Original Article

Celiac-Superior Mesenteric Ganglionectomy, but Not Vagotomy, Suppresses the Sympathoadrenal Response to Insulin-Induced Hypoglycemia

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Afferent innervation of the portal vein has been shown to be critical in hypoglycemic detection, but the neural pathway by which these afferents ascend remains unknown. To ascertain the role of vagal afferents versus spinal afferents in hypoglycemic detection, the catecholamine response to hypoglycemia was assessed in male Wistar rats undergoing hepatic vagotomy (HV), total subdiaphragmatic vagotomy (TSV), or celiac-superior mesenteric ganglionectomy (CSMG). After recovering from the surgery, the animals were exposed to a hyperinsulinemic-hypoglycemic clamp, with glucose infused peripherally via the jugular vein. In all animals, systemic hypoglycemia (2.64 ± 0.03 mmol/l) was induced via jugular vein insulin infusion (25 mU · kg^{-1} · min^{-1}). No significant differences were observed among the groups with respect to arterial glucose or insulin concentration. When hypoglycemia was induced in sham-operated control animals, epinephrine was observed to rise from a basal value of 0.84 ± 0.10 to 25.18 ± 1.24 nmol/l. Neither HV nor TSV had any significant impact on the epinephrine response to hypoglycemia. In contrast, CSMG animals demonstrated a significant suppression in the epinephrine response to whole-body hypoglycemia (11.25 ± 1.21 vs. 22.32 ± 0.86 nmol/l in CSMG vs. controls; $P < 0.05$). The norepinephrine response for controls, 2.00 ± 0.22 at basal and rising to 8.95 ± 0.20 nmol/l in hypoglycemia, was not significantly different from that of the HV and TSV animals. As with epinephrine, the norepinephrine response to hypoglycemia was significantly suppressed in CSMG compared with control animals (4.72 ± 0.48 vs. 7.15 ± 0.76 nmol/l; $P < 0.05$). These findings are consistent with the idea that hypoglycemic detection at the portal vein is mediated by spinal, and not vagal, glucose-sensitive afferents. *Diabetes* 54:3258–3264, 2005

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2-DG, 2-deoxyglucose; CGRP, calcitonin gene-related peptide; CSMG, celiac-superior mesenteric ganglionectomy; HV, hepatic vagotomy; TSV, total subdiaphragmatic vagotomy.

The inability of type 1 diabetic patients to suppress insulin and these patients' diminished glucagon response severely limits their ability to defend against hypoglycemia (1). For these individuals, the sympathoadrenal response (i.e., epinephrine and norepinephrine) constitutes the primary mechanism for counterregulation (2). However, the sympathoadrenal response may also be compromised in these individuals, predisposing them to more severe episodes of hypoglycemia (3). Attempts to effect more rigorous glycemic control have actually exacerbated this problem (4,5). As such, hypoglycemia now constitutes the primary limitation in the treatment of diabetes (6).

The defective sympathoadrenal response for individuals undergoing insulin treatment appears to be due, in part, to a defect in hypoglycemic detection. Once thought to be exclusive to the central nervous system, hypoglycemic detection is now known to also occur in the periphery (7–9). In particular, glucosensors residing in the portal vein appear essential for mediating the full sympathoadrenal response to hypoglycemia (10,11). Normalizing portal vein glycemia in the face of systemic hypoglycemia has been shown to severely blunt the sympathoadrenal response to hypoglycemia (7,8,10–12). This reaction is particularly profound when hypoglycemia develops slowly over the course of several hours (8). Under such conditions, the sympathoadrenal response has been shown to be a function of the portal glycemia and to be independent of brain glycemia (8).

Although portal vein glucose sensing has not been well characterized, we have shown that denervation of the portal vein with phenol substantially impairs the sympathoadrenal response to hypoglycemia and eliminates portal glucose detection (11). The origin of these glucose-sensitive afferents has been the subject of some speculation. It has been known for some time that glucose-sensitive vagal afferents innervate the portohepatis. Niijima (13) observed hepatic vagal afferents that demonstrated firing rates inversely related to the concentration of glucose perfusing the portal vein. This modulation of vagal firing was specific to glucose, as other hexoses or pentoses had no suppressive effect (13,14). Others have demonstrated that the induction of satiety via portal vein glucose infusions could be eliminated via hepatic vagotomy (15,16). These observations have led many to surmise that hypoglycemic detection at the portal vein is mediated by vagal afferents (17,18).

