Effect of hepatic denervation on the counterregulatory response to insulin-induced hypoglycemia in the dog

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Jackson, Patricia A., Sylvain Cardin, Christopher S. Coffey, Doss W. Neal, Eric J. Allen, Angelina R. Penaloz, Wanda L. Snead, and Alan D. Cherrington. Effect of hepatic denervation on the counterregulatory response to insulin-induced hypoglycemia in the dog. Am J Physiol Endocrinol Metab 279: E1249–E1257, 2000.—Our aim was to determine whether complete hepatic denervation would affect the hormonal response to insulin-induced hypoglycemia in dogs. Two weeks before study, dogs underwent either hepatic denervation (DN) or sham denervation (CONT). In addition, all dogs had hollow steel coils placed around their vagus nerves. The CONT dogs were used for a single study in which their coils were perfused with 37°C ethanol. The DN dogs were used for two studies in a random manner, one in which their coils were perfused with 20°C ethanol (DN + COOL) and one in which they were perfused with 37°C ethanol (DN). Insulin was infused to create hypoglycemia (51 ± 3 mg/dl). In response to hypoglycemia in CONT, glucagon, cortisol, epinephrine, norepinephrine, pancreatic polypeptide, glycerol, and hepatic glucose production increased significantly. DN alone had no inhibitory effect on any hormonal or metabolic counterregulatory response to hypoglycemia. Likewise, DN in combination with vagal cooling also had no inhibitory effect on any counterregulatory response except to reduce the arterial plasma pancreatic polypeptide response. These data suggest that afferent signaling from the liver is not required for the normal counterregulatory response to insulin-induced hypoglycemia.

hepatic glucose production; liver nerves; parasympathetic blockade; vagus nerve

LOW BLOOD GLUCOSE results in counterregulatory hormone responses (glucagon, cortisol, epinephrine, norepinephrine, and growth hormone) that increase glucose production and decrease glucose utilization. Although the effects of these hormones have been well characterized, the signal initiating their release remains controversial.

Glucosensors for hypoglycemia have been proposed to lie both within the hepatoportal region and the central nervous system (CNS). Their function is to sense low blood glucose and to signal the brain to coordinate a counterregulatory response. Frizzell et al. (11) brought about cerebral euglycemia during generalized hypoglycemia and almost totally eliminated the counterregulatory response. From these data, they suggested that the brain contained the glucose-sensing sites. In contrast, Donovan et al. (7) conducted a study in which they maintained hepatic euglycemia during generalized hypoglycemia. They observed a 40% decrement in the sympathoadrenal response to hypoglycemia and concluded that the liver contained glucosensors and that they play the dominant role in initiating the counterregulatory response.

It has been suggested that glucose sensors in the hepatoportal region are linked to the brain via afferent fibers traveling within the vagus nerves (1, 23). In a previous study, we blocked vagal afferent signaling during hyperinsulinemic hypoglycemia in conscious dogs (14). We showed that, under those conditions, functioning vagus nerves were not necessary for a complete counterregulatory hormone response to moderate hypoglycemia. It remains possible, however, that hepatic glucosensors communicate with the brain through afferent fibers traveling via nonparasympathetic nerves. In support of this possibility, Lamarche et al. (15), using the anesthetized dog, suggested that liver denervation resulted in a diminution in the normal response of plasma norepinephrine and epinephrine to hypoglycemia. Furthermore, in a recent paper, Hevener et al. (13) suggested that chemical denervation of the hepatic portal vein decreased the sympathoadrenal response to sustained systemic hypoglycemia in rats. The question thus arises as to whether, in the conscious dog, hepatic denervation would result in a diminution of the counterregulatory response to insulin-induced hypoglycemia.

To clearly address the question of afferent neural signaling in the counterregulatory response to hypoglycemia, we conducted the present study using conscious dogs that had undergone hepatic denervation. In addition, we used a vagal cooling technique to ensure the elimination of both parasympathetic and sympa-
thetic signaling, because it is not possible to confirm the former on biopsy. With this model, we were able to completely interrupt neural communication between the hepatoporal region and the brain. The aim of our study, therefore, was to determine whether chronic hepatic denervation alone or in combination with vagal cooling would reduce the counterregulatory response to insulin-induced hypoglycemia in the conscious dog.

