Effect of Vagal Cooling on the Counterregulatory Response to Hypoglycemia Induced by a Low Dose of Insulin in the Conscious Dog

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We previously demonstrated, using a nerve-cooling technique, that the vagus nerves are not essential for the counterregulatory response to hypoglycemia caused by high levels of insulin. Because high insulin levels per se augment the central nervous system response to hypoglycemia, the question arises whether afferent nerve fibers traveling along the vagus nerves would play a role in the defense of hypoglycemia in the presence of a more moderate insulin level. To address this issue, we studied two groups of conscious 18-h–fasted dogs with cooling coils previously placed on both vagus nerves. Each study consisted of a 100-min equilibration period, a 40-min basal period, and a 150-min hypoglycemic period. Glucose was lowered using a glycogen phosphorylase inhibitor and a low dose of insulin infused into the portal vein (0.7 mU kg\(^{-1}\) min\(^{-1}\)). The arterial plasma insulin level increased to 15 ± 2 μU/ml and the plasma glucose level fell to a plateau of 57 ± 3 mg/dl in both groups. The vagal cooling coils were perfused with a 37°C (SHAM COOL; \(n = 7\)) or a −20°C (COOL; \(n = 7\)) ethanol solution for the last 90 min of the study to block parasympathetic afferent fibers. Vagal cooling caused a marked increase in the heart rate and blocked the hypoglycemia-induced increase in the arterial pancreatic polypeptide level. The average increments in glucagon (pg/ml), epinephrine (pg/ml), norepinephrine (pg/ml), cortisol (pg/dl), glucose production (mg · kg\(^{-1}\) · min\(^{-1}\)), and glycerol (μmol/l) in the SHAM COOL group were 53 ± 9, 625 ± 186, 131 ± 48, 4.63 ± 1.05, −0.79 ± 0.24, and 101 ± 18, respectively, and in the COOL group, the increments were 39 ± 7, 837 ± 235, 93 ± 39, 6.28 ± 1.03 (\(P < 0.05\)), −0.80 ± 0.20, and 73 ± 29, respectively. Based on these data, we conclude that, even in the absence of high insulin concentrations, afferent signaling via the vagus nerves is not required for a normal counterregulatory response to hypoglycemia. Diabetes 50: 558–564, 2001

Hypoglycemia is a serious condition that can lead to death if the organism does not respond properly. The physiological response to hypoglycemia involves an increase in the plasma levels of a variety of counterregulatory hormones, including glucagon, cortisol, epinephrine, norepinephrine, and growth hormone. This hormonal response results in an increase in glucose production and a decrease in glucose utilization, thereby defending the organism against the hypoglycemia. Individuals with type 1 diabetes have a blunted counterregulatory response to hypoglycemia, making them more vulnerable to low blood glucose. It is of clinical importance, therefore, to understand which areas of the body are responsible for sensing a decrease in blood glucose. The site of hypoglycemic sensing is controversial. Until recently, the brain was generally accepted as the site of detection of hypoglycemia. In accordance with this, Borg et al. (1) demonstrated in rats that ablation of the ventromedial hypothalamus abolished the counterregulatory response to hypoglycemia. In the conscious dog, the counterregulatory response to insulin-induced hypoglycemia was completely abolished when glucose was infused into the head simultaneously via the vertebral and carotid arteries so as to create euglycemia in the brain during systemic hypoglycemia (2).

