Original Article

Blockade of $GABA_A$ Receptors in the Ventromedial Hypothalamus Further Stimulates Glucagon and Sympathoadrenal but Not the Hypothalamo-Pituitary-Adrenal Response to Hypoglycemia

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Hypoglycemia provokes a multifaceted counterregulatory response involving the sympathoadrenal system, stimulation of glucagon secretion, and the hypothalamo-pituitary-adrenal axis that is commonly impaired in diabetes. We examined whether modulation of inhibitory input from γ-aminobutyric acid (GABA) in the ventromedial hypothalamus (VMH), a major glucose-sensing region within the brain, plays a role in affecting counterregulatory responses to hypoglycemia. Normal Sprague-Dawley rats had carotid artery and jugular vein catheters chronically implanted, as well as bilateral steel microinjection guide cannulas inserted down to the level of the VMH. Seven to 10 days following surgery, the rats were microinjected with artificial extracellular fluid, the $GABA_A$ receptor agonist muscimol (1 nmol/side), or the $GABA_A$ receptor antagonist bicuculline methiodide (12.5 pmol/side) before being subjected to a hyperinsulinemic-hypoglycemic (2.5 mmol/l) glucose clamp for 90 min. Following VMH administration of bicuculline methiodide, glucose infusion rates were significantly suppressed, whereas muscimol raised glucose infusion rates significantly compared with controls. Glucagon and epinephrine responses were elevated with the antagonist and suppressed with the agonist compared with controls. Corticosterone responses, however, were unaffected by either administration of the agonist or antagonist into the VMH. These data demonstrate that modulation of the GABAergic system in the VMH alters both glucagon and sympathoadrenal, but not corticosterone, responses to hypoglycemia. Our findings are consistent with the hypothesis that GABAergic inhibitory tone within the VMH can modulate glucose counterregulatory responses. *Diabetes* 55:1080–1087, 2006

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aECF, artificial extracellular fluid; GABA, γ-aminobutyric acid; HPA, hypothalmo-pituitary-adrenal; K_{ATP} channel, ATP-sensitve K^+ channel; VMH, ventromedial hypothalamus.

Hypoglycemia elicits a multitude of endocrine and neuroendocrine responses that act in concert to raise blood glucose levels to normal. As blood glucose levels start to fall, there is a cessation of insulin secretion, and this is followed shortly by a rise in glucagon and epinephrine levels (1). During prolonged or more severe hypoglycemia, growth hormone and cortisol act to aid in the recovery from hypoglycemia. Normally, this cascade of events works efficiently to restore blood glucose levels to normal; however, in the case of prolonged diabetes or in cases where diabetic patients are exposed to recurrent episodes of hypoglycemia, these mechanisms can be compromised (2–4). The cause of this defect is still unclear. Studies in recent years have focused on how the body detects these falls in blood glucose levels and how counterregulatory responses to hypoglycemia are initiated.

Glucose sensors have been localized in the portal vein (5–7), carotid body (8–10), and brain (11–19). One brain region in particular, the ventromedial hypothalamus (VMH), has been shown to play an important role not only in sensing falls in blood glucose levels but also in initiating counterregulatory responses to hypoglycemia (11,20–23). It has been suggested that this sensing mechanism may be similar to that in the pancreas, whereby glucose is metabolized and changes in the ATP-to-ADP ratio are detected by ATP-sensitve K^+ channels (K_{ATP} channels) in order to ultimately regulate the release of insulin (22,24), or in the case of the brain, most likely detected by neurotransmitters or neuropeptides. Many of the crucial components of the pancreatic glucose-sensing mechanism, such as glucose transporters, glucokinase, and K_{ATP} channels, have been localized to glucose-sensing neurons within the VMH (25). However, the precise mechanism by which hypoglycemia is detected by the central nervous system and, subsequently, how counterregulatory responses are triggered has not been fully understood.

γ-Aminobutyric acid (GABA) is the most ubiquitous inhibitory neurotransmitter in the mammalian central nervous system. More specifically, both GABA and its biosynthetic enzyme GAD, are present in essentially all parts of the hypothalamus (26), and it is clear that the blockade of GABAergic neurons causes hemodynamic, hormonal, and metabolic alterations that mimic the response to stress (27–30). One stimulus in particular, glucoprivation, has been shown to alter GABA levels within the brain (31–33),

but the evidence for its role in glucose counterregulation remains somewhat controversial. Alprazolam, a benzodiazepine analog, has been shown to reduce neuroendocrine and adrenomedullary responses to hypoglycemia in humans and primates (34,35). On the other hand, Modafinil, which is thought to lower brain GABA concentrations, has no significant effect on counterregulatory hormone release but does improve adrenergic sensitivity and some aspects of cognitive function during hypoglycemia (36). Furthermore, regionally specific determinations of GABA levels in VMH microdialysate samples revealed a biphasic rise in GABA levels during glucoprivation (33,37) that may be attributed to a corresponding rise in local norepinephrine concentrations (32).

Thus, the objective of this study was to determine whether manipulation of GABAergic inhibitory tone in the VMH of normal animals, using either the $GABA_A$ receptor agonist muscimol or the $GABA_A$ receptor antagonist bicuculline methiodide affects glucose counterregulation during hypoglycemia. Our data demonstrate that GABAergic tone in the VMH plays an important role in determining the magnitude of glucagon and sympathoadrenal, but not corticosterone, responses to hypoglycemia.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 300–350 g were individually housed in the Yale Animal Resource Center in temperature- (22–23°C) and humidity-controlled rooms. The animals were fed rat chow (Agway Prolab 3000; Syracuse, NY) and water ad libitum and were acclimatized to a 12-h light-dark cycle (lights on between 0700 and 1900) for a period of 1 week before experimental manipulation. Principles of laboratory animal care were followed, and experimental protocols were approved by the Yale University Institutional Animal Care and Use Committee.