MATERIALS AND METHODS

Animal care. Experiments were conducted on 10 conscious mongrel dogs (24.8 ± 1.5 kg) of either sex. The animals were fed once daily with a meat (KalKan; Vernon, CA) and chow (Purina Lab Canine Diet No. 5006, St. Louis, MO) diet (34% protein, 46% carbohydrate, 14.5% fat, and 5.5% fiber based on dry weight). Before study, the dogs were deprived of food for 18 h. The animals were housed in a facility that met the standards of the American Association for the Accreditation of Laboratory Animal Care International, 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% isoflurane) for the entire surgical procedure. All dogs underwent a laparotomy. Five dogs underwent complete surgical denervation of the liver (DN). Nerves were stripped along the common hepatic artery and its branches from the celiac ganglion to the liver. Nerves were also stripped beginning at the entry of the splenic vein into the portal vein and continuing rostrally to the liver. The other five dogs underwent a sham procedure (CONT), in which the structures along the hepatic nerves were manipulated but innervation was not interrupted. The success of the surgical denervation procedure was verified by measuring liver norepinephrine levels after each study. The liver norepinephrine concentration in DN dogs was 2.5 ± 1% of that in normal innervated liver (2). Stainless steel cooling coils, with Silastic extension tubing attached, were placed around the vagus nerves in all dogs as described previously (14). The effectiveness of cooling (COOL) to block parasympathetic signaling was verified by measuring the heart rate and arterial plasma pancreatic polypeptide concentrations (both of which are under vagal control).

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 0.04 in., OD 0.085 in.) was inserted through a small hole in the artery, and its tip was extended to distally. A Silastic catheter (ID 0.04 in., OD 0.085 in.) from the cooling bath and to outflowing lines connecting them to the collection reservoir. Angiocaths (18 gauge; Becton-Dickinson, Sandy, UT) were inserted percutaneously into the left cephalic vein for [3-3H]glucose infusion, the left saphenous vein for insulin infusion, and 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 DN dog was reanesthetized (sodium pentothal, 15 mg/kg) after the experiment, and its femoral artery catheter was filled with heparin. The free ends of the arterial and cooling coil catheters were knotted and 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 in a random manner. Three days before each experiment, the leukocyte count and hematocrit of the animal were again measured. Only dogs which met the study criteria described earlier were reused.

Experimental design. Eighteen-hour-fasted dogs underwent an experiment consisting of a 100-min tracer equilibration period (−40 to −0 min), a 40-min control period (−40 to 0 min) and a 90-min experimental period (0–90 min). A priming dose (33 μCi) of [3-3H]glucose (Du Pont-NEN, Boston, MA) was administered at −140 min, followed by a continuous infusion of 0.29 μCi/min of [3-3H]glucose. This tracer infusion was adjusted as needed in each of the three groups (see below) to clamp the glucose specific activity (SA) at a constant value approximately equal to the basal SA (11,945 ± 1,673 to 9,742 ± 1,234 dpm/mg in CONT, 11,671 ± 1,415 to 9,989 ± 1,045 dpm/mg in DN, and 12,157 ± 1,256 to 10,227 ± 680 dpm/mg in DN + COOL). At time 0, a peripheral porcine insulin infusion (5.0 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 (≥50 mg/dl), glucose (20% dextrose; Baxter, Deerfield, IL) was infused through a leg vein as needed. During CONT and DN experiments, the coils were perfused for the entire experimental period (90 min) with 37°C fluid concurrent with the insulin infusion. In contrast, in the DN + COOL group, a perfusion of −20°C fluid was begun 5 min before the initiation of insulin infusion (time 0) and continued throughout the 90-min experimental period. The order of the DN and DN + COOL protocols was randomly determined. 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 (24). Approximately 8% of the dog’s total blood volume was removed during each study.

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 by a double-antibody radioimmunoassay described previously (20) with interassay coefficients of variation (CV) of 7 and 5%, respectively. Plasma samples used for glucagon determination contained 100,000 kallikrein inhibitor units of aprotinin (Trasylol; Miles, Kankakee, IL) added at collection. Catecholamines were assayed by high-performance liquid chromatography, as previously described (18). The interassay CV for epinephrine and norepinephrine were 7 and 5%, respectively. The samples for catecholamine analysis con-
Hepatic denervation and hypoglycemia.