On the other hand, the hepatoporal region has also been proposed to be a site for the detection of hypoglycemia. This postulate is based on the work of Niijima (3), who showed that infusion of glucose intraduodenally decreased the firing rate of afferent fibers of the hepatic vagus nerve. This indicated that the liver, or more specifically the hepatoporal region, could sense a change in the glucose level and relay that information to the brain. Hamilton-Wessler et al. (4), as well as Donovan et al. (5), reported that maintaining euglycemia at the liver during systemic and central nervous system hypoglycemia blunted the catecholamine response to insulin-induced hypoglycemia by approximately 40%. In agreement with this, Hevener et al. (6) reported that chemical denervation of the hepatic portal vein decreased the sympathoadrenal response to sustained systemic hypoglycemia in rats. Along the same line, Lamarche et al. (7) demonstrated in anesthetized dogs, using a cross-perfusion model, that the creation of local hepatic hypoglycemia during systemic hypoglycemia using a nerve-cooling technique could prevent a blunted counterregulatory response to hypoglycemia.
euglycemia induced an adrenal counterregulatory response. Recently, we undertook a study to investigate the involvement of the hepatoportal region in the initiation of the counterregulatory response to insulin-induced hypoglycemia in conscious dogs. We showed that interrupting vagal transmission using a nerve-cooling technique before or during hypoglycemia induced by high-dose insulin in conscious dogs did not modify the counterregulatory response (8). The results of our experiments argue against a role for vagal afferents in the initiation of the counterregulatory response to insulin-induced hypoglycemia. On the other hand, Davis et al. (9,10) demonstrated that during insulin-induced hypoglycemia in conscious dogs, the brain insulin level is a key determinant of the magnitude of the counterregulatory response, such that higher insulin levels markedly augment the sympathetic response to hypoglycemia. The concept that the insulin concentration can modify the counterregulatory response to hypoglycemia has also been demonstrated in nondiabetic (11,12) and diabetic humans (13,14). It is possible, therefore, that in our previous experiments, the use of high insulin levels emphasized the role of the brain in the response to hypoglycemia and thereby minimized the role of the hepatoportal glucose sensors. To investigate this possibility, we undertook the present study, in which we again used vagal cooling, but during hypoglycemia brought about using a glycogen phosphorylase inhibitor and low-rate insulin infusion. In this way, we were able to examine the role of the vagal afferents in mediating the response to low blood glucose in the presence of low insulin levels.

**RESEARCH DESIGN AND METHODS**

**Animal care.** Experiments were conducted on seven conscious, mongrel dogs (25 ± 1 kg) of either sex. Once daily, the animals were fed meat (KalKan, Vernon, CA) and Dog Chow (Purina Lab Diets, East St. Louis, IL). All animals were housed in a facility that met the standards of the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical School Animal Care Committee.

**Surgical procedures.** Two weeks before the initial experiment, the dogs were intravenously administered a short-acting general anesthetic (thiopental sodium, 15 mg/kg), after which they were intubated and administered an inhalational anesthetic (1% isoflurane) for the entire surgical procedure. All dogs underwent a laparotomy for the placement of catheters in a splenic and jejunal vein for intraportal infusion of insulin (catheter dimensions: inner diameter 0.04 inch; outer diameter 0.085 inch) was inserted through a jejunal vein for intraportal infusion of insulin (catheter dimensions: inner diameter 0.125 inch; outer diameter 0.25 inch) from the cooling bath and to vagal cooling coils were joined to inflowing lines (catheter dimensions: inner diameter 0.04 inch; outer diameter 0.085 inch).

For the placement of a catheter in the femoral artery, a 1-cm incision was made parallel to the vessel in the left inguinal area. The artery was isolated by blunt dissection and ligated distally. A Silastic catheter (catheter dimensions: inner diameter 0.04 inch; outer diameter 0.085 inch) was inserted through a small hole in the wall of the artery and its tip was positioned in the iliac artery. All catheters were secured and filled with heparin (1,000 U/ml; Abbott, North Chicago, IL), and the free ends were knotted. The catheters were then buried in subcutaneous pockets to allow complete closure of the skin incision. Stainless steel cooling coils prepared with Silastic extension tubing were placed around the vagus nerves in all dogs, as described previously (8). A ventral midline incision was made 3 cm superior to the manubrium of the sternum and extended 8 cm rostrally through the sternocleidomastoid muscle. Blunt dissection bilaterally through the anterior fascia and between the sternum and extended 8 cm rostrally through the sternocleidomastoid muscle to create a wall between the nerve and carotid artery at the level of the coil on both sides of the neck. The ends of the Silastic tubes were placed in a subcutaneous pocket and the incision was closed. The effectiveness of cooling in blocking barosympathetic signaling was verified in each study by measuring the heart rate and the hypoglycemia-induced change in the arterial plasma pancreatic polypeptide concentrations (both of which are under vagal control). Penicillin G (100,000 U) was administered intramuscularly (1000 mg/kg) b.i.d. for the first 4 days postoperatively. Each dog was used for an experiment only if it had a leukocyte count <18,000/mm^3, hematocrit >35%, a good appetite, and normal stools.