Hypoglycemic clamp studies. Three groups of rats were used: control rats administered artificial extracellular fluid (aECF) ($n = 8$), rats administered the $GABA_A$ receptor antagonist bicuculline methiodide ($n = 8$), and rats administered the $GABA_A$ receptor agonist muscimol ($n = 10$). On day 0, vascular catheters were placed into the left carotid artery and right jugular vein, as described previously (38). In addition, bilateral stainless steel microinjection guide cannulas were stereotaxically positioned 1 mm dorsal to the VMH according to the coordinates of Paxinos and Watson (from bregma: −2.6 mm anterior posterior, ±3.8 mm medial lateral, and −8.3 mm dorsal ventral, at an angle of 20°) (39). The animals were then allowed 7–10 days to recover from surgery and to acclimatize to being handled. Those animals that did not recover to their presurgery body weights were excluded from the study. Following an overnight fast, the experiments were carried out in conscious and unrestrained animals. Catheters were extended outside of the cage to minimize investigator interaction and were connected to infusion pumps. The animals were allowed ~2–2.5 h to recover from handling before basal hormone samples were collected (between 0930 and 1000), just before the start of the study. Following the collection of basal blood samples, microinjection needles were inserted down to the level of the VMH, and 0.1 μl aECF (135 mmol/l NaCl, 3 mmol/l KCl, 1 mmol/l $MgCl_2$, 1.2 mmol/l $CaCl_2$, and 2 mmol/l Na_2HPO_4) and 0.75 mmol/l glucose (40,41), bicuculline methiodide in aECF (12.5 pmol/side), or muscimol in aECF (1 nmol/side) was delivered over the course of 1 min using a CMA/102 pump (CMA Microdialysis, N. Chelmsford, MA). The dose of bicuculline methiodide was achieved by gradually titrating down a concentration of the compound that caused convulsions until reaching a level that was just below the threshold where convulsions were consistently observed. With muscimol, the lowest dose that produced a consistent effect was used. Pilot studies using India ink verified that a volume of 0.1 μl delivered over 1 min was adequately localized to the VMH region. The rats then underwent a hyperinsulinemic-hypoglycemic glucose clamp. A constant insulin (50 mU · kg^{-1} · min^{-1} regular human insulin; Eli Lilly, Indianapolis, IN) and variable dextrose (20%; Abbott, Chicago, IL) infusion through the jugular vein catheter was used to maintain plasma glucose levels at 2.5 ± 0.3 mmol/l for 90 min. The concentration of glucose was determined every 5 min from a sample of plasma (10 μl) using a Beckman glucose analyzer II (Beckman, Palo Alto, CA). Arterial blood samples were obtained from the carotid catheter at 0, 30, 60, 90, and 120 min following the start of the insulin infusion. For glucagon and insulin measurements, 250 μl whole blood was collected in chilled tubes containing EDTA (J.T. Baker, Phillipsburg, NJ) and Trasylol (Bayer, Tarrytown, NY). Blood samples for catecholamines (250 μl) were collected in chilled tubes containing 4 μl reduced glutathione/EGTA (Amersham, Arlington Heights, IL). For corticosterone measurements, 250 μl whole blood was collected into an eppendorf tube and serum separated from the erythrocytes. The plasma and serum were aliquoted into storage tubes and stored at −20°C (or −80°C for catecholamine determination). Erythrocytes, after removal of plasma, were resuspended in an equivalent volume of sterile artificial plasma (115 mmol/l NaCl, 5.9 mmol/l KCl, 1.2 mmol/l $MgCl_2$, 1.2 mmol/l NaH_2PO_4, 1.2 mmol/l Na_2SO_4, 2.5 mmol/l $CaCl_2$, 25 mmol/l $NaHCO_3$, and 4% BSA, pH 7.45) and reinfused after each blood sampling to prevent volume depletion and anemia.

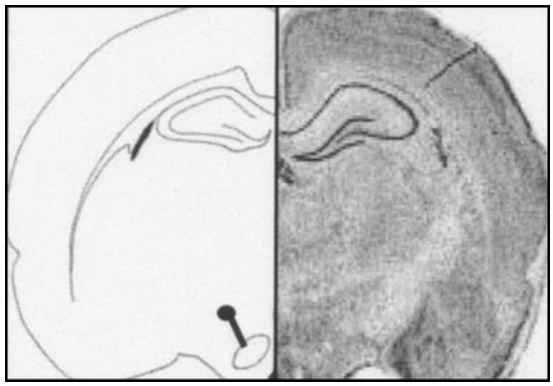

FIG. 1. Representative picture of a coronal rat brain section depicting the typical position of microinjection probes. In the *left panel*, the ball indicates the end of the guide cannula and the small bar indicates the 1-mm extension of the microinjection needle.

Euglycemic clamp studies. To test the specificity of our manipulations on counterregulatory responses to hypoglycemia, we performed a set of hyperinsulinemic-euglycemic (6.4 ± 0.6 mmol/l) clamps in two groups of animals: control rats administered aECF ($n = 5$) and rats administered the $GABA_A$ receptor agonist muscimol ($n = 6$). As described above, these compounds were microinjected into the VMH just before the start of constant insulin (50 mU · kg^{-1} · min^{-1}) and variable dextrose (50%; Abbott) infusions. The same blood sampling protocol as described above was followed.

Probe placement. At the end of the experiments, the rats were killed with an overdose of sodium pentobarbital (Sleepaway; Fort Dodge Animal Health, Fort Dodge, IO). Brains were removed, frozen, and stored at −80°C until analysis. Accuracy of microinjection needle placements were verified histologically in coronally sectioned brain slices at the end of the study. A schematic of typical needle placements is shown in Fig. 1. Only data collected from those animals with probes in the appropriate target area were included for statistical analysis.

Plasma hormone and catecholamine determination. Plasma insulin (Linco Research, St. Charles, MO), corticosterone (Diagnostic Products, Los Angeles, CA), and glucagon (Linco Research) concentrations were determined using commercially available radioimmunoassay kits. Plasma epinephrine and norepinephrine concentrations were analyzed by high-performance liquid chromatography using electrochemical detection (ESA, Acton, MA).

Data analysis. Hormonal data are presented as means ± SE. Statistical analysis was performed by one- or two-way ANOVA for independent or repeated measures, as appropriate, followed by post hoc analysis using

TABLE 1
Baseline hormone concentrations for the hypoglycemic clamp study

	Control	Bicuculline	Muscimol
n	8	8	10
Body weight (g)	334 ± 7	330 ± 8	334 ± 14
Plasma glucose (mmol/l)	6.3 ± 0.1	6.1 ± 0.2	6.2 ± 0.2
Corticosterone (ng/ml)	63 ± 14	89 ± 23	67 ± 30
Glucagon (ng/l)	39 ± 5	37 ± 2	44 ± 5
Epinephrine (pg/ml)	87 ± 27	95 ± 35	51 ± 14
Norepinephrine (pg/ml)	87 ± 32	112 ± 18	121 ± 14
Insulin (μU/ml)	11 ± 5	18 ± 10	10 ± 2

Data are means ± SE.

