Effects of vagal blockade on the counterregulatory response to insulin-induced hypoglycemia in the dog

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Effects of vagal blockade on the counterregulatory response to insulin-induced hypoglycemia in the dog. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1178–E1788, 1997.—Our aim was to determine whether vagal transmission is required for the hormonal response to insulin-induced hypoglycemia in 18-h-fasted conscious dogs. Hollow coils were placed around the vagus nerves, with animals under general anesthesia, 2 wk before an experiment. On the day of the study they were perfused with −15°C ethanol for the purpose of blocking vagal transmission, either coincident with the onset of insulin-induced hypoglycemia or after 2 h of established hypoglycemia. In a separate study the coils were perfused with 37°C ethanol in a sham cooling experiment. The following parameters were measured: heart rate, arterial plasma glucose, insulin, pancreatic polypeptide, glucagon, cortisol, epinephrine, norepinephrine, glycerol, free fatty acids, and endogenous glucose production. In response to insulin-induced hypoglycemia (42 mg/dl), plasma glucagon peaked at a level that was double the basal level, and plasma cortisol levels quadrupled. Plasma epinephrine and norepinephrine levels both rose considerably to 2,135 ± 314 and 537 ± 122 pg/ml, respectively, as did plasma glycerol (330 ± 60%) and endogenous glucose production (150 ± 20%). Plasma free fatty acids peaked at 150 ± 20% and then returned to basal levels by the end of the study. The hypoglycemia-induced changes were not different when vagal cooling was initiated after the prior establishment of hypoglycemia. Similarly, when vagal cooling occurred concurrently with the initiation of insulin-induced hypoglycemia (46 mg/dl), there were no significant differences in any of the parameters measured compared with the control. Thus vagal blockade did not prevent the effect on either the hormonal or metabolic responses to low blood sugar. Functioning vagal afferent nerves are not required for a normal response to insulin-induced hypoglycemia.

hepatic glucose production; vagus nerve

INSULIN-INDUCED HYPOGLYCERIA leads to a counterregulatory response involving the action of a number of hormones (4, 9, 17, 21, 42), neural signals (14, 20, 29, 48, 49, 50, 51), and autoregulatory responses (3, 22, 32, 45) that act in concert to increase glucose production and decrease glucose utilization. The counterregulatory hormonal changes are known (9, 17, 21, 42, 46) to be responsible for the majority of the metabolic response to insulin-induced hypoglycemia. The hormones that are involved include glucagon, epinephrine, norepinephrine, cortisol, and growth hormone. Although a great deal of research has established the effects of each of these counterregulatory hormones during insulin-induced hypoglycemia (9, 21, 42), a controversy still exists regarding the site at which the change in the plasma glucose level is sensed. The brain and the liver have both been postulated to contain glucoreceptors that are responsible for triggering the counterregulatory response.

The classic studies of Claude Bernard (1) were the first to suggest a role for the central nervous system (CNS) in glucose maintenance. More recent research has demonstrated that neural pathways linking the brain and endocrine organs are involved in the control of secretion of the counterregulatory hormones (19, 27, 37–39). In a study by Biggers et al. (2), peripheral hypoglycemia was maintained while cerebral hypoglycemia was selectively eliminated by the infusion of glucose into the carotid and vertebral arteries of conscious dogs. These authors observed that the counterregulatory response to hypoglycemia was almost eliminated when cerebral euglycemia was maintained. A study by Cane et al. (8), on the other hand, showed that the counterregulatory response to insulin-induced hypoglycemia was not significantly reduced by intracarotid glucose infusion. This apparent discrepancy was explained by Frizzell et al. (18), who showed that the brain contains redundant sites for glucose sensing such that glucose infusion into either the carotid or vertebral arteries alone produced little blunting of the counterregulatory response but that simultaneous infusion of glucose into the carotid and vertebral arteries brought about an almost complete elimination of the response. In further support of a glucose-sensing role for the brain, Borg and co-workers (5, 6) conducted several studies in the conscious rat. They first observed that bilateral lesions of the ventromedial hypothalamus (VMH) abolished the counterregulatory response to hypoglycemia (5). They then created localized glucopenia within the VMH and observed the initiation of a counterregulatory response in the absence of peripheral hypoglycemia (6). Taken together, these studies provide evidence to support the hypothesis that the counterregulatory hormone response to hypoglycemia is initiated in, or at least involves, the brain.