On the day of the study, the femoral artery, gastric, and venous catheters, as well as the Silastic tubes connected to the cooling coils, were removed from their subcutaneous pockets under local anesthesia (2% lidocaine; Astra Pharmaceutical Products, Worcester, MA). The contents of the femoral catheter were aspirated and heparinized saline (1 U/ml) was slowly infused through it during the study. The ends of the Silastic tubes connected to the vagal cooling coils were joined to inflowing lines (catheter dimensions: inner diameter 0.125 inch; outer diameter 0.25 inch) from the cooling bath and to outflowing lines linking them to the collection reservoir. Angioplasts (18-gauge; Becton Dickinson, Sandy, UT) were inserted percutaneously into the left cephalic vein for [3-H]glucose infusion and in the right cephalic vein for [3-H]dextrose (as a tracer) infusion (0.5 mg/kg/h). The dog was allowed to stand calmly in a Pavlov harness for 30 min before the start of the experiment. Each dog was reanesthetized (sodium pentothal, 15 mg/kg) after the experiment, and the catheters were filled with heparin. The free ends of the catheters were knotted and the catheters were placed into new subcutaneous pockets. The incisions were closed and antibiotics were administered as described earlier. The animals were then studied 14 days later. Three days before each experiment, the leukocyte count and hematocrit were again measured. Only dogs that met the study criteria described earlier were reused.

**Experimental design.** The experiment performed on 18-h–fasted dogs consisted of a 100-min tracer equilibration period (–140 to –40 min), a 40-min control period (–40 to 0 min), a 60-min period during which the plasma glucose level was allowed to decrease (0 to 60 min), and a 90-min experimental cooling or sham cooling period (60 to 150 min). A priming dose (33 μCi) of [3-H]glucose (Du Pont-NEN, Boston, MA) was administered at –140 min, followed by a continuous infusion of 0.29 μCi/min of [3-H]glucose. This tracer infusion was adjusted as needed in each group to clamp the glucose specific activity at a constant value approximately equal to the basal specific activity. At time 0, a glycogen phosphorylase inhibitor (BAY R3401; 10 mg/kg in a 0.5% NaCl solution) was infused intravenously through a femoral vein. At approximately 140 min, all dogs were infused through a leg vein as needed. Beginning at 60 min, the coils were perfused either with a 37°C (SHAM COOL) or a –20°C (COOL) solution (creating a 2°C temperature at the vagus nerve level in the COOL group). The order of the SHAM COOL and COOL protocols was randomized. Arterial blood was sampled every 10 min during the basal period and every 15 min thereafter. The collection and processing of blood samples have been described previously (15). Approximately 8% of the dog's total blood volume was removed during each study and was replaced with two volumes of saline.

**Hormone and metabolic assays.** Plasma glucose levels were assayed using the glucose-oxidase method with a Beckman glucose analyzer. Small blood samples were taken every 5 min to measure the glucose concentration so that exogenous glucose could be administered as needed to maintain the hypoglycemic clamp. Plasma insulin and glucagon were measured using a double-antibody radioimmunoassay described previously (16) with interassay coefficients of variation (CVs) of 7% and 5%, respectively. Plasma samples used for glucagon determination contained 100,000 KIU aprotinin (Trasylol; Miles, Kankakee, IL) added at collection. Catecholamines were assayed using high-performance liquid chromatography as previously described (17). The concentrations of norepinephrine, epinephrine, and total norepinephrine were expressed as mg/liter. The results were plotted as percentages above or below basal values. The area under the curve (AUC) was calculated with the trapezoidal rule.

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LOW-DOSE INSULIN AND HYPOGLYCEMIA

FIG. 1. Arterial plasma insulin and glucose during the basal period (−40 to 0 min) and the period (0–150 min) during which hypoglycemia was brought about by administration of a glucose phosphorylase inhibitor (10 mg/kg) orally and insulin intraportally (700 mU · kg⁻¹ · min⁻¹). The vagus nerves were either kept at 37°C (SHAM COOL) or cooled to 2°C (COOL) to interrupt vagal transmission in conscious, overnight-fasted dogs. Data points represent means ± SE for seven dogs in each protocol.

during the hypoglycemic clamp period was subtracted from tracer-determined glucose production.

Statistical analysis. Data are expressed as means ± SE. The net areas under the curve (AUCs) were calculated by subtracting the 60-min time value multiplied by 90 from the AUC during the experimental period or by subtracting the average basal period value multiplied by 90 from the AUC during the experimental period. The statistical comparisons among and between groups were made using analysis of variance with repeated measures. Post hoc analysis was performed using a universal F test. When two averages were compared, a Student’s t test was used. Significance was presumed at P < 0.05.