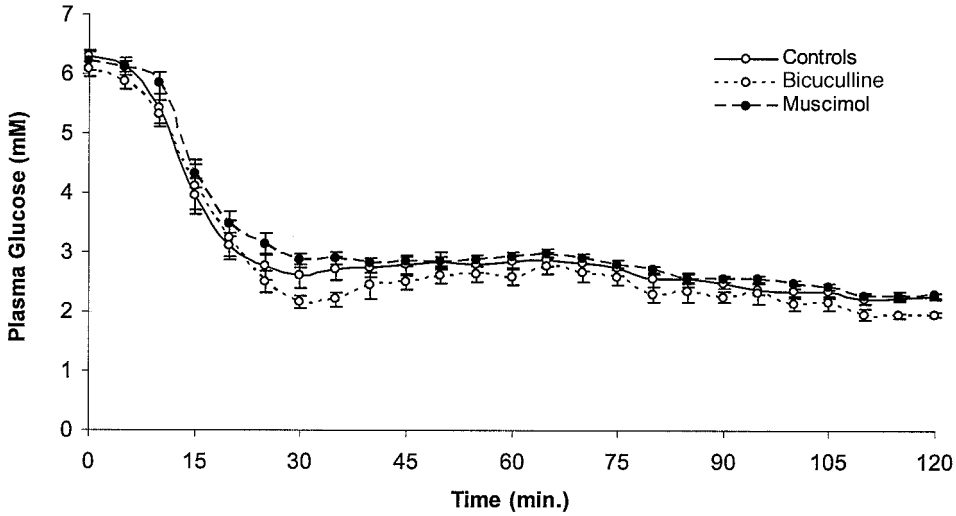

FIG. 2. Plasma glucose concentrations of rats receiving the artificial ECF vehicle (control; $n = 8$), the GABA antagonist bicuculline methiodide ($n = 8$), and the GABA agonist muscimol ($n = 10$) during the hyperinsulinemic-hypoglycemic glucose clamp.

Statistica 6.0 (StatSoft, Tulsa, OK) for personal computers. $P < 0.05$ was set as the criterion for statistical significance.

RESULTS

Hypoglycemia studies—plasma hormones and glucose infusion rates. Plasma hormone concentrations at baseline were not significantly different among experimental treatment groups (Table 1). During the insulin infusion, plasma glucose was reduced to similar levels in all three treatment groups (Fig. 2), and average plasma insulin concentrations during the clamping period were also similar between treatment groups (Fig. 3*A*). Nevertheless, the exogenous glucose infusion rates required to achieve this level of glycemia were significantly altered by local changes in GABAergic tone within the VMH region. Significantly less ($P < 0.05$) glucose was needed in animals treated with the $GABA_A$ receptor antagonist bicuculline methiodide, and significantly more ($P < 0.05$) was needed in animals receiving the $GABA_A$ receptor agonist muscimol, as compared with the control group (Fig. 3*B*).

VMH delivery of the $GABA_A$ receptor antagonist increased peak plasma glucagon responses by 62% ($P < 0.04$), whereas VMH delivery of the $GABA_A$ receptor agonist reduced peak glucagon responses by 44% ($P < 0.05$) compared with vehicle controls (Fig. 4). A similar pattern was observed for epinephrine. Peak epinephrine responses were significantly higher ($P < 0.001$) with bicuculline methiodide and lower with muscimol ($P < 0.05$) injection compared with controls (Fig. 5). On the other hand, plasma norepinephrine concentrations were not significantly affected.

Peak plasma corticosterone concentrations did not dif-

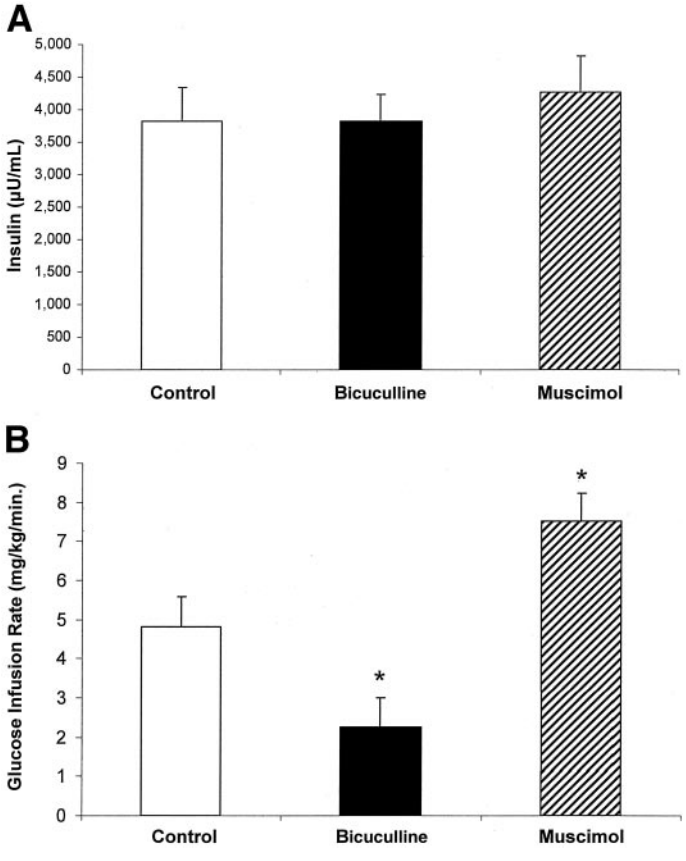

FIG. 3. Average plasma insulin concentrations (*A*) and glucose infusion rates (*B*) during the final 90 minutes of the hypoglycemic clamp for rats receiving the artificial ECF vehicle (control; $n = 8$), the GABA antagonist bicuculline methiodide ($n = 8$), and the GABA agonist muscimol ($n = 10$). Results are presented as means ± SE. *$P < 0.05$ vs. controls.