However, recent studies in which vagal firing was inter-

rupted at the neck level have cast doubt on the relevance of vagal afferents to hypoglycemic detection (19,20). Cooling the vagus nerve to 2°C, sufficient to interrupt nerve firing, had no impact on the counterregulatory response to hypoglycemia (19). This finding led the investigators to question the role of not only vagal afferents but also portal vein glucosensors in hypoglycemic detection; however, such a conclusion ignores the possibility that portal vein glucose-sensitive afferents may be nonvagal in origin (e.g., they may be spinal afferents). Visceral spinal afferents, although generally associated with nociception, are now recognized to support a variety of functions, including metabolic sensing (17). The vasculature of the gut, including the portal vein, has been shown to be richly innervated by afferents emerging from the dorsal root ganglia at T7–T13 (21).

In the current study, we examined the impact of selective vagotomies (hepatic vagotomy [HV] and total subdiaphragmatic vagotomy [TSV]) versus celiac-superior mesenteric ganglionectomy (CSMG) on the subsequent sympathoadrenal response to insulin-induced hypoglycemia. Denervated and sham control animals were exposed to identical hyperinsulinemic-hypoglycemic clamps, and their subsequent sympathoadrenal responses were compared. Hypoglycemia was allowed to develop slowly over the course of 1 h, a time course that appears to maximize the contribution of portal vein glucosensors. As noted previously for dogs, vagotomized rats demonstrated the full sympathoadrenal response to hypoglycemia with no obvious counterregulatory impairment. In contrast, animals undergoing CSMG demonstrated a substantially blunted sympathoadrenal response, comparable with that observed previously for denervation of the portal vein (11). These findings provide the first evidence that hypoglycemic detection at the portal vein is mediated by nonvagal, most likely spinal, afferents that traverse the celiac-superior mesenteric ganglion complex.

RESEARCH DESIGN AND METHODS

Experiments were conducted on male Wistar rats (weight 244.7 ± 21.3 g; n = 25) in the conscious relaxed state. All surgical and experimental procedures were preapproved by the University of Southern California Institutional Animal Care and Use Committee. Animals were chronically cannulated under single-dose anesthesia (3:3:1 ketamine HCl, xylazine, acepromazine maleate; 0.10 ml/100 g body wt, i.m.). Cannulas were placed in the carotid artery (Clay Adams, PE-50) for arterial blood sampling and in the jugular vein (dual cannula silastic, 0.012 cm internal diameter) for peripheral infusion of insulin and glucose. All cannulas were tunneled subcutaneously, exteriorized at the back of the neck, and connected to a dual channel swivel via a tethering system (Instech, Plymouth Meeting, PA).

The surgical procedures for HV and TSV have been previously described in detail (22,23). Briefly, for HV, the abdominal cavity was exposed through a ventral midline incision and the intestines were reflected to the right side. The subdiaphragmatic portion of the esophagus was exposed and the anterior trunk of the vagus nerve was identified. The median and caudal lobes of the liver were then deflected to allow visualization of the hepatic vagus branch, which makes a distinct bifurcation from the anterior vagal trunk several millimeters proximal to the cardia. The hepatic branch of the vagus, along with the fascia surrounding the nerve, was completely sectioned 10 mm distal to its origin. To effect a TSV, both the anterior and posterior trunks were located on the esophagus and 10 mm segments of both trunks were removed immediately caudal to the diaphragm, with the hepatic branch of vagus being cut as described above. Control animals underwent identical surgical procedures, including isolation of the hepatic and subdiaphragmatic trunks, but the nerves were left intact.

Extirpation of the celiac and superior mesenteric ganglia was initiated via a ventral midline incision, exposing the abdominal cavity with the intestines reflected to the right side. The celiac and superior mesenteric ganglia were then located under microscope on the descending aorta at the branch points between the celiac and superior mesenteric arteries. Both ganglia were then gently removed by blunt dissection and severed from all visible connections to the splanchnic nerve trunks (23–26). Control animals were subjected to an identical surgical procedure except for the actual denervation.

After all surgical procedures, wounds were closed via individual sutures and the animals were given 6 days to recover and regain their body weight. Total subdiaphragmatic denervation leads to impaired digestion, intestinal motility, and appetite. Therefore, to ensure adequate postsurgery weight gain in TSV rats, their diets were supplemented with a mixture of sucrose and milk, an easily digested and palatable mixture. Because HV rats become hyperphagic and gain body weight when given the same sweet milk diet (22), HV and control animals were provided with a standard rat diet and water after surgery. Consequently, no significant differences in body weight were observed in any experimental or control group on the day of the experiment (242.2 ± 24.3, 254.8 ± 19.1, and 257.3 ± 28.0 g for HV, TSV, and control animals, respectively). Although we did not measure or manipulate food intake, no significant differences in body weight were observed between the CSMG and control animals on the day of the experiment (250.1 ± 13.7 and 258.8 ± 13.0 g).

