Noradrenergic and GABAAergic systems in the medial hypothalamus are activated during hypoglycemia

J. LEE BEVERLY,1,2,3 MARTIN G. De VRIES,3 STEPHAN D. BOUMAN,4 AND LINDA M. ARSENEAU1

1Department of Animal Sciences, 2Division of Nutritional Sciences, and 3Program in Neuroscience, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801; and 4Department of Animal Physiology, University of Groningen, 9750 AA Haren, The Netherlands

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Noradrenergic and GABAAergic systems in the medial hypothalamus are activated during hypoglycemia. Am J Physiol Regulatory Integrative Comp Physiol 280: R563–R569, 2001.—Noradrenergic and GABAAergic systems in the medial hypothalamus influence plasma glucose and may be activated during glucoprivation. Microdialysis probes were placed into the ventromedial nucleus (VMH), lateral hypothalamus (LHA), and paraventricular nucleus (PVH) of male Sprague-Dawley rats to monitor extracellular concentrations of norepinephrine (NE) and GABA. During systemic hypoglycemia, induced by insulin (1.0 U/kg), NE concentrations increased in the VMH (P < 0.05) and PVH (P = 0.06) in a bimodal fashion during the first 10 min and 20–30 min after insulin administration. In the VMH, GABA concentrations increased (P < 0.05) in a similar manner as NE. Extracellular NE concentrations in the LHA were slightly lower (P = 0.13), and GABA levels remained at baseline. The increases in NE and GABA in the VMH were absent during euglycemic clamp; however, NE in the PVH still increased, reflecting a direct response to hyperinsulinemia. On the basis of these data, we propose that the activity of noradrenergic afferents to the medial hypothalamus is increased during hypoglycemia and influences the activity of local GABAAergic systems to activate appropriate physiological compensatory mechanisms.

THE VENTRAL HYPOTHALAMUS is a critical brain area for maintaining plasma glucose concentrations. This brain area influences the activity of parts of the sympathetic nervous system that directly affect glycemic state (17, 38). Within the ventromedial portion of the hypothalamus (VMH), the firing rates of some neurons are influenced by glucose availability and affect the activity of the sympathetic nervous system (23, 28). Lesions to the VMH abolished compensatory responses to systemic hypoglycemia (6), and confining glucoprivation to the VMH by direct application of the glucose analog 2-deoxy-d-glucose (2-DG), increased plasma concentrations of epinephrine, norepinephrine, and glucagon (7). Conversely, maintaining glucose levels within the VMH during systemic hypoglycemia reduced the hypoglycemia-associated increases in plasma catecholamines and glucagon (5).

Noradrenergic systems in the hypothalamus are likely to be involved in compensatory responses to glucoprivation. During hypoglycemia, increased norepinephrine (NE) turnover in the mediobasal hypothalamus, which includes the VMH, has been consistently described (1, 29, 37). The influence of noradrenergic systems in the VMH in glucose regulation is well established. Concentrations of glucose and glucose-mobilizing hormones in the circulation are increased in response to application of NE into the VMH (8, 30, 38). Studies using microdialysis or push-pull perfusion to directly measure extracellular NE in the VMH during hypoglycemia are less consistent. Using microdialysis probes in the VMH, Shimizu and Bray (33) reported an increase in extracellular NE 30–60 min after systemic hypoglycemia, yet no change in NE release to insulin was identified using push-pull perfusion (18). Several studies measuring tissue content of NE and its metabolites reported increased NE turnover in the VMH after a glucoprivic episode induced by 2-DG (27, 36, 37). In response to a 2-DG-induced glucoprivic challenge, concentrations of the neurotransmitter GABA and NE were increased in the VMH (2, 3). The increase in NE was reported to mediate the increase in GABA after 2-DG (3).

The first objective of the present study was to characterize noradrenergic activity and GABAAergic activity in discrete hypothalamic areas during a period of hypoglycemia. Microdialysis probes were used to monitor NE or GABA synaptic overflow in the VMH, the adjacent lateral hypothalamic area (LHA), and paraventricular nucleus (PVH) of rats during a period of insulin-induced hypoglycemia. A euglycemic clamp procedure was used to differentiate the responses to hypoglycemia from hyperinsulinemia. The results were similar to those observed after 2-DG-induced glucoprivation. Extracellular NE and GABA concentrations in the VMH were increased during hypoglycemia and followed the same profile measured after 2-DG.

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RESEARCH DESIGN AND METHODS

This study was approved by the Laboratory Animal Care Advisory Committee of the University of Illinois. Male Sprague-Dawley rats, ~250 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 (26 ± 2°C)-controlled room. Fresh water and rodent diet (Harlan Teklabs, Madison, WI) were available at all times, except during sample collection.