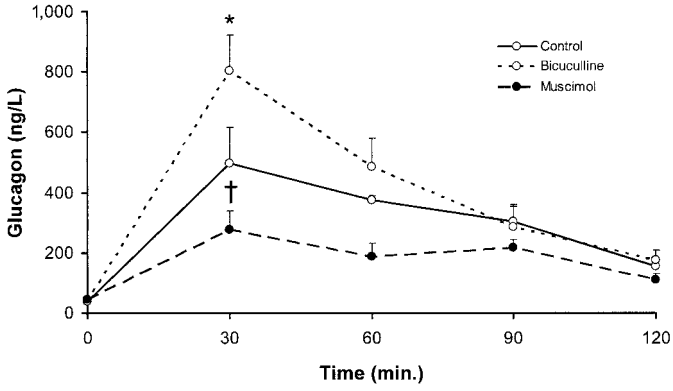

FIG. 4. Plasma glucose responses during the hypoglycemic clamp for rats receiving the artificial ECF vehicle (control; $n = 8$), the GABA antagonist bicuculline methiodide ($n = 8$), or the GABA agonist muscimol ($n = 10$). Results are presented as means ± SE. *$P < 0.04$ vs. controls; †$P < 0.05$ vs. controls.

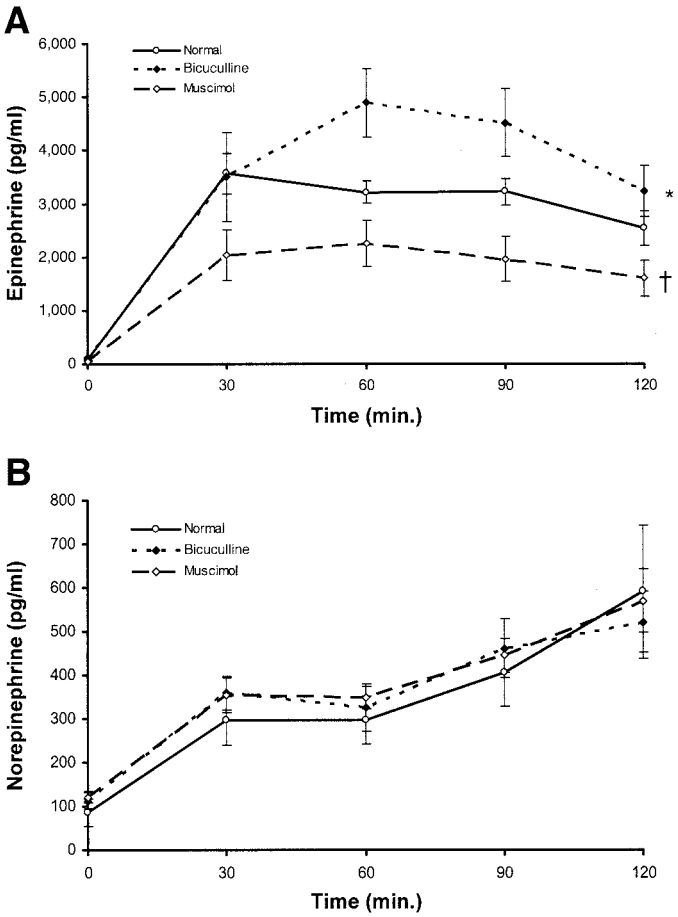

FIG. 5. Epinephrine (*A*) and norepinephrine (*B*) responses during the hypoglycemic clamp for rats receiving the artificial ECF vehicle (control; $n = 8$), the GABA antagonist bicuculline methiodide ($n = 8$), and the GABA agonist muscimol ($n = 10$). Results are presented as means ± SE. *$P < 0.001$ vs. controls; †$P < 0.05$ vs. controls.

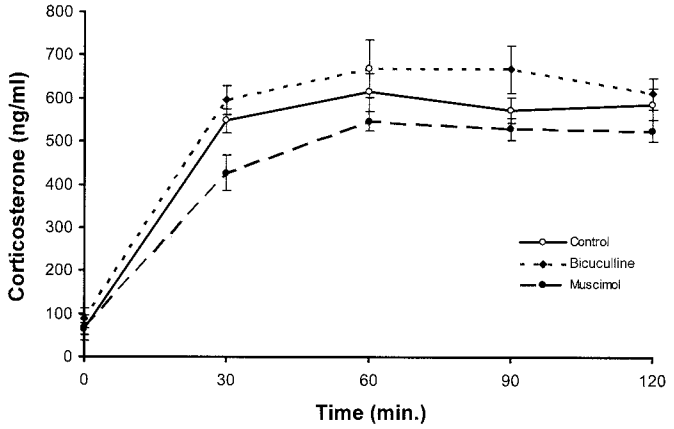

FIG. 6. Plasma cortisone concentrations during the hypoglycemic clamp for rats receiving the artificial ECF vehicle (control; $n = 8$), the GABA antagonist bicuculline methiodide ($n = 8$), or the GABA agonist muscimol ($n = 10$). Results are presented as means ± SE.

fer significantly between the three treatment groups during the hypoglycemic clamp (Fig. 6).

Euglycemia studies. Baseline plasma hormone concentrations were not significantly different between the two experimental groups (Table 2). Plasma glucose levels in the two treatment groups were maintained at similar levels during the euglycemic clamp (Fig. 7). Neither average insulin concentrations (control 4,071 ± 129 and muscimol 4,168 ± 388 μU/ml) nor glucose infusion rates (controls 33 ± 1 and muscimol 32 ± 2 mg · kg^{-1} · min^{-1}) differed between the two groups during the euglycemic clamping period.

Local delivery of the $GABA_A$ receptor agonist muscimol into the VMH did not significantly affect any of the counterregulatory hormone responses during the hyperinsulinemic-euglycemic clamp (Figs. 8–10).