RESULTS

Insulin and glucose. With portal insulin infusion, the arterial plasma insulin levels rose similarly in both groups from basal levels up to average values during the last 90 min of 15 ± 1 μU/ml and 15 ± 2 μU/ml in the SHAM COOL and COOL groups, respectively (Fig. 1). The average basal arterial glucose levels were 111 ± 2 mg/dl in both protocols (Fig. 1). After insulin infusion and drug administration, the arterial plasma glucose level slowly declined to 68 ± 3 and 70 ± 2 mg/dl at 60 min, averaging 57 ± 3 and 58 ± 3 mg/dl, in the SHAM COOL and COOL groups, respectively. There were no significant differences in insulin or glucose between protocols at any time.

Heart rate and pancreatic polypeptide. The increase in heart rate (Fig. 2) caused by hypoglycemia was modest (∆18 ± 10 beats/min) in the SHAM COOL group. During the coil perfusion in the COOL group, the heart rate increased significantly (∆79 ± 12 beats/min; P < 0.05). Hypoglycemia induced an increase in pancreatic polypeptide levels from an average of 122 ± 25 pg/ml in the basal period to an experimental period average of 372 ± 42 pg/ml in the SHAM COOL group (Fig. 2). This increase in the pancreatic polypeptide level was completely abolished by the vagal cooling procedure such that the average value in the experimental period (112 ± 18 pg/ml) was below the baseline value (134 ± 28 pg/ml). The increase in heart rate and the decrease in pancreatic polypeptide levels during the experimental period attest to the effectiveness of the cooling procedure.

Glucagon and cortisol. Arterial glucagon peaked by 90 min of hypoglycemia (108 ± 24 and 86 ± 10 pg/ml in the SHAM COOL and COOL groups, respectively) and then began to decline as hypoglycemia continued (Fig. 3). There were no significant differences in glucagon levels between groups. Similarly, the average values during the last 45 min of the coil perfusion period were not statistically different (86 ± 10 and 70 ± 5 pg/ml for the SHAM COOL and COOL groups, respectively). Likewise, the net AUCs for the 90-min experimental period were not significantly different (2,395 ± 712 and 1,909 ± 704 pg/ml for the SHAM COOL and COOL groups, respectively; P = 0.41). Even when the net AUC was calculated relative to the basal period, there was no significant difference in the two responses. It is apparent, therefore, that vagal cooling (COOL) did not significantly reduce the response of the α-cells to hypoglycemia.

The arterial plasma cortisol levels rose to averages of 7.34 ± 0.84 and 9.46 ± 1.28 μg/dl in the SHAM COOL and COOL groups, respectively, for the final 45 min of hypoglycemia (P > 0.05) (Fig. 3). Although there was a tendency for the levels to be higher in the COOL group, the net AUC for plasma cortisol was not significantly different.
between the SHAM COOL (262 ± 65 mg/dl) and the COOL group (413 ± 106 mg/dl). This was the case even when we used the basal period value to calculate the net AUC. Therefore, it is clear that vagal cooling (COOL) did not decrease the cortisol response to hypoglycemia.

Epinephrine and norepinephrine. The arterial plasma epinephrine level (Fig. 4) increased to averages of 887 ± 197 and 1,020 ± 274 pg/ml for the final 45 min of the SHAM COOL and COOL protocols, respectively. There was no significant difference between the groups when comparing the net AUCs (48,880 ± 13,092 [SHAM COOL] and 61,765 ± 14,571 pg/ml [COOL]; P = 0.28). Similarly, when the net AUC was calculated relative to the baseline value, rather than the 60-min value, the plasma norepinephrine response was 10% less in the COOL group (P = 0.04). Despite this, it is clear that no meaningful differences were found between the two groups with regard to epinephrine or norepinephrine, showing that vagal cooling did little to reduce the sympathetic nervous system response to hypoglycemia.