Hyperinsulinemic-hypoglycemic clamp. All food was removed from rats' cages 24 h before the experiment. On the day of the experiment, all animals were exposed to the same general protocol for the induction of hypoglycemia. Jugular catheter extensions from a dual-channel infusion swivel were connected to infusion pumps for insulin and glucose infusion. Animals were then allowed 30 min of rest before sampling was initiated (−60 to −30 min). Basal arterial samples were then drawn via the carotid cannula at −30 and 0 min for the analysis of glucose and catecholamines. At 0 min, after arterial sampling, insulin (25 mU · kg^{-1} · min^{-1}) and glucose infusions (variable) were initiated and maintained for the 105 min of the hypoglycemic clamp. Glucose infusion was decreased slowly with time to achieve deep hypoglycemia (~2.5 mmol/l) between the 60th and 75th min. During this period, serial sampling for glucose was performed at 10-min intervals to maintain the integrity of the hypoglycemic clamp and accurately control the rate of fall in blood glucose. In all cases, deep hypoglycemia (~2.5 mmol/l) was achieved by the 75th min; the glucose infusion was adjusted thereafter to sustain this level of glycemia for the remaining 30 min of the experiment (75–105 min). Arterial plasma catecholamine and glucose samples were taken at the 60th, 75th, 90th, and 105th min of the hypoglycemic clamp. Additional arterial samples were drawn at 0 and 105 min to assay insulin concentrations.

Analytical procedures. Glucose was assayed with the glucose oxidase method using a fixed enzyme analyzer (YSI, Yellow Springs, OH). Epinephrine and norepinephrine concentrations were assayed using a single-isotope radioenzymatic approach (27). Insulin samples were assayed via radioimmunoassay using a commercially available kit (Linco Research, St. Charles, MO).

Statistical analysis. Data are expressed as means ± SE. Comparisons of animal characteristics between groups were made using one-way ANOVA for independent groups. Comparisons among treatments over time were made by repeated-measures ANOVA using Tukey's test for post hoc analysis. In all cases, the significance was set at $P < 0.05$.

Verification of denervation procedures. In all cases, we were careful to clearly identify the site of denervation before continuing with the surgery. If any ambiguities existed with respect to the identification of the respective vagal trunks/branches or celiac and superior mesenteric ganglia, the animal was excluded from the experiment. At the conclusion of each experiment, rats were killed via an intravenous infusion of sodium pentobarbital (overdose), and a ventral midline incision was made to expose the abdominal cavity. The intestines were then reflected, the respective site of denervation was isolated, and the denervation procedure (HG, TSV, or CSMG) was visually verified under an operating microscope.

For the vagotomies that appeared to have no impact on the sympathoadrenal response, we sought further confirmation of the efficacy of our denervation surgeries. Rats undergoing TSV have been reported to demonstrate an impairment of gastrointestinal transit and absorption when fed normal pellets and water (22). We confirmed this for our procedures in a separate group of animals (n = 4) in which all animals demonstrated this impairment, as verified by changes in eating behavior, body weight gain, and 1-week postmortem inspection of the intestines. To confirm the efficacy of our HV procedure, we also used a separate group of adrenalectomized rats in which the insulin response to an acute HV was assessed under anesthesia (28–31). Based on electrophysiological studies, it has been postulated that the efferent vagal system, which stimulates insulin secretion, receives tonic inhibition by afferent discharges from the hepatic vagal branch when the portal glucose concentration is low (14). Therefore, acute HV in overnight-fasted adrenalectomized rats should lead to a decrease in the inhibitory signal to the celiac vagus, with a subsequent increase in insulin secretion, an observation that has been reported by several investigators (28–31).