DISCUSSION

The mechanisms underlying blood glucose sensing in the brain remain elusive. Recent studies indicate that several regions within the brain may serve as sensors to detect changes in glucose levels, including the paraventricular nucleus, the arcuate nucleus, and the VMH (11–13,19,42). The latter two regions contain neurons that not only possess much of the glucose-sensing machinery (i.e., glucokinase, glucose transporters, and K_{ATP} channels) but also adjust their firing rates in accordance with changes in ambient glucose levels, either increasing (glucose-excited neurons) or decreasing (glucose-inhibited neurons) rates in response to high glucose levels (18,42,43). In studies conducted by Kang et al. (25) to characterize VMH neurons, it was noted that 56% of glucose-excited and 36% of glucose-inhibited neurons expressed GAD, the rate-limiting enzyme in GABA synthesis. This observation suggests that GABA could be a potential candidate neurotransmitter modulating brain glucose sensing and, in turn, glucose homeostasis. Early studies showed that hepatic glucose production in the basal state can be enhanced with central GABA antagonism (44), providing some evidence for the role of GABA in regulating glucose homeostasis. Whether GABA plays a predominant role in modulating glucose counterregulatory responses to hypoglycemia is still unclear. Measurements of GABA in the brain during hypoglycemia show varying results that may be indicative of regionally specific regulation. For example, perfusion of 2-deoxyglucose or systemic insulin-induced hypoglycemia produced a 45 and 49% decrease, respectively, in microdialysate GABA concentration in the substantia nigra (31). On the other hand, insulin-induced hypoglycemia has been reported to cause a 47% increase in hippocampal GABA levels (45). In the VMH, a biphasic increase in extracellular GABA levels was reported following 2-deoxyglucose administration and insulin-induced hypoglycemia (32,33). Thus, although it is likely that hypoglycemia elicits a multifaceted change in GABAergic tone within the central nervous system, the reason for these differing responses remains to be established.

To clarify the role of GABA in hypoglycemic counter-

TABLE 2
Baseline hormone concentrations for the euglycemic clamp study

	Control	Muscimol
n	5	6
Body weight (g)	338 ± 7	314 ± 1
Plasma glucose (mmol/l)	5.8 ± 0.2	5.9 ± 0.5
Corticosterone (ng/ml)	29 ± 3	44 ± 6
Glucagon (ng/l)	38 ± 5	41 ± 12
Epinephrine (pg/ml)	52 ± 1	61 ± 24
Norepinephrine (pg/ml)	75 ± 16	80 ± 19
Insulin (μU/ml)	28 ± 9	10 ± 4

Data are means ± SE.

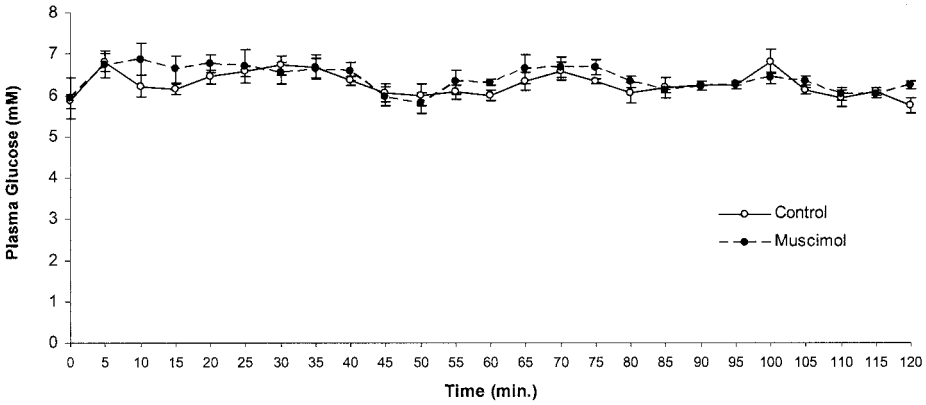

FIG. 7. Plasma glucose concentrations of rats receiving the artificial ECF vehicle (control; $n = 5$) and the GABA agonist muscimol ($n = 6$) during the hyperinsulinemic-euglycemic glucose clamp.

regulation, we microinjected a GABA receptor agonist and antagonist specifically into the VMH to establish whether changes in GABAergic inhibitory tone within this region can affect glucagon and sympathoadrenal responses to hypoglycemia. Our data demonstrate that local VMH administration of the GABA receptor antagonist amplified glucagon and epinephrine responses during hypoglycemia, whereas the agonist suppressed them. Norepinephrine responses, on the other hand, were not affected by either the antagonist or agonist. These results are consistent with previous studies conducted under basal conditions that revealed a rise in glucagon and epinephrine levels when bicuculline methiodide was injected into the third ventricle (29). Although this study did not localize the brain region responsible for changes in glucoregulatory hormones, our data suggest that the GABAergic system within the VMH is primarily responsible for these effects. However, we cannot establish with certainty how the GABAergic system is integrated into the entire glucose-sensing system or, more specifically, how its effects on glucagon and epinephrine secretion are mediated. It is notable that Nonogaki et al. (29) suggested that the rise in plasma glucagon levels following bicuculline methiodide administration may have been mediated indirectly by a corresponding increase in epinephrine secretion, since no significant changes in glucagon secretion were observed when the experiment was repeated in adrenalectomized rats. In contrast to these observations, DeRosa and Cryer (46) reported that the glucagon response to hypoglycemia is still intact in bilaterally adrenalectomized patients, suggesting that epinephrine may not be an absolute requirement for glucagon release in humans. Although species differences could account for the conflicting results in the two studies, we hypothesize that GABA released from glucose-sensing neurons within the VMH may act to modulate sympathetic nervous system output to pancreatic α-cells and the adrenal medulla and thereby play a role in determining the magnitude of these counterregulatory responses to hypoglycemia. Whereas our data provides evidence for the role of GABA in the VMH in modulating glucagon and sympathoadrenal responses to hypoglycemia, it does not necessarily exclude other brain regions or even other substrates or neurotransmitters from playing a contributory role to glucose counterregulation.

There is currently little information available regarding the specific role of glutamate, an excitatory neurotrans-

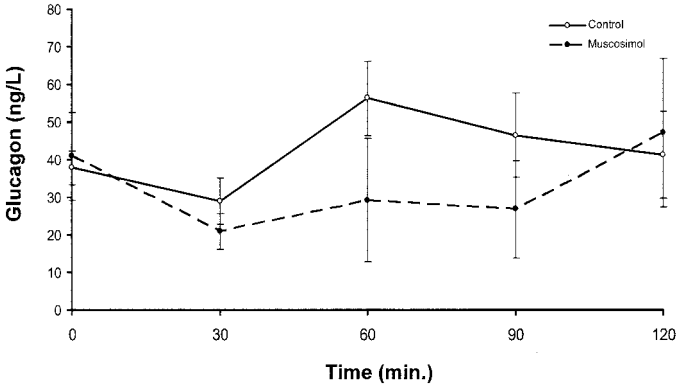

FIG. 8. Plasma glucagon responses during the euglycemic clamp for rats receiving the artificial ECF vehicle (control; $n = 5$) and the GABA agonist muscimol ($n = 6$). Results are presented as means ± SE.