Other research supports the hypothesis that the liver plays a key role in glucose sensing. Russek (44) first proposed the existence of portohepatic receptors that could detect glucose levels. He further suggested that the receptors send their afferent signal regarding the glucose concentration to the CNS via the vagus nerves (45). In support of this hypothesis, electrophysiological studies demonstrated that an increase in the glucose concentration in the portal vein was associated with a decrease in the firing rate of the afferent fibers in the hepatic branch of the vagus nerve (36, 40). These studies proved the existence of neural pathways con-
necting the liver to the CNS, but they did not offer evidence of their function during hypoglycemia. To define a role of the proposed hepatic glucoreceptors in that regard, Donovan and co-workers (12, 13) conducted a hyperinsulinemic-hypoglycemic clamp in conscious dogs. They maintained systemic hypoglycemia while concurrently infusing glucose into the portal vein to selectively create euglycemia at the liver. Liver glucose clamping brought about a 40% suppression in the sympathoadrenal response to hypoglycemia. From these results the authors concluded that hepatic hypoglycemia is required to obtain a full sympathoadrenal response to low blood sugar. In a related study by Lamarche et al. (28), acute hepatic denervation was performed in anesthetized dogs. The sympathoadrenal response to insulin-induced hypoglycemia was markedly reduced, again suggesting that hepatic afferent nerves are involved in the counterregulatory response. Collectively, the above findings support the hypothesis that hepatic glucoreceptors are linked by an afferent pathway, presumably the vagus nerve, to the CNS as part of a hepatic glucoregulatory reflex that ultimately can affect the release of counterregulatory hormones.

The aim of the present study, therefore, was to determine whether the counterregulatory hormone response to insulin-induced hypoglycemia would be reduced by acutely blocking vagal transmission either during preexisting hypoglycemia or coincident with the induction of insulin-induced hypoglycemia in the conscious dog.

MATERIALS AND METHODS

Animal Care

Experiments were conducted on five conscious mongrel dogs (19.9 ± 1.5 kg) of either sex. The animals were fed once daily with meat (KalKan; Vernon, CA) and chow (Purina Lab Canine Diet no. 5006, St. Louis, MO). Before study, the dogs were deprived of food for 18 h. They were housed in a surgical 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 injected intravenously with a short-acting general anesthetic (sodium pentothal, 15 mg/kg), after which they were intubated and placed on an inhalation anesthetic (1% isofluorane) for the entire surgical procedure. A ventral midline incision was made 3 cm superior to the manubrium of the sternum that extended 8 cm rostrally through the sternocephalicus muscle. Blunt dissection bilaterally through the anterior fascia and between the sternocleidomastoid and sternohyoid muscles provided exposure of the carotid sheaths. Each vagus nerve was carefully isolated from the carotid artery over a length of 4 cm. It was then elevated with umbilical tape to facilitate placement of the coil. Hollow stainless steel coils of ~1.5 cm in length and five complete revolutions (ID 0.04 in., OD 0.625 in.) were placed around each vagus nerve (35). This length of coil was great enough to prevent saltation of nerve transmission over the block. Silastic tubing (ID 0.04 in., OD 0.085 in.) was secured to each end of each coil. The coils were then insulated with tygon tubing (ID 0.375 in., OD 0.129 in.) to prevent cooling of surrounding tissue and carotid blood. The sternocephalicus muscle was sutured to the sternothyroideus muscle to create a wall between the nerve and carotid artery at the level of the coil on both sides of the neck (Fig. 1). The ends of the Silastic tubes were placed in a subcutaneous pocket and the incision was closed.

A cut-down procedure was used for the placement of a femoral artery catheter. 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 (ID

Fig. 1. Placement of vagal cooling coils and insulator in muscle layers of neck of dog.
anesthetized dog

Table 1. Temperature and heart rate measurements in a preliminary study of vagal cooling in the anesthetized dog

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Bath</th>
<th>Vagal nerve</th>
<th>Carotid artery</th>
<th>Heart Rate, beats/min</th>
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<tr>
<td>37</td>
<td>36.5</td>
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<td>5</td>
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<td>115</td>
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<td>−7</td>
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<td>35</td>
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<td>−12 + Atropine</td>
<td>2</td>
<td>35</td>
<td>35</td>
<td>181</td>
</tr>
</tbody>
</table>

Assessment of temperature was made using temperature probes placed in cooling bath, inside cooling coil, and into vagus nerve and next to carotid artery in an anesthetized dog connected to a heart monitor for heart rate measurements. Assessment of heart rate at −15°C was made during vagal cooling in a conscious dog.

rate, as an index of vagal cooling, because PP release is known to be under vagal control (43).