For the CSMG animals, there was some concern that our surgical procedures might compromise the efferent signal to the adrenal gland, thereby

blunting the response to hypoglycemia. Innervation of the adrenal medulla by preganglionic neurons of the splanchnic nerve occurs rostral to the celiac ganglion, and there is no evidence for postganglionic innervation of the adrenal gland from the celiac ganglion (32). However, the surgical procedure does remove tissue immediately adjacent to the suprarenal ganglia, which innervates the adrenal, although this appears to be largely associated with the adrenal cortex (32). Thus, to confirm that the efferent aspect of the sympathoadrenal response remained intact in rats subjected to CSMG, glucopenia was induced via peripheral 2-deoxyglucose (2-DG) injection. It is well known that the epinephrine response to hypoglycemia and 2-DG–induced glucopenia requires intact innervation of the adrenal gland (33–35). Experiments were conducted on male Wistar rats (weight 251.0 ± 22.8 g; $n = 11$) in the conscious relaxed state. At 1 week before the experiments, animals were chronically cannulated in the carotid artery under single-dose anesthesia, as described above, for 2-DG injection and subsequent arterial sampling. While under anesthesia, animals also underwent a laparotomy in which either the celiac and superior mesenteric ganglia were removed (CSMG) or a sham operation (control), performed as described above. Animals were then allowed 6 days after surgery to recover and regain body weight. All food was removed from rats' cages 24 h before the experiment. On the day of the experiment, the animal's cannula was attached to a fluid swivel and the animal was given 30 min (−60 to −30 min) to acclimate. Basal samples were drawn at −30 and 0 min for analysis of glucose and catecholamines. At 0 min, after arterial sampling, a single dose of 2-DG (500 mg/kg dissolved in 0.5 ml saline) was injected. Serial arterial sampling for glucose was performed every 10 min for the next 60 min, with sampling for catecholamines at 15, 30, 45, and 60 min. According to the working hypothesis, if innervation of the adrenal gland is intact in CSMG animals, their catecholamine response to 2-DG–induced glucopenia should be similar to that of controls.

RESULTS

Vagotomies. Basal plasma insulin values (20.62 ± 3.40, 19.16 ± 2.56, and 20.22 ± 3.63 μU/ml for HV, TSV, and control, respectively) and glucose values (6.83 ± 0.12, 6.51 ± 0.19, and 6.32 ± 0.16 mmol/l for HV, TSV, and control) were not significantly different among the groups. Insulin infusion, initiated at 0 min, increased plasma insulin concentrations significantly to 886 ± 94, 986 ± 123, and 962 ± 125 μU/ml for HV, TSV, and control animals. No significant differences in elevated plasma insulin concentrations were observed among the three groups. During insulin infusion, arterial glucose was reduced from a basal value of 6.55 ± 0.10 mmol/l to a hypoglycemic nadir of 2.61 ± 0.03 mmol/l by the 75th min, a level that was maintained to the 105th min. By design, the arterial glucose concentration was matched in all three groups over the course of the experiment, with no statistical differences observed at any time point (Fig. 1).

Basal epinephrine (0.80 ± 0.09, 1.12 ± 0.08, and 0.84 ± 0.10 nmol/l for HV, TSV, and control) and norepinephrine (1.78 ± 0.11, 2.08 ± 0.17, and 2.00 ± 0.22 nmol/l for HV, TSV, and control) values were not different among the three groups (Fig. 2*A* and *B*). In response to whole-body hypoglycemia, epinephrine concentrations increased throughout the experiment, reaching peak values of 24.6 ± 3.38, 24.19 ± 3.81, and 25.18 ± 1.24 nmol/l by the 105th min for HV, TSV, and control animals (NS). There were no statistical differences in epinephrine concentrations among the groups at any time point. The norepinephrine concentration increased 4.5-fold above the basal level, reaching 9.55 ± 1.49, 9.52 ± 0.58, and 8.95 ± 0.20 nmol/l by the 105th min in the HV, TSV, and control groups. No statistical differences in norepinephrine concentrations were observed among the groups at any time point (Fig. 2*B*).

Celiac-superior mesenteric ganglionectomy. Insulin infusion increased the plasma insulin concentration from a basal value of 13 ± 1.4 μU/ml to a hyperinsulinemic concentration of 706 ± 31 μU/ml during the hypoglycemic clamp. No significant differences in basal or elevated plasma insulin concentrations were observed between the CSMG and control groups. By design, arterial glucose fell from 6.19 ± 0.08 mmol/l to a nadir of 2.68 ± 0.05 mmol/l by the 75th min, which was sustained for the remainder of the hypoglycemic clamp. Arterial glucose concentrations were matched between the groups over the course of the hypoglycemic clamps, with no significant differences in