Surgical procedures. After a 1-wk acclimation period, rats were anesthetized with a mixture of ketamine HCl, xylazine HCl, and acepromazine (30:6:1 mg/kg im). The level of anesthesia was monitored and maintained at an appropriate level throughout the surgical procedure. The top of the head and the neck of each rat were shaved, and the skin was washed with povidone-iodine 10% (Betadine). A jugular vein catheter was aseptically placed through a vertical incision in the neck. The right external jugular vein was isolated, and a 4-cm segment of Silastic tubing (0.025 in. ID × 0.047 in. OD) was inserted toward the heart. The catheter was secured with 5–0 suture, tunneled under the skin, and exteriorized through an incision on top of the head before the skin in the neck was closed with wound staples. Some animals also received a femoral vein catheter. A section of the left femoral vein was isolated, and a 1-cm segment of Silastic tubing (0.020 in. ID × 0.035 in. OD) was inserted into the vein, tunneled under the skin, and exteriorized through the incision on top of the head. The incision on the lower abdomen was closed with wound staples. A piece of 21- or 23-gauge stainless steel tubing was inserted onto the end of each catheter, and the catheters were filled with a 40% polyvinylpyrrolidone (PVP) solution containing 500 U heparin/ml and capped with a sealed piece of Tygon tubing to maintain patency. A catheter was attached to the stainless steel tubing at the end of the catheter. Dialysate samples were collected at 10-min intervals and immediately frozen at the end of each sample period and maintained at −84°C until assayed. Samples for catecholamine analysis were collected into microtubes containing 0.2 μl 0.1 N perchloric acid. Blood samples (~20 μl) were collected at regular intervals, and plasma glucose concentration was measured on a handheld glucose monitor (Boehringer Manheim).

Effect of insulin-induced hypoglycemia on NE and GABA. To establish basal concentrations of NE and GABA, three 10-min dialysate samples were collected before administering 1.0 U/kg regular insulin (HumulinR, Eli Lilly, Indianapolis, IN) was infused into the jugular vein catheter (n = 6–8 rats per brain area). The insulin was diluted in sterile saline and delivered at a volume of 1 ml/kg body wt (n = 5 rats per brain area). An equal volume of sterile saline was used as a control. Dialysates were collected at 10-min intervals over the next 60 min. Blood glucose concentrations were determined at the midpoint of each 10-min sample. Separate animals were used for analysis of NE and GABA. To distinguish whether the responses in NE and GABA were due to hypoglycemia or hyperinsulinemia, a euglycemic clamp procedure was used. Baseline neurotransmitter and plasma glucose concentrations were determined as in the first experiment. The third baseline sample was collected and 3–5 min before administering 1.0 U/kg regular insulin (HumulinR), sterile saline or a 20% glucose solution (in sterile saline) was infused into the femoral vein at a constant rate (20 μl/min). Blood glucose levels were checked at 2-min intervals for the next 30 min, then at 5-min intervals until the end of the study. The infusion rate of the glucose solution (or saline) was adjusted to maintain blood glucose concentrations near baseline for 60 min after insulin infusion. Dialysate samples were collected at 10-min intervals for 90 min after insulin administration. At the end of the experiments, rats were anesthetized, and the heart was exposed. The right auricle was punctured, and ~60 ml of chilled saline followed by ~60 ml of 10% formalin solution was perfused through the brain via the left ventricle. The formalin-fixed brain was removed from the skull, and intrahypothalamic cannula position was verified histologically.

Sample analysis. Catecholamines were analyzed on a Dynamax SD-200 system (Varian Instruments, Woburn, MA) by reverse-phase HPLC and electrochemical detection. Samples (5–10 μl) were injected onto a 150 × 2-mm C18 (3 μm) Hypersil column fitted with a 2-mm C18 (3 μm) Hypersil j rival guard column (Keystone Scientific, Bellfonte, PA). Mobile phase (pH 3.0) was 75 mM NaH2PO4, 1.7 mM L-ascorbic acid, 10% (vol/vol) acetonitrile, and 0.1% (vol/vol) tetrahydrofuran. A DECADE electrochemical detector fitted with a VT-03 glassy carbon electrode (Antec Leyden, Leiden, The Netherlands) set at +0.75 V was used with Dynamax MacIntegrator II and “C” module programs (Rainin Instruments, Woburn, MA) for peak integration and quantification. With this method, sensitivity for dialysate samples (peak height twice baseline) was 0.1 nM, with an interassay coefficient of variation of ≤3%.
GABA was analyzed on a BAS 480 Analyzer (Bioanalytical Systems) by a reverse-phase HPLC method, using a modified isocratic procedure (2). Sample (20 µl) was mixed with 2.5 µl of derivatization reagent (11 mg O-phthalaldehyde in 5 ml of a 0.1 M sodium borate buffer (pH 9.2) containing 5% methanol and 250 µl of 0.03 M sodium sulfate) and heated at 35°C for 5 min before injection onto a 100 x 4-mm C18 (3 µm) reverse-phase Microsorb column and 5 x 4-mm C18 guard column (Varian Instruments). Mobile phase (pH 5.0) was 0.1 M sodium phosphate buffer containing 0.1 mM EDTA and 15% (vol/vol) methanol. Quantitation was by electrochemical detection (BAS LC-4C, Bioanalytical Systems), using a glassy carbon electrode set at +0.85 V. Sensitivity was 2.5 nM. Data were collected and analyzed using Chromgraph software (Bioanalytical Systems).