Blood glycerol and endogenous glucose production. Arterial blood glycerol levels (Fig. 5) rose to averages of 283 ± 44 and 250 ± 42 pg/ml for the final 45 min in SHAM COOL and COOL protocols, respectively. Again, the AUCs were not significantly different (7,753 ± 3,202 and 4,525 ± 3,532 pg/ml for SHAM COOL and COOL groups, respectively; P = 0.18). In contrast to epinephrine, when the net AUC was calculated relative to the baseline value rather than the 60-min value, the plasma norepinephrine response was 10% less in the COOL group (P = 0.04). Despite this, it is clear that no meaningful differences were found between the two groups with regard to epinephrine or norepinephrine, showing that vagal cooling did little to reduce the sympathetic nervous system response to hypoglycemia.
Mia was thus unaffected by vagal cooling. Endogenous glucose production began to decrease concomitantly with the decrease in blood glucose (Fig. 5), from basal average values of $2.5 \pm 0.14$ and $2.7 \pm 0.20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the SHAM COOL and COOL groups, respectively, to average values of $1.57 \pm 0.19$ and $1.74 \pm 0.18 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in the two groups, respectively, during the last 45 min of the study. Glucose production and arterial blood glycerol were not significantly different between the two groups at any time.

**DISCUSSION**

Some investigators have suggested that the counterregulatory responses to hypoglycemia are, at least in part, initiated by hepatoportal glucose sensors. It is known that glucose sensors in the portal vein send afferent messages to the central nervous system via the vagus nerves (4, 5, 7, 25). We have previously suggested, however, that vagal afferents are not involved in the transmission of the hypoglycemic signal to the brain (8). In our earlier experiments, we used high levels of insulin to create hypoglycemia and showed that complete vagal blockade failed to alter the counterregulatory response. Davis et al. (9), however, have shown, using their head insulin infusion model, that in the presence of the same systemic glycemia ($\sim 57 \text{ mg/dl}$) and insulinemia ($\sim 15 \mu\text{U/ml}$) conditions in conscious, overnight-fasted dogs. Data points represent means $\pm$ SE for seven dogs in each protocol.

**FIG. 4.** Effects of vagal cooling on arterial plasma catecholamine, epinephrine, and norepinephrine concentrations during equivalent hypoglycemic ($\sim 57 \text{ mg/dl}$) and hyperinsulinemic ($\sim 15 \mu\text{U/ml}$) conditions in conscious, overnight-fasted dogs. Data points represent means $\pm$ SE for seven dogs in each protocol.

**FIG. 5.** Effects of vagal cooling on arterial blood glycerol and tracer-determined endogenous glucose production during equivalent hypoglycemic ($\sim 57 \text{ mg/dl}$) and insulinemic ($\sim 15 \mu\text{U/ml}$) conditions in conscious, overnight-fasted dogs. Data points represent means $\pm$ SE for seven dogs in each protocol.
The present study was thus designed to create hypoglycemia without the use of high levels of insulin to further assess the role of afferent fibers traveling along the parasympathetic vagal trunk in initiation of the counterregulatory response to low blood glucose. This was achieved by intragastric administration of a glycogen phosphorylase inhibitor (BAY R3401, which is known to suppress hepatic glycogenolysis by allosteric inhibition and dephosphorylation of phosphorylase a [26]), along with a low-rate portal insulin. Moderate hypoglycemia (~58 mg/dl) was again achieved, but in this case, it occurred in the presence of only slight hyperinsulinemia (15 µU/ml). Despite the low insulin level, vagal cooling did not result in a significant decrease in the response of any counterregulatory hormone or in any metabolic response to hypoglycemia. This was not the result of an inability of vagal cooling to block nerve transmission. The effectiveness of our vagal cooling technique was demonstrated by the absence of a hypoglycemia-induced increase in arterial pancreatic polypeptide levels and the presence of a marked increase in the heart rate. Because such changes are similar in magnitude to those caused by maximal atropine administration, we can conclude that vagal firing was effectively blocked.

Clearly, the response of epinephrine to hypoglycemia was not altered by vagal cooling. In fact, the similarity of the catecholamine responses in the two groups was remarkable. Given the variance of the increase in epinephrine, however, we cannot definitively rule out the possibility that we missed a small change in the epinephrine response caused by vagal cooling. For this parameter, the statistical power that this experiment provided, to avoid a type 2 error, was 0.67, taking into account that the P value was fixed at 0.1. In the case of norepinephrine, no difference was evident in the two groups when the net AUC was calculated relative to the 60-min value, but a 10% decrease was seen when the net AUC was calculated relative to the baseline value. For this parameter, the statistical power that this experiment provided, to avoid a type 2 error, was 0.85, taking into account that the P value was fixed at 0.1. Whether this difference represents a real decrease or a random event is not clear. Even if it were real, however, it was much less than the ~40% decrease reported by Donovan and colleagues (4–6,27). It is important to note that in previous studies, when the vagal cooling technique was used in the presence of basal pancreatic hormonal replacement, there was no increase in the plasma level of either catecholamine. It can be concluded, therefore, that any stress associated with vagal cooling per se was not enough to increase the plasma catecholamine levels. Such an increase could not, therefore, have masked a hypothetical decrease caused by the block in vagal afferent firing.