RESULTS

Insulin and glucose. With peripheral insulin infusion, plasma insulin levels rose similarly in all groups from basal levels (CONT, 13 ± 1; DN, 10 ± 1; DN + COOL, 12 ± 2 μU/ml) to average values during the last hour of 297 ± 42, 296 ± 50, and 338 ± 39 μU/ml in CONT, DN, and DN + COOL, respectively (Fig. 1). The average basal glucose levels were 112 ± 2, 108 ± 2, and 107 ± 4 mg/dl in the three protocols (CONT, DN, and DN + COOL, respectively; Fig. 1). Upon insulin infusion, the glucose level quickly declined to a hypoglycemic plateau (51 ± 3, 50 ± 2, and 48 ± 2 mg/dl during the final hour in CONT, DN, and DN + COOL, respectively). There were no significant differences among the glucose levels in the three protocols at any time.

Heart rate and pancreatic polypeptide. The heart rate (Fig. 2) increased significantly from basal in both CONT and DN in the hypoglycemic period (averaging 113 ± 10 and 108 ± 11 beats/min, respectively, during the last hour). The addition of vagal cooling resulted in a marked increase in heart rate to 211 ± 16 beats/min within 15 min. Pancreatic polypeptide levels increased significantly in the CONT and DN groups to 941 ± 304 and 871 ± 277 pg/ml, respectively, and then fell slightly as hypoglycemia continued (Fig. 2). In the DN + COOL group, there was a slight decrease from basal during the experimental period, attesting to the effectiveness of cooling in preventing parasympathetic input to the pancreas.

Glucagon and cortisol. Glucagon peaked by 30 or 45 min of hypoglycemia (101 ± 18, 120 ± 41, and 97 ± 19 pg/ml in CONT, DN, and DN + COOL groups, respectively) and then began to decline as the hypoglycemia continued (Fig. 3). There were no significant differences in glucagon levels among groups. Similarly, AUC measurements showed no significant differences in arterial plasma glucagon levels (1,087 ± 457, 1,483 ± 327, and 2,329 ± 523 pg/ml for CONT, DN, and DN + COOL, respectively), although there was a tendency for the glucagon change to be greater in the presence of combined denervation and cooling than in the other two groups. Clearly, hepatic denervation (DN) did not significantly reduce the glucagon response to hypoglycemia.
The plasma cortisol levels rose to averages of 7.8 ± 1.3, 9.6 ± 1.5, and 10.1 ± 1.5 μg/dl in CONT, DN, and DN + COOL, respectively, for the final 45 min of hypoglycemia (Fig. 3). AUCs for plasma cortisol were 237 ± 38, 373 ± 60, and 475 ± 71 μg/dl in CONT, DN, and DN + COOL, respectively. In this case, the AUC in the DN + COOL protocol was significantly higher than in the CONT group. It can still be concluded, however, that hepatic denervation (DN) did not decrease the cortisol response to hypoglycemia.

Epinephrine and norepinephrine. The plasma epi-


erine level (Fig. 4) increased markedly during the hyperinsulinemic hypoglycemic period to averages of 1,492 ± 263, 1,778 ± 449, and 1,945 ± 839 pg/ml for the final 30 min of the CONT, DN, and DN + COOL protocols, respectively. The AUCs were 63,228 ± 10,825, 68,520 ± 12,136, and 89,480 ± 27,862 pg/ml for each protocol, respectively. The levels were not significantly different among protocols, indicating that nei-


er denervation nor denervation plus vagal cooling significantly altered the adrenal response to hypoglycemia. Similarly, plasma norepinephrine levels (Fig. 4) rose to averages of 400 ± 34, 494 ± 79, and 514 ± 165 pg/ml for the final 30 min in CONT, DN, and DN + COOL protocols, respectively. The AUCs were, respectively, 14,039 ± 2,508, 13,049 ± 666, and 15,457 ± 5,445 pg/ml in each protocol. Neither denervation nor denervation plus vagal cooling significantly reduced the sympathetic nervous system’s response to hypoglycemia.