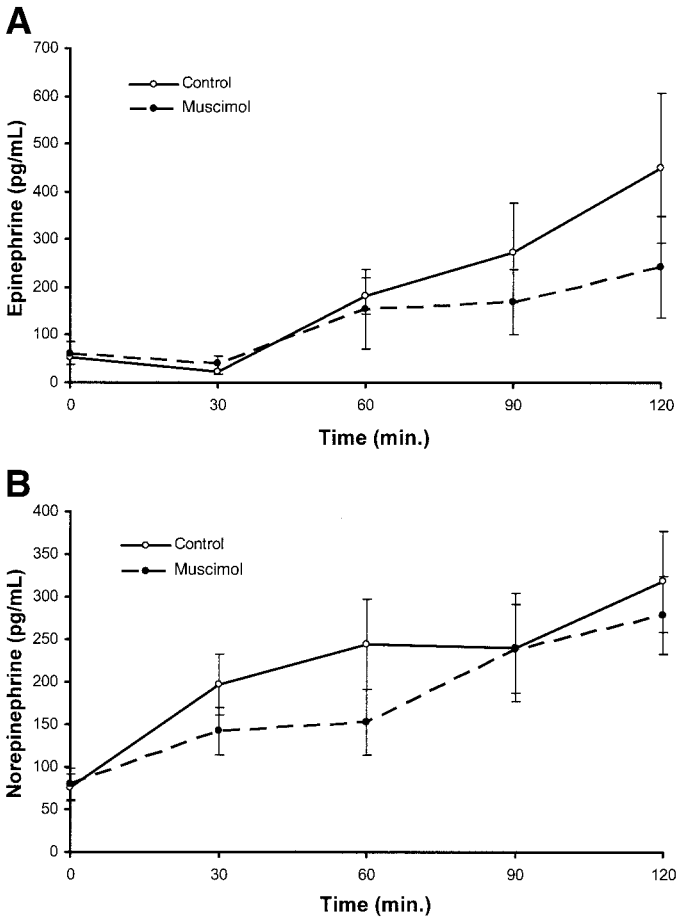

FIG. 9. Epinephrine (*A*) and norepinephrine (*B*) responses during the euglycemic clamp for rats receiving the artificial ECF vehicle (control; $n = 5$) and the GABA agonist muscimol ($n = 6$). Results are presented as means ± SE.

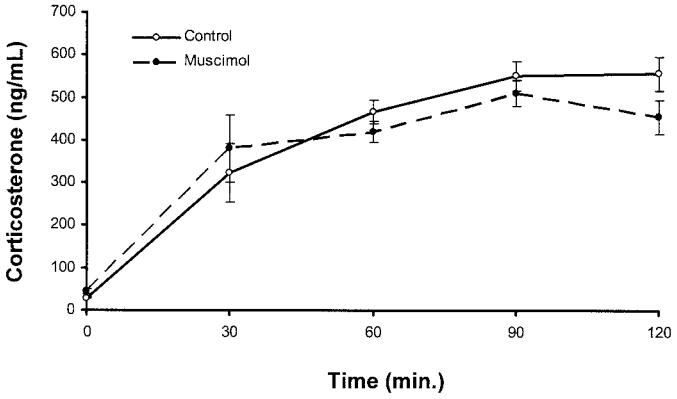

FIG. 10. Plasma corticosterone concentrations during the hypoglycemic clamp for rats receiving the artificial ECF vehicle (control; $n = 5$) and the GABA agonist muscimol ($n = 6$). Results are presented as means ± SE.

mitter, in regulating counterregulatory responses to hypoglycemia. Studies have reported that stimulation of glutamate receptors in the VMH can increase peripheral glucose disposal (47) and activate sympathetic nervous activity in intrascapular brown adipose tissue (48). These studies suggest that glutamate could potentially have a role in the regulation of glucose and energy homeostasis. In fact, GABAergic neurons in the VMH not only synapse on other neurons immunoreactive for GABA, but they also synapse on other as yet unidentified neurons. It is possible that glutamatergic tone may also contribute to modulation of counterregulatory responses. Although the study of glutamate is beyond the scope of the current study, its contribution to glucose counterregulation warrants further investigation.

While plasma glucose levels fell at similar rates in the three different treatment groups in the current study, local VMH delivery of a GABA receptor antagonist lowered and a GABA agonist raised glucose infusion rates. These observations are consistent with data showing that intracerebroventricular bicuculline methiodide administration altered baseline glucose homeostasis, producing hyperglycemia in a dose-dependent manner (28,29) and stimulating endogenous glucose production (44). Our data further extend the findings of these studies by examining the GABAergic system under conditions of hypoglycemia and, more importantly, implicate the VMH as the brain center responsible for these effects. We postulate that the observed changes in glucose infusion rates with GABA agonism and antagonism are likely due in part to the effects of glucagon and epinephrine on peripheral glucose production and utilization.

Neither VMH pretreatment with the $GABA_A$ receptor agonist or antagonist altered pituitary-adrenal responses to hypoglycemia. In contrast, Giordano et al. (34) reported that activation of GABA receptors with the benzodiazepine Alprazolam can suppress ACTH and cortisol responses to hypoglycemia in humans when taken systemically. Taken systemically, the benzodiazepine can potentially activate GABAergic receptors in other sites within the central nervous system. More specifically, if activated, GABA receptors in the hypothalamic paraventricular nucleus, which is believed to be the locus for initiation of the stress response, can suppress hypothalmo-pituitary-adrenal (HPA) activity (35). Our findings suggest that GABAergic tone within the VMH may not play a crucial role in regulating the HPA response to hypoglycemia. In agreement with this, Evans et al. (49) demonstrated that lidocaine-induced inactivation of neurons within the dorsomedial hypothalamus was sufficient to inhibit HPA responses, but not the glucagon or epinephrine responses, to hypoglycemia. These data are consistent with the idea that activation of the HPA axis in response to hypoglycemia may require signals that emanate from neurons residing outside of the VMH and possibly from the dorsomedial nucleus and/or the paraventricular nucleus itself. In accordance with this hypothesis, we noted that manipulation of GABAergic tone within the hypothalamic paraventricular nucleus specifically altered adrenocortical responses to hypoglycemia and not to those of glucagon or epinephrine (O.C., unpublished observations). This may indicate that activation of the HPA axis and the sympathoadrenal system in response to hypoglycemia may be regulated by different regions within the brain, consistent with the idea of a redundant failsafe mechanism.