On the day of the study, the femoral artery catheter and the Silastic tubes connected to the cooling coils were exteriorized 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 either connected to inflowing lines (ID 0.125 in., OD 0.25 in.) from the cooling bath or to outflowing lines to the collection reservoir. Angiocaths (18 gauge; Becton-Dickinson, Sandy, UT) were inserted percutaneously into the left cephalic vein for [3-H]glucose infusion and the left saphenous vein for insulin infusion. An angiocath was placed in the right cephalic vein for peripheral glucose infusion (20% dextrose) as needed. After coil preparation, the dog was allowed to stand calmly in a Pavlov harness for 30 min before the start of the experiment. Each dog was anesthetized (sodium pentothal, 15 mg/kg) following the experiment, and its femoral artery catheter was filled with heparin. The free ends of the catheter was knotted and placed into a new subcutaneous pocket. The tubing connected to each coil was also placed into a new subcutaneous pocket. The incisions were closed, and antibiotics were administered as described earlier. The animals were then studied 7–10 days later. Three days before each experiment, the leukocyte count and hematocrit of the animal were again measured. Only dogs that met the study criteria described earlier were reused.

Experimental Design

Each experiment consisted of a 100-min tracer equilibration period (−140 min to −40 min), a 40-min control period (−40 min to 0 min), and either a 90-min experimental period (0 min to 90 min) or a 210-min experimental period (0 min to 210 min). A priming dose (33 μCi) of [3-H]glucose 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 (based on preliminary experiments) to clamp the glucose specific activity (SA) at a constant value [experimental period mean: 15,556 ± 1,930, 12,689 ± 1,350, 14,159 ± 1,230, and 13,410 ± 986 disintegrations (dpm) mg⁻¹ in control 1, control 2, and control 2 groups, respectively] approximately equal to the mean basal SA (14,185 ± 1,853, 13,330 ± 1,350, 13,788 ± 1,600, and 13,941 ± 1,375 dpm/mg) in control 1, control 2, and cool 2 groups, respectively). At time 0, a peripheral insulin infusion (3.5 μU·kg⁻¹·min⁻¹; Eli Lilly, Indianapolis, IN) was started and continued for the entirety of the experiment in all protocols. To maintain a hypoglycemic clamp (−45 mg/dl), glucose (20% dextrose; Baxter Healthcare, Deerfield, IL) was infused peripherally as needed. In one pair of studies designed to determine whether the counterregulatory response to insulin-induced hypoglycemia could be reversed by vagal cooling, the vagal coils were perfused with either a −15°C or a 37°C alcohol-saline solution for 90 min beginning after 2 h of preexisting hypoglycemia. The 37°C group will be referred to as the control group, and the −15°C group will be referred to as the first test group, or cool 1. In a third group, perfusion of the coils with the −15°C solution was started 5 min before the start of insulin infusion (time 0); it is referred to as the second test group, or cool 2. The goal of this protocol was to determine whether counterregulation could be prevented if vagal blockade began before the induction of insulin-induced hypoglycemia. To provide control data for this protocol, we averaged the data from the 0- to 90-min period from the first pair of protocols, and they will be referred to as control 2. Each dog
participated in random order in all three limbs of the study over a period of 1 mo. 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 (52). Fifteen percent of the dog's total blood volume (250 ml) was removed in cool 1 and control 1 groups, whereas 145 ml (9%) were removed for the cool 2 group. Each volume of blood removed was replaced with two volumes of saline. The hematocrit was measured at the start of each study and averaged 41 ± 2, 40 ± 2, and 41 ± 3% in the control 1, cool 1, and cool 2 groups, respectively. Over the course of each of the three studies, the hematocrit fell by only 2, 2, and 1%, respectively.