**Blood glycerol and endogenous glucose production.** Arterial blood glycerol levels (Fig. 5) began to rise after the first 15 min of the hypoglycemic period in all groups, reaching a similar plateau in the last 30 min of the study (194 ± 19, 229 ± 61, and 198 ± 53 μmol/l in CONT, DN, and DN + COOL, respectively). The lipolytic response to hypoglycemia was unaffected by hepatic denervation or vagal cooling. Figure 5 shows that endogenous glucose production be-


gan to rise by 30 min of hypoglycemia and averaged
4.7 $\pm$ 0.7, 5.7 $\pm$ 0.5, and 5.8 $\pm$ 0.6 mg·kg$^{-1}$·min$^{-1}$ in
CONT, DN, and DN + COOL, respectively, during
the last hour of the study. Glucose production was
not significantly different from the control response
when the liver was denervated or when denervation
was combined with vagal blockade.

When one examines the AUCs (Fig. 6) for the last 15
min of the experiment, it is clear that there was no
significant difference among the three groups with
regard to arterial plasma epinephrine or norepineph-
rine. Similarly, there was no significant difference
among the three groups with regard to endogenous
glucose production or the arterial blood glycerol level.

Substrates. Plasma free fatty acids, blood alanine,
lactate, and β-hydroxybutyrate are shown in Table 1.
ANOVA failed to show any period or treatment effects
for free fatty acids or β-hydroxybutyrate. There was a
period effect for alanine that failed to reach signifi-
cance when post hoc analysis was applied. There was a
significant increase in blood lactate levels during the
hypoglycemic period in the DN and DN + COOL
groups ($P < 0.05$).

DISCUSSION

It has been suggested that the counterregulatory
hormone responses to hypoglycemia are, at least in
part, initiated by hepatic glucoreceptors that send af-
ferrrent messages to the CNS (7, 8). We have previously
shown that the vagus nerves are not involved in the
transmission of this signal (14). In the present study,
we attempted to define the role of afferent nerves, not
traveling along the parasympathetic system, in trans-
mitting signals from the liver to the brain in response
to hypoglycemia. This is particularly important in light
of recent suggestions that the afferent signals may not
travel along the vagal trunk. The present results dem-
strate that afferent nerves originating in the hepato-
portal region, regardless of their path to the brain,
are not necessary for a complete counterregulatory
response to the insulin-induced hypoglycemic challenge we presented.

To examine the effects of afferent nerve transmission from the liver to the CNS, we chronically denervated the liver. This surgical technique has been utilized previously in our laboratory (2, 19), and as before, its effectiveness was determined by measuring liver norepinephrine concentrations from each of the seven liver lobes and comparing them with levels in normal dog liver. In the present study, the norepinephrine content of the liver decreased to $2.5 \pm 1.0\%$ of normal concentration, thus indicating complete denervation. Because we could not directly measure liver acetylcholine, we further ensured blockade of afferent signals by combining our previously described vagal cooling technique (14) with liver denervation. The completeness of parasymathetic blockade was confirmed by measuring plasma pancreatic polypeptide levels and heart rate. The former failed to rise in response to hypoglycemia, whereas the heart rate increased twofold.

A similar hypoglycemic nadir ($\approx 50 \text{ mg/dl}$) was achieved in all three protocols. Likewise, hyperinsulinemia ($\approx 310 \text{ mU/ml}$) was similar in all three groups. Hepatic denervation (DN) caused no impairment in the hypoglycemia-induced elevations in plasma norepinephrine, epinephrine, cortisol, or glucagon. This is most easily demonstrated by our observation that the increase in AUC for each of the above hormones was, if anything, slightly greater in the DN group than in the CONT group. Despite the lack of evidence of a decreased response, it is possible that we could have missed a small change in the counterregulatory hor-

Table 1. Effects of hepatic denervation and hepatic denervation plus vagal cooling on free fatty acids, alanine, lactate, and $\beta$-hydroxybutyrate during average basal and average last 60 min of experimental periods