While feeding behavior was not examined in the current study, it is well known that insulin-induced hypoglycemia and 2-deoxyglucose elicit very clear hyperphagic responses. Although GABA in the VMH does appear to have a role in stimulating feeding behavior (50), GABAergic neurons in the VMH form many different synaptic connections that do not all originate from glucose-sensitive neurons. It is unclear whether the neurons involved in stimulation of counterregulatory responses are the same neurons that regulate feeding. It is possible that feeding behavior and modulation of counterregulatory responses are regulated by different subpopulations of GABAergic neurons within the VMH.

Interestingly, our euglycemia studies revealed that increasing GABAergic tone in the VMH under conditions of hyperinsulinemic-euglycemia does not significantly alter the secretion of counterregulatory hormones. This data suggests that counterregulatory responses may be under tonic inhibition under conditions of euglycemia. We hypothesize that changes in tonic inhibition during hypoglycemia may be one means by which the brain modulates the magnitude of counterregulatory responses when glucose levels fall.

Taken together, our data suggest that the GABAergic system within the VMH plays an important role in modulating the magnitude of glucagon and epinephrine responses to insulin-induced hypoglycemia. It is foreseeable that in situations where this system is disrupted or is ineffective, problems can potentially arise in one's ability to mount an adequate counterregulatory response. Hence, this study has important implications in conditions such as long-standing diabetes, where both glucagon and catecholamine responses to hypoglycemia are either absent or significantly compromised. It is conceivable that an increase in GABAergic tone in the VMH, such as in the case of experimental diabetes (51), and/or an inability to relieve inhibitory tone from this hypothalamic region may contribute to impairment of the primary mechanisms that defend the body from hypoglycemia. As hypoglycemia remains the limiting factor in the proper glycemic management of patients with diabetes (2), understanding the mechanisms underlying impaired counterregulation may lead to the development of new treatment strategies that will minimize or prevent exposure to hypoglycemia. We speculate that minimizing GABAergic inhibitory tone in the VMH may be one means to improve counterregulatory responses in patients with diabetes.