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 radioimmunoassy (RIA), as described previously (34), with interassay coefficients of variation (CV) of 11 and 8%, respectively. Plasma samples for glucagon determination contained 50 µl of 100,000 kallikrein-inhibitor units aprotinin (TrasyloI; Miles; Kankakee, IL) added at collection. Catecholamines were assayed using high-performance liquid chromatography, as previously described (33). The interassay CVs for epinephrine and norepinephrine were 7 and 5%, respectively. The samples for catecholamines contained 60 µl glutathione-ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid added at collection. Plasma cortisol was assayed using the Clinical Assays Gamma Coat RIA kit with an interassay CV of 6% (15). PP was assayed using the method described by Hagopian et al. (23), with an interassay CV of 8%. Whole blood levels of lactate, glycerol, alanine, and β-hydroxybutyrate were determined from perchloric acid-treated samples according to the method of Lloyd et al. (30) as adapted to the Monarch 2000 centrifugal analyzer (Lexington, MA). Plasma nonesterified fatty acids were measured using a Wako FFA C test kit (Wako Chemicals, Richmond, VA) also applied to the Monarch 2000 centrifugal analyzer.

Measurement of Endogenous Glucose Production

The total rate of glucose production was determined by means of a primed tracer infusion. The data were calculated according to the method of Wall et al. (54), as simplified by DeBodo et al. (10), and also according to a two-compartment model described by Mari (31) with use of parameters for the dog as determined by Dobbins et al. (11). The results obtained with the two methods were not significantly different, and we chose to display the data from the two-compartment model in Figs. 6 and 12. To obtain endogenous glucose production, the amount of glucose infused during the hypoglycemic clamp was subtracted from total glucose production.

Statistical Analysis

Data are expressed as means ± SE. Statistical comparisons among groups and between groups were made using analysis of variance with repeated measures (53). Post hoc analysis was performed using universal F-tests or paired t-tests as appropriate. Significance was presumed at P < 0.05.

RESULTS

Reversal of the Counterregulatory Response to Hypoglycemia

Glucose and insulin. The basal glucose levels in the control and first test groups were 109 ± 1 and 108 ± 1 mg/dl, respectively (Fig 2). On insulin infusion, the glucose level declined quickly and similarly in both groups (2.0 ± 0.2 and 2.0 ± 0.1 mg·dl⁻¹·min⁻¹), reaching a hypoglycemic plateau within ~1 h. There was no significant difference between the glucose levels in the two groups at any time. Plasma insulin levels rose similarly (9 ± 1 to 127 ± 11 and 6 ± 1 to 136 ± 19 μU/ml) in both groups (Fig. 2).

Heart rate and PP. The heart rate increased significantly to 109 ± 16 beats/min in the control group and to 101 ± 11 beats/min in the test group by 2 h of hypoglycemia (Fig. 3). On cooling, there was a further increase in the heart rate to 181 ± 14 beats/min, whereas sham cooling had no effect. In the control study PP levels (Fig. 3) increased from 99 ± 13 pg/ml to a peak of 569 ± 192 pg/ml (75 min) in response to hypoglycemia but then began to fall, eventually reaching 252 ± 25 pg/ml (final 30 min). There was a similar increase in PP, from 120 ± 32 to a peak of 728 ± 325 pg/ml (90 min) in the test group. As in the control group, PP levels then began to fall, but when the vaga...
blockade was initiated, the rate of fall was increased and the PP levels quickly declined to baseline (105 ± 16 pg/ml).

Glucagon and cortisol. Glucagon peaked after 45 min of hypoglycemia and then fell to a plateau (50 ± 8 and 64 ± 15 pg/ml, respectively) between 90 and 120 min in both groups (Fig. 4). There was a slight fall thereafter, but it was similar in both groups, indicating that vagal blockade had no detectable effect on the plasma glucagon level.

The plasma cortisol levels rose to averages of 7.8 ± 0.7 and 9.8 ± 1.3 µg/dl in the two groups before the cooling period. They then declined slightly, so that by the final 30 min of the study they were 5.2 ± 0.9 and 8.2 ± 2.1 µg/ml in the control and cool groups, respectively (Fig. 4). Although not statistically significant, there appears to be a slightly higher level of cortisol in the animals subjected to vagal cooling, but the difference was apparent before cooling. It can thus again be concluded that vagal blockade had no inhibitory effect on the cortisol response to hypoglycemia.

Epinephrine and norepinephrine. Plasma epinephrine (Fig. 5) increased to 2,135 ± 314 and 1,756 ± 363 pg/ml before the cooling period in the control and cool groups, respectively. The levels did not change significantly in response to coil perfusion, indicating that vagal cooling had no effect on adrenal epinephrine.
release. Plasma norepinephrine levels (Fig. 5) rose to 537 ± 122 and 493 ± 75 pg/ml in the control and cool groups, respectively, before the cooling period. Again neither cooling nor sham cooling altered the norepinephrine response, indicating that vagal blockade was also without effect on the sympathetic nervous system's response to hypoglycemia.