<table>
<thead>
<tr>
<th>Periods</th>
<th>CONT Average</th>
<th>CONT Average</th>
<th>DN Average</th>
<th>DN Average</th>
<th>DN+COOL Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal (last 60 min)</td>
<td>Experimental, Experimental, (last 60 min)</td>
<td>Basal (last 60 min)</td>
<td>Experimental, Experimental, (last 60 min)</td>
<td>Basal (last 60 min)</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>$509 \pm 96$</td>
<td>$726 \pm 103$</td>
<td>$665 \pm 88$</td>
<td>$749 \pm 216$</td>
<td>$600 \pm 87$</td>
</tr>
<tr>
<td>Alanine</td>
<td>$359 \pm 49$</td>
<td>$294 \pm 14$</td>
<td>$340 \pm 43$</td>
<td>$239 \pm 28$</td>
<td>$403 \pm 43$</td>
</tr>
<tr>
<td>Lactate</td>
<td>$869 \pm 154$</td>
<td>$1,512 \pm 233$</td>
<td>$667 \pm 73$</td>
<td>$1,497 \pm 116^*$</td>
<td>$828 \pm 107$</td>
</tr>
<tr>
<td>$\beta$-Hydroxybutyrate</td>
<td>$35 \pm 18$</td>
<td>$24 \pm 4$</td>
<td>$19 \pm 2$</td>
<td>$16 \pm 3$</td>
<td>$17 \pm 4$</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE expressed in $\mu\text{mol/l}$. CONT, control group; DN, liver-denervated group; DN+COOL, DN + cooling of vagus nerves. $^*$Significant difference from the respective control group.
mone response due to the variation in the data. With regard to norepinephrine, we had excellent power and could have detected a change of 75 pg/ml with a power of 0.95. With epinephrine, the variation was greater, thereby lessening our precision; nevertheless, we could have detected a change in epinephrine of 800 pg/ml with a power of 0.80.

Because there was a possibility of residual parasympathetic activity at the liver (upon biopsy, we could not rule out the presence of parasympathetic nerve endings), we utilized our vagal cooling technique in addition to hepatic denervation (DN + COOL) to ensure complete neural isolation of the liver. Even in the absence of both sympathetic and parasympathetic signaling, there was still no impairment in any counterregulatory response to hypoglycemia. The addition of vagal nerve cooling to liver denervation (DN + COOL) caused a significant rise (≈5 μg/dl) in cortisol levels at the initial 15-min time point that was not evident in the CONT group. As a result, the AUC for the plasma cortisol level was significantly greater with the DN + COOL treatment than in DN. We noted such a change previously on two occasions (14, 27), and it probably represents a response to a mild stress created by the cooling per se. In a previous study (27), we showed that under euglycemic conditions with insulin and glucagon clamped at basal values, vagal cooling resulted in a ≈3 μg/dl increase in the plasma cortisol level that was evident for the 90 min of the experiment. When one calculates the AUC for cortisol in the present study (DN + COOL) and subtracts the part probably attributable to the cooling stress (assuming it was sustained for 90 min) there was no difference in the cortisol response to hypoglycemia in the control and DN + COOL protocols.

In the present study, we also observed a small initial rise in plasma glucagon in response to cooling that was not evident in the control protocol. Glucagon secretion, therefore, may also have been stimulated by the cooling stress. It should be noted, however, that this phenomenon was not observed in our earlier euglycemic experiments because we used somatostatin to clamp plasma glucagon (27). Because we do not know the time course of any potential effect of cooling on glucagon secretion, it is difficult to interpret the glucagon data for the DN + COOL protocol. It should be remembered, however, that liver denervation had no inhibitory effect on hypoglycemia-induced glucagon secretion in the present study and that in none of the studies by Donovan and colleagues (7, 8, 13) was there any evidence for an alteration in the response of glucagon to hypoglycemia. Interestingly, as we showed previously and as is evident here, neither epinephrine nor norepinephrine appeared to show any increase in response to the stress of vagal cooling per se.

We also assessed the effect of hepatic denervation on the metabolic responses to insulin-induced hypoglycemia. Not only was there no diminution in the counterregulatory hormone responses to low plasma glucose when the liver was denervated, there was also no decrease in the increment in glucose production. Removal of both parasympathetic and sympathetic nerves to the liver had no apparent effect on its ability to produce glucose in response to hypoglycemia. Similarly, the lipolytic response to hypoglycemia, as reflected in the plasma glycerol level, was also unaffected by hepatic denervation with or without the addition of vagal cooling.