Data analysis. The effect of hypoglycemia (i.e., saline vs. insulin or clamp vs. non-clamp) on extracellular neurotransmitter concentration within a brain area was analyzed by repeated-measures ANOVA. Changes in the response at individual time points, within a treatment, were determined by ANOVA and Scheffe’s multiple-comparison test. Post hoc analysis of a significant ANOVA was by Scheffe’s multiple-comparison test. Results are presented as means ± SE. Plasma glucose concentrations were analyzed by repeated-measures ANOVA and Scheffe’s multiple-comparison test.

Supplies. Ketamine, acepromazine, and butorphanol 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

In response to insulin-induced hypoglycemia, extracellular NE concentrations changed in the VMH [F(1,10) = 7.97, P = 0.02] and PVH [F(1,10) = 6.98, P = 0.02] but not the LHA [F(1,10) = 2.26, P = 0.16]. During hypoglycemia, extracellular NE in both the VMH and PVH were elevated in a bimodal fashion [Fig. 1; F(8,54) = 2.90, P < 0.01 and F(8,54) = 2.46, P = 0.02, respectively]. Concentrations of NE in VMH were increased to 165 ± 27% during the first 10 min after insulin and 165 ± 22% during the 20- to 30-min period. In the PVH, NE increased to 148 ± 16 and 146 ± 16% during the 0- to 10-min and 20- to 30-min sample periods, respectively. In the LHA, NE concentrations during hypoglycemia were lower than baseline as extracellular NE was reduced to 79 ± 6% of baseline during the 20- to 30-min collection period after insulin. Baseline concentrations of NE in dialysates were 0.25 ± 0.10, 0.33 ± 0.15, and 0.74 ± 0.14 nM in the VMH, LHA, and PVH, respectively, after standardizing to in vitro recovery of 5.0% (in vitro recoveries were 4.9 ± 0.4%). Blood glucose concentrations during the baseline period were 6.1 ± 0.4 mM across brain areas and reached a nadir of 2.8 ± 0.4 mM by 15 min after insulin administration.

There was a significant difference among treatment groups in the NE response in both the VMH [F(1,9) = 23.99, P < 0.01] and PVH [F(1,8) = 7.07, P = 0.03] in the glucose clamp study. As observed in the first set of experiments, extracellular NE concentrations in the VMH [F(11,36) = 2.97, P < 0.01] and PVH [F(11,36) = 2.68, P = 0.01] increased in a bimodal fashion (Fig. 3) in control animals receiving saline infusions into the femoral vein. When plasma glucose concentrations were maintained at baseline levels, the increase in NE was absent in the VMH [F(11,72) = 1.04, P = 0.42] but not the PVH [F(11,60) = 1.80, P = 0.07]. In the PVH, the initial increase in NE after insulin was still apparent in rats during the euglycemic clamp procedure, although the timing of the changes was different [F(11,88) = 2.62, P < 0.01]. In this set of animals, the second peak increase in NE (136 ± 14% of baseline) was apparent in the 30- to 40-min sample period and there was a third peak (129 ± 9% of baseline) during the 60- to 70-min time period. The increase in GABA concentrations in the VMH after insulin administration was also absent in animals during the euglycemic clamp procedure [Fig. 4; F(1,9) = 9.07, P = 0.01].
was also a difference among treatment groups \([F(1,9) = 19.96, P < 0.01]\) in the euglycemic clamp study when GABA was measured in the VMH (Fig. 4). The bimodal increase in GABA concentrations in the VMH was apparent in saline-infused control animals \([F(11,36) = 2.74, P = 0.01]\) but was absent during euglycemic clamp \([F(11,72) = 1.04, P = 0.42]\). During euglycemic clamp studies, glucose concentrations were maintained near baseline concentrations \((6.2 \pm 0.4 \text{ mM})\) for 60 min after insulin (Fig. 5). Blood glucose concentrations fell to \(45 \pm 7\%\) of baseline in saline-infused animals.

