivation (3). To our knowledge, the issue of what neurotransmitter system impinges upon the noradrenergic nerve terminals remains to be elucidated.

In summary, increased NE release in the VMH to insulin-induced hypoglycemia is inhibited when this area is locally perfused with glucose to keep it euglycemic in the face of systemic hypoglycemia. Apparently, the importance of the VMH in generation of appropriate compensatory hormonal responses is not only based on its responsiveness to integratedafferent input but also on the primacy of glucoprivation-detection to take place specifically in this brain area. Detection of glucoprivation in the VMH is essential for increased neuroendocrine and sympathoadrenal responses to hypoglycemia, possibly by acting presynaptically upon noradrenergic nerve terminals.

ACKNOWLEDGMENTS

The authors are grateful for the assistance of Dolores Doane, Zach Stahl-schmidt, and Meredith Barnes in completing this study.

Current address of M. G. de Vries is Dept. of Biomonitoring and Sensing, Univ. of Groningen Center for Pharmacology, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands.

GRANT

This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Research Grant DK-59755.

REFERENCES

6. Borg MA, Borg WP, Tamborlane WV, Brines ML, Shulman GI, and Sherwin RS. Chronic hypoglycemia and diabetes impair counterregu-