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REFERENCES

1. Cryer PE: Glucose counterregulation: prevention and correction of hypoglycemia in humans. *Am J Physiol* 264:E149–E155, 1993
2. Cryer PE: Banting Lecture: Hypoglycemia: the limiting factor in the management of IDDM. *Diabetes* 43:1378–1389, 1994
3. Cryer PE: Hypoglycaemia: the limiting factor in the glycaemic management of type I and type II diabetes. *Diabetologia* 45:937–948, 2002
4. Gerich JE, Mokan M, Veneman T, Korytkowski M, Mitrakou A: Hypoglycemia unawareness. *Endocrine Rev* 12:356–371, 1991
5. Donovan CM: Portal vein glucose sensing. *Diabetes Nutr Metab* 15:308–312, 2002
6. Hevener AL, Bergman RN, Donovan CM: Novel glucosensor for hypoglycemic detection localized to the portal vein. *Diabetes* 46:1521–1525, 1997
7. Hevener AL, Bergman RN, Donovan CM: Portal vein afferents are critical for the sympathoadrenal response to hypoglycemia. *Diabetes* 49:8–12, 2000
8. Garcia-Fernandez M, Ortega-Saenz P, Pardal R, Lopez-Barneo J: Glucose sensing cells in the carotid body. *Adv Exp Med Biol* 536:47–53, 2003
9. Lopez-Barneo J: Oxygen and glucose sensing by carotid body glomus cells. *Curr Opin Neurobiol* 13:493–499, 2003
10. Pardal R, Lopez-Barneo J: Low glucose-sensing cells in the carotid body. *Nat Neurosci* 5:197–198, 2002
11. Borg MA, Sherwin RS, Borg WP, Tamborlane WV, Shulman GI: Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. *J Clin Invest* 99:361–365, 1997
12. Borg MA, Borg WP, Tamborlane WV, Brines ML, Shulman GI, Sherwin RS: Chronic hypoglycemia and diabetes impair counterregulation induced by localized 2-deoxyglucose perfusion of the ventromedial hypothalamus in rats. *Diabetes* 48:584–587, 1999
13. Dunn-Meynell AA, Govek E, Levin BE: Intracarotid glucose selectively increases Fos-like immunoreactivity in paraventricular, ventromedial and dorsomedial nuclei neurons. *Brain Res* 748:100–106, 1997
14. Frizzell RT, Jones EM, Davis SN, Biggers DW, Myers SR, Connolly CC, Neal DW, Jaspan JB, Cherrington AD: Counterregulation during hypoglycemia is directed by widespread brain regions. *Diabetes* 42:1253–1261, 1993
15. Mobbs CV, Kow LM, Yang XJ: Brain glucose-sensing mechanisms: ubiquitous silencing by aglycemia vs. hypothalamic neuroendocrine responses. *Am J Physiol Endocrinol Metab* 281:E649–E654, 2001
16. Ritter S, Dinh TT, Zhang Y: Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Res* 856:37–47, 2000
17. Sanders NM, Ritter S: Repeated 2-deoxy-D-glucose–induced glucoprivation attenuates Fos expression and glucoregulatory responses during subsequent glucoprivation. *Diabetes* 49:1865–1874, 2000
18. Song Z, Levin BE, McArdle JJ, Bakhos N, Routh VH: Convergence of pre- and postsynaptic influences on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes* 50:2673–2681, 2001
19. Wang R, Liu X, Hentges ST, Dunn-Meynell AA, Levin BE, Wang W, Routh VH: The regulation of glucose-excited neurons in the hypothalamic arcuate nucleus by glucose and feeding-relevant peptides. *Diabetes* 53:1959–1965, 2004
20. Borg MA, Tamborlane WV, Shulman GI, Sherwin RS: Local lactate perfusion of the ventromedial hypothalamus suppresses hypoglycemic counterregulation. *Diabetes* 52:663–666, 2003
21. Evans ML, McCrimmon RJ, Flanagan DE, Keshavarz T, Fan X, McNay EC, Jacob RJ, Sherwin RS: Hypothalamic ATP-sensitive K^+ channels play a key role in sensing hypoglycemia and triggering counterregulatory epinephrine and glucagon responses. *Diabetes* 53:2542–2551, 2004
22. McCrimmon RJ, Fan X, Ding Y, Zhu W, Jacob RJ, Sherwin RS: Potential role for AMP-activated protein kinase in hypoglycemia sensing in the ventromedial hypothalamus. *Diabetes* 53:1953–1958, 2004
23. Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M, Ashcroft F, Minokoshi Y, Roeper J, Seino S: ATP-sensitive K+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. *Nat Neurosci* 4:507–512, 2001
24. Schuit FC, Huypens P, Heimberg H, Pipeleers DG: Glucose sensing in pancreatic β-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. *Diabetes* 50:1–11, 2001
25. Kang L, Routh VH, Kuzhikandathil EV, Gaspers LD, Levin BE: Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. *Diabetes* 53:549–559, 2004
26. Tappaz ML, Brownstein MJ, Kopin IJ: Glutamate decarboxylase (GAD) and gamma-aminobutyric acid (GABA) in discrete nuclei of hypothalamus and substantia nigra. *Brain Res* 125:109–121, 1977
27. McCann SM, Rettori V: Gamma amino butyric acid (GABA) controls anterior pituitary hormone secretion. *Adv Biochem Psychopharmacol* 42:173–189, 1986
28. Narita K, Nishihara M, Takahashi M: Concomitant regulation of running activity and metabolic change by the ventromedial nucleus of the hypothalamus. *Brain Res* 642:290–296, 1994
29. Nonogaki K, Iguchi A, Sakamoto N: Bicuculline methiodide influences the central nervous system to produce hyperglycemia in rats. *J Neuroendocrinol* 6:443–446, 1994
30. Soltis RP, DiMicco JA: Interaction of hypothalamic GABAA and excitatory amino acid receptors controlling heart rate in rats. *Am J Physiol* 261:R427–R433, 1991
31. During MJ, Leone P, Davis KE, Kerr D, Sherwin RS: Glucose modulates rat substantia nigra GABA release in vivo via ATP-sensitive potassium channels. *J Clin Invest* 95:2403–2408, 1995
32. Beverly JL, de Vries MG, Beverly MF, Arseneau LM: Norepinephrine mediates glucoprivic-induced increase in GABA in the ventromedial hypothalamus of rats. *Am J Physiol Regul Integr Comp Physiol* 279:R990–R996, 2000
33. Beverly JL, de Vries MG, Bouman SD, Arseneau LM: Noradrenergic and GABAergic systems in the medial hypothalamus are activated during hypoglycemia. *Am J Physiol Regul Integr Comp Physiol* 280:R563–R569, 2001
34. Giordano R, Grottoli S, Brossa P, Pellegrino M, Destefanis S, Lanfranco F, Gianotti L, Ghigo E, Arvat E: Alprazolam (a benzodiazepine activating GABA receptor) reduces the neuroendocrine responses to insulin-induced hypoglycaemia in humans. *Clin Endocrinol (Oxf)* 59:314–320, 2003
35. Van Vugt DA, Washburn DL, Farley AE, Reid RL: Hypoglycemia-induced inhibition of LH and stimulation of ACTH secretion in the rhesus monkey is blocked by alprazolam. *Neuroendocrinology* 65:344–352, 1997
36. Smith D, Pernet A, Rosenthal JM, Bingham EM, Reid H, Macdonald IA, Amiel SA: The effect of modafinil on counter-regulatory and cognitive responses to hypoglycaemia. *Diabetologia* 47:1704–1711, 2004
37. Beverly JL, Beverly MF, Meguid,MM: Alterations in extracellular GABA in the ventral hypothalamus of rats in response to acute glucoprivation. *Am J Physiol* 269:R1174–R1178, 1995
38. Chan O, Chan S, Inouye K, Shum K, Matthews SG, Vranic M: Diabetes impairs hypothalamo-pituitary-adrenal (HPA) responses to hypoglycemia, and insulin treatment normalizes HPA but not epinephrine responses. *Diabetes* 51:1681–1689, 2002
39. Paxinos G, Watson C: *The Rat Brain in Stereotaxic Coordinates*. 3rd ed. San Diego, CA, Academic Press, 1997
40. de Vries MG, Arseneau LM, Lawson ME, Beverly JL: Extracellular glucose in rat ventromedial hypothalamus during acute and recurrent hypoglycemia. *Diabetes* 52:2767–2773, 2003
41. McNay EC, Sherwin RS: From artificial cerebro-spinal fluid (aCSF) to artificial extracellular fluid (aECF): microdialysis perfusate composition effects on in vivo brain ECF glucose measurements. *J Neurosci Methods* 132:35–43, 2004
42. Routh VH: Glucosensing neurons in the ventromedial hypothalamic nucleus (VMN) and hypoglycemia-associated autonomic failure (HAAF). *Diabete Metab Res Rev* 19:348–356, 2003
43. Routh VH: Glucose-sensing neurons: are they physiologically relevant? *Physiol Behav* 76:403–413, 2002
44. Lang CH: Inhibition of central GABAA receptors enhances hepatic glucose production and peripheral glucose uptake. *Brain Res Bull* 37:611–616, 1995
45. Shuaib A, Ijaz MS, Waqar T, Voll C, Kanthan R, Miyashita H, Liu L: Insulin elevates hippocampal GABA levels during ischemia: this is independent of its hypoglycemic effect. *Neuroscience* 67:809–814, 1995
46. DeRosa MA, Cryer PE: Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation. *Am J Physiol Endocrinol Metab* 287:E32–E41, 2004
47. Sudo M, Minokoshi Y, Shimazu T: Ventromedial hypothalamic stimulation

enhances peripheral glucose uptake in anesthetized rats. *Am J Physiol* 261:E298–E303, 1991
48. Yoshimatsu H, Egawa M, Bray GA: Sympathetic nerve activity after discrete hypothalamic injections of L-glutamate. *Brain Res* 601:121–128, 1993
49. Evans SB, Wilkinson CW, Gronbeck P, Bennett JL, Zavosh A, Taborsky GJ Jr, Figlewicz DP: Inactivation of the DMH selectively inhibits the ACTH and corticosterone responses to hypoglycemia. *Am J Physiol Regul Integr Comp Physiol* 286:R123–R128, 2004
50. Tsujii S, Bray GA: GABA-related feeding control in genetically obese rats. *Brain Res* 540:48–54, 1991
51. Ohtani N, Ohta M, Sugano T: Microdialysis study of modification of hypothalamic neurotransmitters in streptozotocin-diabetic rats. *J Neurochem* 69:1622–1628, 1997