**DISCUSSION**

Noradrenergic and GABAergic activity in the medial hypothalamus are increased during an acute hypoglycemic episode. A bimodal increase in extracellular NE in both the VMH and PVH was consistently recorded during the first 30 min of induced hypoglycemia. The pattern of increased NE release was consistent across experiments, occurring in 80% of the rats tested, differing only in the timing of the second peak in the PVH during the euglycemic clamp procedure. The present results differ from earlier reports in either the timing or direction of NE release in medial hypothalamic sites (18, 33). The difference in the timing of the increase in NE concentration may be due to the rate hypoglycemia is induced, inasmuch as the intravenous administration of insulin in the present study would be expected to elicit a quicker decline in plasma glucose than the intraperitoneal route used in the previous reports. There is also disagreement among reports on the NE response in the LHA. During hypoglycemia, there was a slight decrease in NE concentrations in the present study and decreased NE release in a push-pull perfusion study (18). However, extracellular NE concentrations in the LHA were reported to be higher after insulin administration in a microdialysis study (33). In the present study, the decrease in NE in the LHA was apparent in \(-50\%\) of rats tested and occurred during the same time interval as a decrease in extracellular GABA in the LHA after 2-DG (2).
The increases in extracellular GABA and NE in the VMH appear to be common responses to glucoprivation. The bimodal increases during hypoglycemia mirrored those observed in the VMH after cellular glucopenia induced by 2-DG (2, 3). The increase in extracellular NE concentrations in the VMH and PVH during either glucoprivic episode is consistent with reports of increased NE turnover in the hypothalamus after either insulin (1, 35) or 2-DG (27, 36). The increased extracellular GABA during hypoglycemia is also in agreement with reports of increased GABA content (15) and increased GABA synthesis (4) in the VMH during hypoglycemia. Of interest is the common observation that NE in the medial hypothalamus remained elevated when animals were not allowed to eat after a glucoprivic episode. Extracellular NE concentrations in the VMH remained above baseline up to 90 min after insulin administration in the present study and were still elevated 6 h after insulin administration in an earlier study (33), returning to baseline when rats were allowed to eat. Extracellular GABA concentrations in the VMH also remained above baseline at least 60 min after insulin (this study) or after 2-DG administration (2) in animals not allowed to eat. When rats were allowed to eat after 2-DG, GABA concentrations returned to baseline levels after 30 min (2). Because the pattern of NE and GABA release after both hypoglycemia and 2-DG was similar, it is unlikely that the increases in either neurotransmitter were due to increased glucose concentrations after 2-DG administration.

The increases in noradrenergic and GABAergic activity in the VMH are consistent with their being involved in the compensatory responses to hypoglycemia. Electrical or chemical stimulation of the VMH increased sympathetic nerve activity (43) and plasma glucose concentrations (40, 41). Conversely, lesions of the VMH reduced the characteristic increases in plasma catecholamines and glucagon in response to hypoglycemia (6). Involvement of NE in the VMH is suggested by reports of increased NE release in the VMH after stimulation of the VMH (41) and increases in the activity of sympathetic efferents (28), plasma glucagon (9), and plasma glucose concentrations (8, 36, 38) after microinjection of NE into the VMH. Increases in blood glucose to a glucoprivic challenge (20, 36) or exercise (30, 38) were also blunted by disruption of NE activity in the medial hypothalamus. In addition, the feeding response to glucoprivation was absent after depletion of NE in the hypothalamus (20, 27, 36).

The functional significance of the bimodal pattern in neurotransmitter activity in the medial hypothalamus is unclear. The two peaks may be involved in different aspects of glucose mobilization. Scheurink et al. (30) noted differences in the role of VMH α- and β-adrenoceptors on sympathetic activity during exercise. An exercise-induced increase in plasma epinephrine was reduced by β-blockade in the VMH, whereas the increase in plasma NE concentration during exercise was reduced when timolol, a β-adrenoceptor antagonist, was administered into the VMH. The necessity of a functional GABAergic system in the VMH for the feeding response to hypoglycemia was demonstrated when application of the GABA-receptor antagonist bicuculline into the VMH blocked insulin-induced feeding (14).