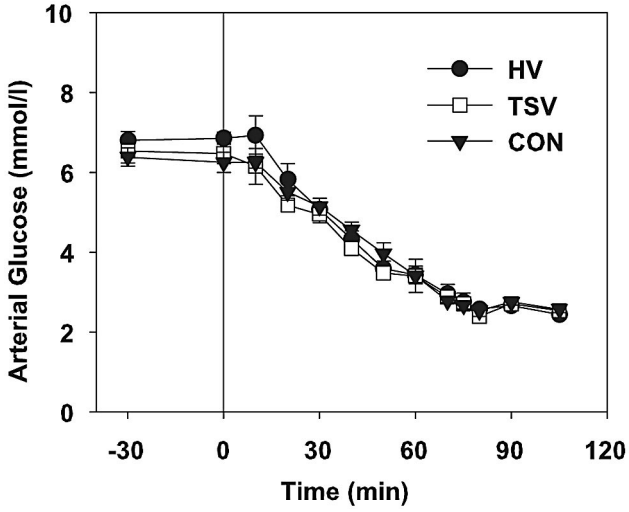

FIG. 1. Arterial glucose concentration at basal and during the hyperinsulinemic-hypoglycemic clamp for the HV, TSV, and control (CON) groups. Data are means ± SE.

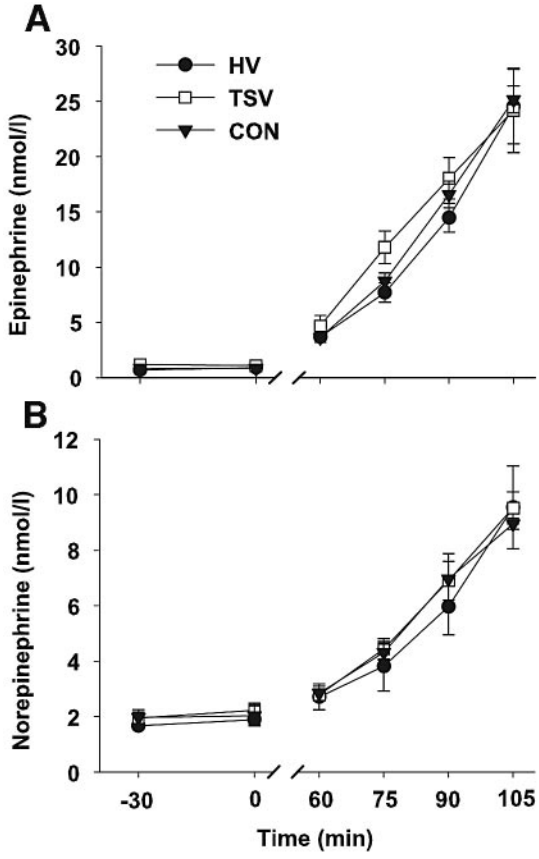

FIG. 2. Epinephrine (*A*) and norepinephrine (*B*) concentrations at basal and during sustained deep hypoglycemia for HV, TSV, and control (CON) groups. Data are means ± SE.

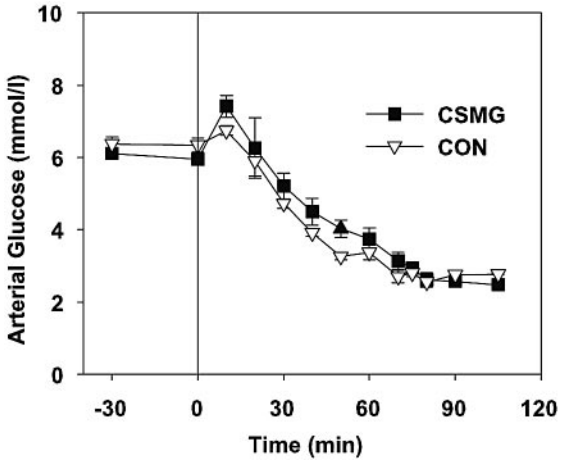

FIG. 3. Arterial glucose concentration at basal and during the hyperinsulinemic-hypoglycemic clamp for control (CON) and CSMG groups. Data are means ± SE.

arterial glucose concentrations observed at any time during the experiment (Fig. 3).

Basal epinephrine (0.43 ± 0.08 and 0.57 ± 0.13 nmol/l for CSMG and control) and norepinephrine (1.48 ± 0.13 and 1.61 ± 0.25 nmol/l for CSMG and control) values were not different between CSMG and control animals (Fig. 4*A* and *B*). In response to whole-body hypoglycemia, plasma epinephrine in control animals increased throughout the experiment, reaching a peak value of 22.32 ± 0.86 nmol/l by the 105th min. In contrast to HV or TSV rats, the epinephrine response of CSMG rats to hypoglycemia was suppressed by 50% when compared with control animals, reaching a peak value of 11.25 ± 1.21 nmol/l at the 105th min ($P < 0.05$) (Fig. 4*A*). Norepinephrine concentrations demonstrated a similar response, with control values increasing 4.5-fold above basal to 7.15 ± 0.76 nmol/l by the 105th min. Again, CSMG rats demonstrated significantly suppressed peak norepinephrine concentrations during hypoglycemia (4.72 ± 0.48 nmol/l; $P < 0.05$) when compared with control rats (Fig. 4*B*).