Endogenous glucose production. Figure 6 shows that hepatic glucose production decreased initially by 1.2 ± 0.2 and 0.8 ± 0.2 mg·kg⁻¹·min⁻¹ in response to insulin in the control and cool groups, respectively. This was followed by a rapid increase in glucose production to averages of 3.6 ± 0.5 and 4.7 ± 0.3 mg·kg⁻¹·min⁻¹, respectively, by 45 min. Glucose production then decreased to plateaus of 3.3 ± 0.3 and 3.6 ± 0.4 mg·kg⁻¹·min⁻¹ between 90 and 120 min in the two groups. It did not change in response to coil perfusion in either group. Vagal blockade was therefore also without effect on the hypoglycemia-induced increase in glucose production.

Glycerol and free fatty acids. The arterial blood glycerol level increased to plateaus of 279 ± 25 and 267 ± 20 µmol/l (90–120 min) in the control and cool groups, respectively. Thereafter, the glycerol levels declined slightly but equivalently in both groups. Vagal cooling was without effect on the plasma glycerol level. Arterial plasma free fatty acids (FFA) rose to 1,072 ± 122 and 1,373 ± 218 µmol/l before coil perfusion in the control and cool groups, respectively (Fig. 7). In the cooling period, the FFA levels returned to baseline with or without vagal blockade. Vagal cooling was thus also without effect on the lipolytic response to hypoglycemia.

Prevention of the Counterregulatory Response to Hypoglycemia

Glucose and insulin. In the basal period, the arterial plasma glucose levels were 108 ± 1 and 104 ± 2 mg/dl in the control and cool groups, respectively (Fig. 8). The glucose level decreased after the start of insulin infusion at a similar rate (2.0 ± 0.1 mg·dl⁻¹·min⁻¹) in both groups. A hypoglycemic plateau was reached in the last hour of the study. Plasma insulin levels rose from 8 ± 1 to 141 ± 4 µU/ml in the control group and from 8 ± 1 to 141 ± 12 µU/ml in the presence of vagal cooling (Fig. 8).

Heart rate and PP. The heart rate (Fig. 9) in the control study rose slightly (75 ± 5 to 103 ± 13 beats/min) during hypoglycemia. In contrast, in the presence of vagal cooling, the heart rate increased to almost 200 beats/min (P < 0.05). The PP levels rose significantly in response to hypoglycemia (109 ± 20 pg/ml to 600 ± 207 pg/ml) in the presence of sham cooling. In contrast, the plasma PP levels did not change in the presence of vagal cooling (110 ± 32 vs. 105 ± 23 pg/ml; Fig. 9).

Glucagon and cortisol. Plasma glucagon levels (Fig. 10) peaked at 45 min in both groups and then began to fall to final levels 1.5 times basal (58 ± 9 and 65 ± 10 pg/ml in control and cool groups, respectively). Plasma cortisol levels (Fig. 10) increased similarly in both groups in response to hypoglycemia (1.8 ± 0.4 to 7.4 ± 0.3 µg/ml and 1.3 ± 0.4 to 8.5 ± 1.1 µg/ml in the absence and presence of vagal blockade, respectively). Cooling may have been associated with a slightly earlier increase in plasma cortisol and glucagon, but the differences between the two groups were not significant.

Epinephrine and norepinephrine. Plasma epinephrine increased from a basal level of 53 ± 11 pg/ml to 1,946 ± 458 pg/ml in the sham-cooled group (Fig. 11). A slightly smaller response was noted in the presence of vagal cooling (29 ± 10 to 1,376 ± 303 pg/ml), but the differences between groups were not significant. Plasma

![Fig. 6. Effects of vagal cooling (2°C) vs. control (37°C) on tracer-determined endogenous glucose production during equivalent hypoglycemic, hyperinsulinemic conditions in conscious 18-h-fasted dogs. Values are means ± SE; n = 5/group.](https://ajpendo.physiology.org/doi/10.1152/ajpendo.00363.2017)
norepinephrine levels rose similarly in the absence or presence of vagal cooling (Fig. 11).

Endogenous glucose production. Glucose production decreased initially in response to insulin (Fig. 12). It then increased to $4.3 \pm 0.3$ mg·kg$^{-1}$·min$^{-1}$ (control group) and $4.0 \pm 0.4$ mg·kg$^{-1}$·min$^{-1}$ (cool group). The hypoglycemia-induced increase in hepatic glucose production was therefore not significantly altered by vagal blockade.