Because the increases in extracellular NE and GABA in the VMH were absent during the euglycemic clamp procedure, the increases in these neurotransmitters were due to hypoglycemia. Although it is likely that peripheral glucose-responsive elements influence hypothalamic activity (13, 21), the increased NE and GABA may be primarily affected by decreased extracellular glucose in the medial hypothalamus. Neural activity in and functional output from the hypothalamus are directly affected by glucose availability. Extracellular glucose concentrations in the brain reflect circulating glucose concentrations, being higher during hyperglycemia and lower during hypoglycemia (34). In the medial and lateral hypothalamus, 20–40% of isolated neurons were responsive to extracellular glucose concentrations (23), and glucose infusions into the carotid artery affected the activity of neurons in medial hypothalamus (10). Adding glucose to perfusion buffer inhibited NE release in the VMH (19) and reduced GABA release from medial hypothalamic pieces (11). The suppressive effect of glucose on NE release was also apparent in hyperglycemic streptozotocin-induced diabetic rats (22, 32). The reduced extracellular NE concentration in VMH of diabetic rats was normalized when glucose levels were maintained by supplemental insulin (22). Finally, maintaining extracellular glucose concentrations in the VMH during a glucoprivic chal-
lence blocked the counterregulatory responses to systemic hypoglycemia (5).

The relationship between NE and GABA in the VMH during hypoglycemia is likely to be the same as during 2-DG-induced glucoprivation. The increase in GABA concentrations in the VMH in response to 2-DG was promoted by the increase in NE (3). The initial peak in GABA release after 2-DG was regulated by α-adrenoceptors, and the second peak in GABA release was regulated by β-adrenoceptors (3). The noradrenergic nerve terminals in the hypothalamus extend from hindbrain areas (25), whereas GABA is likely to be from local interneurons in postsynaptic contact with NE neurons. Noradrenergic neurons innervating the preoptic hypothalamus have been demonstrated to regulate GABA release by direct synaptic connection (12). It remains to be determined what neurons are immediately postsynaptic to GABA and where these neurons extend.

The changes in NE concentrations in the PVH may have been due to a direct effect of insulin and not a response to glucoprivation. Unlike the response in the VMH, the increase in extracellular NE after insulin administration was still apparent during the euglycemic clamp procedure. The increase in NE being due to insulin may explain why there was no increase in extracellular NE in the PVH after 2-DG administration (3). A direct effect of insulin was also indicated when insulin perfused directly into the medial hypothalamus, during push-pull perfusion, increased NE release (19), and intracerebroventricular insulin administration increased NE turnover in the hypothalamus (29). In addition, NE release from hypothalamic brain slices was increased when insulin was added to the incubation medium (29). Smythe et al. (35) suggested that the increase in NE turnover in the medial hypothalamus during hypoglycemia was due to a direct effect of insulin. Circulating insulin is taken up into the brain (24, 31, 39) and binds to insulin receptors present throughout the medial hypothalamus (42). Although insulin does have a direct effect on glucose-responsive neurons in the hypothalamus (23), it is unclear whether the initial increase in NE in PVH in the present study was due to the injected insulin. The uptake of insulin from the circulation into the cerebral spinal fluid was reported to be longer than 30 min (31, 39). However, using a sensitive insulin assay and microdialysis probes, Oroso et al. (24) measured insulin in the VMH and PVH during the first 30 min of a meal.

In summary, noradrenergic and GABAergic activities in medial hypothalamic areas were activated during hypoglycemia. This response is consistent with in vitro evidence of increased NE turnover (36, 37) and GABA synthesis (4) as well as early in vivo measures of increased NE activity in the medial hypothalamus (18, 33). The bimodal increases in GABA and NE activity in the VMH followed the same pattern previously measured after 2-DG (2, 3) and is suggestive of a common response to glucoprivic episodes. Because glucoprivation localized to the VMH was sufficient to induce compensatory responses (7), the VMH is a critical site for monitoring glucose status. The changes in activity of these two neurotransmitter systems are likely to be parts of a mechanism by which changes in circulating glucose are translated into neurochemical activity.

**Perspectives**

The mechanisms by which the brain monitors plasma glucose status are unknown. The activity of 30–50% of the neurons in the VMH is affected by local glucose concentrations (reviewed in Ref. 16). As plasma glucose concentrations decline, glucose concentrations in the hypothalamus and other sensory sites influence the activity of noradrenergic and GABAergic elements in the medial hypothalamus. A role of NE and GABA in the medial hypothalamus in behaviors, endocrine, and autonomic function has been documented and is consistent with our suggestion that increased activity of noradrenergic afferents to the medial hypothalamus work through local GABAergic systems to activate appropriate physiological systems to increase circulating glucose. The nature of the local control in the hypothalamus, the systems activated by GABA, and other modulating factors remain to be determined.

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