In a study by Connolly et al. (3), dogs were adrenalectomized and given somatostatin to completely block the hormonal response to insulin-induced hypoglycemia (≈36 mg/dl). When the dogs were given glucose via carotid and vertebral catheters to maintain cerebral euglycemia, there was little or no blunting of the hypoglycemia-induced increase in glucose production. It was concluded, therefore, that the liver either senses the hypoglycemia and directly responds to it or it transmits a signal to the brain with a subsequent relay back to the liver (despite euglycemia in the brain). Because we know that 50% of the increase in hepatic glucose production during insulin-induced hypoglycemia is dependent on hormonal response (10), and because in the present study we found that neither the counterregulatory hormone response nor the increase in hepatic glucose production changed when the liver was denervated, it would seem that neural input to the liver is of little significance to the hepatic response. This would suggest that, in the study of Connolly et al., the liver increased glucose production by responding directly to the low glucose concentration and not by responding reflexly to neural input from the CNS.

Although we have demonstrated that there appears to be no transmission of signals from the liver to the brain via sympathetic or parasympathetic nerves, it must be noted that our finding may be specific to our experimental conditions, in which hypoglycemia (50 mg/dl) was induced by a high dose of insulin (5.0 mU·kg⁻¹·min⁻¹ administered via a peripheral leg vein). It is possible that the liver plays a role in initiating counterregulation at different levels of hypoglycemia or hyperinsulinemia. For example, in the study of Donovan et al. (7), a peripheral insulin infusion of 3–3.5 mU·kg⁻¹·min⁻¹ was used to create a milder hypoglycemia (≈58 mg/dl). When liver euglycemia (≈95 mg/dl) was maintained by portal glucose infusion, they observed a decrement of ≈40% in the sympathoadrenal response to hypoglycemia. The discrepancy between our findings and theirs may relate to the different levels of insulin and hypoglycemia used, although this seems unlikely given the relatively small differences in the experimental conditions that were used. A more plausible explanation is that, in the study of Donovan et al., a feeding signal was created by the establishment of a negative arterioporal glucose gradient (22). This has been shown to be associated with a decrease in afferent nerve firing to the adrenal glands (21), thus potentially explaining the decrease in the sympathoadrenal response seen by Donovan et al. 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 paper,
Hevener et al. (13) diminished the magnitude of the sympathoadrenal response to insulin-induced hypoglycemia in rats by chemically denervating the portal vein. They therefore concluded that attributing the diminished sympathoadrenal response to a negative arteriportal glucose difference is not valid. The difficulty with their experimental design is that, by chronically denervating the portal vein, the input from the afferent fibers to the brain is eliminated, thereby simulating a high glucose level in the portal vein (an increase in the portal vein glucose concentration decreases afferent vagal firing rates). Their results are, therefore, not surprising and can be interpreted as supporting the concept of a feeding signal reducing the response to hypoglycemia.

Hevener et al. (13) suggested that we might not have completely stripped the nerves of the portal vein for a sufficient distance to completely remove all the hepatopetal sensors. This seems unlikely, given the nature of the surgical procedure, and because in a previous publication (2) we abolished the increase in net hepatic glucose uptake induced by the portal signal by using the same denervation procedure. This suggests that our denervation technique is sufficient to eliminate glucose sensors within the hepatopetal region. It is possible, on the other hand, that, after chronic denervation, the organ develops an adaptive mechanism to react to hypoglycemia, thus explaining why we did not see any differences among the groups in the present study.

It is also possible that, in the presence of very high insulin levels, the brain can integrate neural signals differently. It is known that the brain is responsive to the insulin level in plasma per se, but the significance of this finding is unknown. Davis et al. (4) showed that, with a selective physiological increase in the plasma insulin level in the carotid and vertebral arteries (≈200 μU/ml), the counterregulatory response to hypoglycemia (≈57 mg/dl) was amplified relative to that seen with the same glycemia but less insulin. The increase in hepatic glucose production during insulin-induced hypoglycemia (≈200 μU/ml), the counterregulatory response to hypoglycemia.


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