Glycerol and FFA. The levels of arterial blood glycerol began to rise after the start of the hypoglycemia (Fig. 13). By the final 30 min of the study, glycerol levels had reached $253 \pm 12$ µmol/l in the presence of sham cooling and $226 \pm 40$ µmol/l in the presence of vagal cooling. With the start of insulin infusion, there was an initial drop in FFA levels followed by a steady rise to $1,244 \pm 80$ and $1,236 \pm 246$ µmol/l in the control and cool groups, respectively (Fig. 13). Vagal cooling thus had no effect on the hypoglycemia-induced lipolytic response.

DISCUSSION

It has been suggested that the counterregulatory hormone response to hypoglycemia is initiated by hepatic glucoreceptors that send afferent messages to the CNS (12, 13), most likely by way of the vagus nerve. In the present study, we attempted to define the role of the vagus nerve in response to hypoglycemia. Our results clearly demonstrate that the vagus nerves were not

Fig. 9. Effects of vagal cooling (2°C) vs. control (37°C) on arterial plasma glucagon and cortisol during equivalent hypoglycemic, hyperinsulinemic conditions in conscious 18-h-fasted dogs. Values are means ± SE; n = 5/group.

Fig. 10. Effects of vagal cooling (2°C) vs. control (37°C) on arterial plasma glucagon and cortisol during equivalent hypoglycemic, hyperinsulinemic conditions in conscious 18-h-fasted dogs. Values are means ± SE; n = 5/group.
necessary for a complete counterregulatory response to insulin-induced hypoglycemia under the conditions of our study.

To determine the acute effects of vagus nerve transmission on the counterregulatory response to hypoglycemia, we employed a vagal cooling technique first described by Fishman et al. (16). In our preliminary studies, we confirmed that a 2°C temperature at the vagus nerve created a complete blockade of transmission. We observed that the maximal increase in heart rate (to 180 beats/min) caused by cooling was not further augmented by the addition of the muscarinic blocker atropine. In addition, further cooling was without additional effect on the heart rate. It has been shown previously that, under physiological conditions, the release of PP from the pancreas is under vagal control (43, 47, 48) and can be separated from the regulation of insulin and glucagon secretion (57). In the present study we observed a rapid reversal of the hypoglycemia-induced increase in plasma PP once vagal cooling was started. In addition, we observed a complete inhibition of PP release when the vagus was cooled before the initiation of hypoglycemia. These results confirm that the vagal cooling was effective in blocking nerve transmission.

The possibility existed that vagal cooling per se would create a mild stress. If such were to be the case, we might have simultaneously eliminated the role of the vagal afferents while substituting a nonspecific stress response. To avoid this possibility we maximized the insulation of the cooling coils and eliminated the cooling of surrounding tissues. To further assess the potential stress of cooling, we examined the effect of vagal cooling in conscious dogs maintained on a pancreatic clamp (fixed basal insulin and glucagon levels). Cooling caused a transient small rise in cortisol (\( \Delta 2.0 \mu g/dl \)) that did not reach significance (55). In the present study, there was also a small rise in cortisol each time cooling was initiated, but after 15 min, the rates of change in plasma cortisol were similar in the cooled and noncooled groups. This transient mild stress might also have been responsible for the initial (15-min) increase in glucagon (\( \Delta 18 \) pg/ml) seen in the cooled, as opposed to the sham-cooled, group in the

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Fig. 11. Effects of vagal cooling (2°C) vs. control (37°C) on arterial plasma epinephrine and norepinephrine during equivalent hypoglycemic, hyperinsulinemic conditions in conscious 18-h-fasted dogs. Values are means ± SE; n = 5/group.

Fig. 12. Effects of vagal cooling (2°C) vs. control (37°C) on arterial plasma norepinephrine during equivalent hypoglycemic, hyperinsulinemic conditions in conscious 18-h-fasted dogs. Values are means ± SE; n = 5/group.

Fig. 13. Effect of vagal cooling (2°C) vs. control (37°C) on arterial blood glycerol and arterial plasma free fatty acids during equivalent hypoglycemic, hyperinsulinemic conditions in conscious 18-h-fasted dogs. Values are means ± SE; n = 5/group.