The small nonspecific stress response associated with vagal cooling, on the other hand, was enough to cause a small but rapid increase in the arterial plasma cortisol level. After this rapid increase (~2 µg/dl more than in the SHAM COOL group), the response of cortisol to hypoglycemia remained the same in the two groups. We have noted such a stress response previously on two occasions (8,28). In an earlier study (28), we showed that under euglycemic conditions in which a pancreatic clamp was used to keep insulin and glucagon at basal levels, vagal cooling resulted in a ~3 µg/dl increase in the plasma cortisol level, which was evident for the 90-min experimental period. When the AUC was calculated for cortisol in the present study (COOL) and the contribution of the cooling stress was subtracted (assuming it was sustained for 90 min), there was no difference in the cortisol response to hypoglycemia in the SHAM COOL and COOL protocols.

Vagal cooling did not significantly reduce the glucagon response to hypoglycemia. This confirmed our previous data (8). Given the magnitude and the variance of the plasma glucagon response, however, it would not have been possible to detect a modest change even if it had occurred. Because Donovan et al. (27) and Hamilton-Wessler et al. (4) have not reported a decrease in plasma glucagon levels in response to hepatic glucose clamping in the presence of hypoglycemia, this deficit is of little consequence.

We also assessed the effect of vagal cooling on the overall metabolic responses to insulin-induced hypoglycemia. Not only was there no diminution in the counterregulatory hormone responses to low plasma glucose when the vagus nerves were cooled, there was also no significant difference in glucose production in the two groups. In our previous study (8), glucose production increased in response to similar hypoglycemia (~3 mg · kg⁻¹ · min⁻¹), even in the presence of a higher insulin level. In the present study, it decreased, but it must be kept in mind that in the present study, we used a glycogen phosphorylase inhibitor to decrease hepatic glucose production and create hypoglycemia. As a result, glycogenolysis, which is normally an important source of glucose during hypoglycemia, could not increase normally. The lipolytic response to hypoglycemia was also unaffected by vagal cooling, as indicated by the arterial plasma glycerol levels.

The discrepancy between our findings and those of Donovan et al. (27), as well as those of Hamilton-Wessler et al. (4), are unlikely to be related to subtle differences in insulin or glucose levels. A more plausible explanation is that in their studies, the investigators created a feeding signal by the establishment of a negative arterial-portal glucose gradient. This has been shown to be associated with a decrease in effluent nerve firing to the adrenal glands (29), thus potentially explaining the decrease in the sympathoadrenal response seen by these authors. Because the portal glucose level does not exceed the arterial glucose level in most hypoglycemic settings, this mechanism would not normally play a part in the defense against low blood glucose. In a recent study, Heyvener et al. (6) diminished the magnitude of the sympathoadrenal response to insulin-induced hypoglycemia in rats by chemically denervating the portal vein. These authors concluded that the above “feeding signal” explanation for the diminished sympathoadrenal response is not valid. However, by chronically denervating the portal vein with phenol, afferent signaling to the brain was chronically shut down, thereby simulating a high glucose level in the portal vein. In a way, therefore, denervation of the portal vein created a permanent negative arterial-portal gradient and, as result, a constant feeding signal. The data of Heyvener et al. (6) therefore support the concept that diminution in
sympathetic firing resulting from portal glucose infusion during hypoglycemia is the result of a feeding signal.

In summary, although in our previous study (8) we showed that reducing the activity of vagal afferents did not blunt the counterregulatory response to hypoglycemia, it is possible that we created a scenario in which the brain was dominant by choosing a high rate of insulin infusion. The absence of any differences in the counterregulatory hormone or metabolic responses to hypoglycemia, in response to vagal cooling in the presence of minimal hyperinsulinemia in the present study, clearly demonstrates that the level of insulin in the brain cannot explain the discrepancies between our results and those of Donován and colleagues (4,5,6,27). We therefore conclude that vagal afferent signaling is not involved in the counterregulatory hormonal response to hypoglycemia, regardless of the extent of hyperinsulinemia that accompanies the low blood glucose level.

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REFERENCES