Fig. 14. Effect of vagal cooling (2°C) vs. control (37°C) on arterial blood glycerol and arterial plasma free fatty acids during equivalent hypoglycemic, hyperinsulinemic conditions in conscious 18-h-fasted dogs. Values are means ± SE; n = 5/group.

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second data set. The effect of vagal cooling on glucagon secretion could not be measured by Walmsley et al. (55), because their study design required the use of somatostatin, which inhibited endogenous glucagon release. Because these nonspecific responses to vagal cooling were small and did not persist, they did not adversely affect data interpretation. Because our dogs could only be used three times, we did not include a sham-perfused group as the control for the group in which cooling was started before hypoglycemia. Instead we used the data from the initial part of the studies, in which the cooling period was started at 120 min. When we compared the response to hypoglycemia in the study in which sham cooling occurred after the establishment of hypoglycemia to the response observed by Biggers et al. (2) under the same hyperinsulinemic and hypoglycemic conditions without coil perfusion, it was observed that they were identical. Similarly, in the study of Walmsley et al., sham cooling was without effect on any parameter measured. It is unlikely that sham cooling would have altered the response.

Cooling the vagus nerves, either in the midst of hypoglycemia or before the induction of hypoglycemia, had no impact on the counterregulatory changes in plasma norepinephrine or cortisol. The hypoglycemia-induced increase in plasma epinephrine was slightly reduced by vagal cooling only when cooling preceded the development of hypoglycemia. In terms of area under the curve, the hypoglycemia-induced excision in epinephrine was reduced by 19% in the presence of vagal blockade, but the change was not significant. Variation of this magnitude could result by chance. For example, in the first data set both groups received identical treatment for 2 h, and yet the epinephrine level was 15% higher in one group than the other. The present data therefore indicate that vagal afferents were not required for a normal sympathoadrenal response to hypoglycemia under the conditions of this study.

It has been difficult to characterize the stimulus for glucagon secretion during insulin-induced hypoglycemia because of the convergence of many signals on the pancreatic α-cell (25, 56). It is believed that these signals are part of a redundant control system used in regulating glucagon release, as shown in the rat (24). Havel et al. (26) blocked both the parasympathetic and sympathetic inputs to the pancreas in the dog and observed that the glucagon response to hypoglycemia was substantially diminished, suggesting that the autonomic nervous system is a major contributor to glucagon release. Their study is consistent with the results of Biggers et al. (2), which showed that cerebral euglycemia created a substantial reduction in the autonomic nervous system response to peripheral hypoglycemia and eliminated the normal increase in glucagon secretion. In the present study, whereas the other counterregulatory hormones remained at a maximal level for a prolonged period, glucagon peaked at 45 min and then fell back toward basal levels. A transient increase in glucagon has also been seen in other studies (2, 8, 18). As a result of the fact that the plasma glucagon levels had returned close to baseline values by the start of cooling in the first data set, we were not able to determine whether vagal blockade modified α-cell secretion. However, in the second data set, cooling was started before the induction of hypoglycemia. Blocking vagal transmission did not prevent or diminish the glucagon response. These data also support the hypothesis of redundant control mechanisms for glucagon secretion, because the inhibition of parasympathetic input to the α-cell did not produce a net alteration in glucagon secretion in response to hypoglycemia.

We also assessed the effect of vagal blockade on the metabolic response to hypoglycemia. A major consequence of the rise in counterregulatory hormones is an increase in glucose production. If the vagus nerves are essential in sending a signal from the liver to the CNS to elicit the hypoglycemia-induced counterregulatory hormone response, then one would expect to see a decrease in the hypoglycemia-induced augmentation of hepatic glucose production with vagal cooling. Furthermore, removal of parasympathetic input to the liver in the presence of continued or enhanced sympathetic input would be expected to augment glucose production. We observed no such effect. This is consistent with the unchanged hormone levels, and it suggests that alterations in parasympathetic tone to the liver are of little metabolic consequence during insulin-induced hypoglycemia. Similarly, the lipolytic response to hypoglycemia, which is evident from the change in the blood glycerol level, was unaffected by vagal cooling. Thus vagal blockade failed to have any impact on the metabolic responses of the liver or adipose tissue to insulin-induced hypoglycemia.

The data from the present study suggest that the vagus nerves are not involved in the counterregulatory response to hypoglycemia. As such, they support the conclusions of Biggers et al. (2) and Frizzell et al. (18), who suggested that the counterregulatory response to low blood sugar does not occur in the absence of brain hypoglycemia. They showed that maintenance of brain euglycemia (by carotid and vertebral artery glucose infusion) in the presence of liver hypoglycemia (≤50 mg/dl; 3.5 mU·kg·min) virtually eliminated the counterregulatory hormone response. The present results do not correlate with the observations of Donovan and co-workers (12, 13), which suggested that hepatic glucoreceptors are essential in sensing hypoglycemia and initiating the sympathoadrenal response. In the latter study, a systemic hypoglycemia (∼50 mg/dl) was induced by peripheral insulin infusion (3–3.5 mU·kg·min). Glucose was infused intraperitoneally to maintain liver euglycemia (∼95 mg/dl). These authors observed a decrease of ∼40% in the sympathoadrenal response to hypoglycemia when the liver was selectively euglycemic. They attributed the decrement to the effect of maintaining euglycemia at the liver. Taken together, the work of Frizzell et al. and Donovan and co-workers would support the hypothesis that there are two sites (liver and brain) for sensing hypoglycemia such that, when either is euglycemic, the response to hypoglycemia in the other is attenuated. If this were the case, however, in the present study blockade of afferent signaling from the liver to the CNS should
have accomplished the same thing as portal glucose infusion and reduced the counterregulatory response, and it did not. On the other hand, there is also another possible explanation for the findings of Donovan and co-workers. By maintaining euglycemia in the portal vein in the presence of hypoglycemia in the periphery (i.e., artery), a negative arterial-portal glucose gradient was established. This simulates a condition seen after feeding, when the glucose level is higher in the portal vein than in the arterial circulation (41). A rise in portal vein glucose (which was associated with a negative arterial-portal glucose gradient) has been shown to be associated with a decrease in the firing rates of vagal afferents and adrenal nerves (38). Therefore, the decrease in the sympathoadrenal response observed by Donovan and co-workers might actually have been expected and may represent a feeding response. If such is the case, it should be remembered that the portal glucose level usually does not exceed the arterial glucose level during insulin-induced hypoglycemia. The portal glucose receptors would thus not be activated unless the subject consumed glucose.

In an in vivo study in anesthetized dogs, Lamarche et al. (28) showed that acute hepatic denervation hinders the adrenal catecholamine response to insulin-induced hypoglycemia (≈55 mg/dl; 0.15 IU/kg iv). The maximum catecholamine response to insulin injection in these dogs was significantly attenuated (90% for epinephrine and 82% for norepinephrine) relative to that seen in sham-denervated dogs. This study supports the concept that a hepatoadrenal reflex is involved with the release of epinephrine and norepinephrine in response to hypoglycemia. It must be remembered, however, that, in the study by Lamarche et al., both parasympathetic and sympathetic nerves were acutely severed and the animals were anesthetized as well as surgically stressed. Therefore, the reduction in the catecholamine response to hypoglycemia could be due to the removal of nonvagal afferents, or it could relate to an effect specific to the anesthetized animal. Although it is believed that the putative hepatic glucosensors are linked to the brain via the vagal afferent fibers, the possibility that the hepatic glucosensors send the information concerning the hepatic glucose level via afferent fibers traveling along sympathetic nerves (such as the splanchnic nerves) to the brain cannot be ruled out. In the present study, only the fibers traveling with the vagus nerves have been disrupted, possibly leaving afferent fibers traveling within the sympathetic system to communicate the glucose level information to the CNS. This could explain the discrepancy between the results of Lamarche et al. and our results. In addition, in this particular study we tested the importance of the parasympathetic nervous system in a hyperinsulinemic hypoglycemic setting, and this should not be used as evidence to minimize its importance in other circumstances.

In summary, the present study has shown that the hepatic vagal nerves were not involved in the normal counterregulatory response to insulin-induced hypoglycemia under the conditions of our study (glucose = 45 mg/dl; insulin = 140 pU/ml). These data thus do not support the hypothesis that liver glucose sensors are involved in the normal response to hypoglycemia. The possibility remains that, under other conditions (i.e., different insulin and/or glucose levels), they may play a role. It is also possible that the liver glucose sensors communicate with the brain via a nonvagal mechanism.

The authors acknowledge the excellent technical assistance of M. Scott, P. Venson, E. Allen, and Wanda Snead and the excellent secretarial support of Patsy Raymer.

The work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants 2R01 DK-18243 and SP60 DK-20593.

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Received 22 January 1997; accepted in final form 11 September 1997.

**REFERENCES**