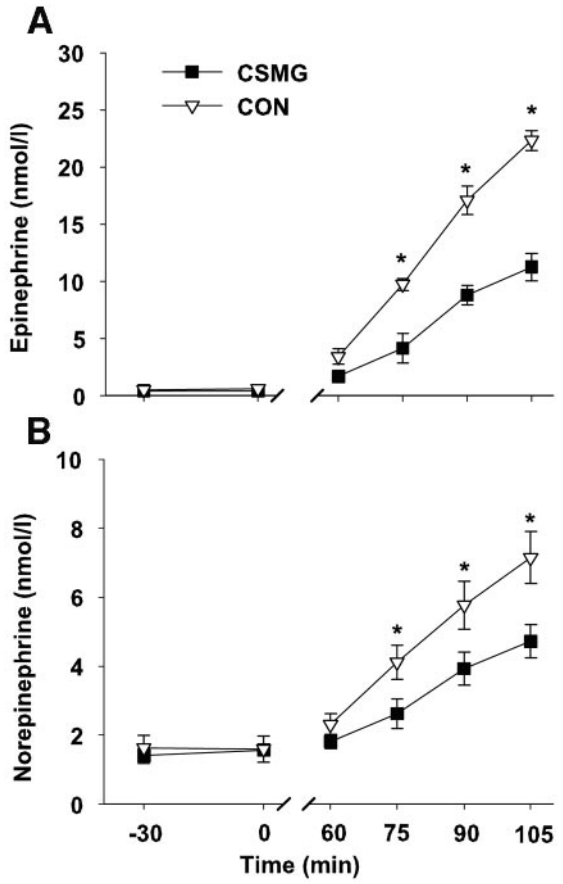

FIG. 4. Epinephrine (*A*) and norepinephrine (*B*) concentrations at basal and during sustained deep hypoglycemia for control (CON) and CSMG groups. Data are means ± SE. *$P < 0.05$ for CSMG vs. CON.

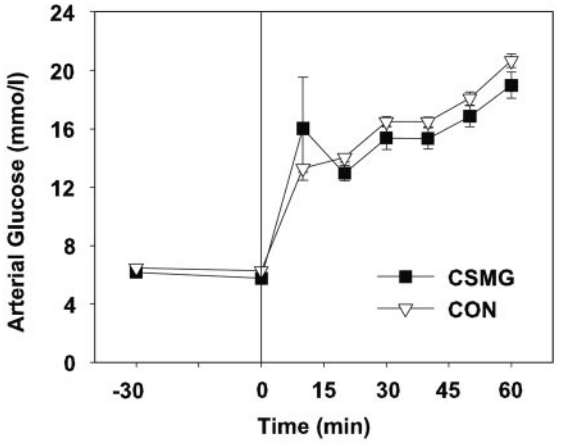

FIG. 5. Arterial glucose concentration at basal and in response to 2-DG injection for control (CON) and CSMG groups. Data are means ± SE.

Verification experiments

2-DG experiments. The basal glucose concentration (6.18 ± 0.09 mmol/l) was not significantly different between CSMG and control animals. In response to the injection of 2-DG, the arterial glucose concentration increased progressively throughout the experiment, reaching a value of 19.89 ± 0.52 mmol/l by the 60th min (Fig. 5). No significant difference in glucose concentration was observed between the groups in response to 2-DG at any time point. Basal insulin concentrations were similar between the groups, averaging 16.16 ± 1.88 μU/ml. The injection of 2-DG had no effect on the plasma insulin concentration in either of the groups (17.27 ± 3.25 and 16.08 ± 2.76 μU/ml for CSMG and control).

Basal epinephrine concentrations were not different between the groups (0.80 ± 0.50 and 0.64 ± 0.55 nmol/l for CSMG and control) (Fig. 6*A* and *B*). Epinephrine concentrations rose significantly after 2-DG injection, attaining peak values of 43.73 ± 2.60 and 52.68 ± 6.08 nmol/l for CSMG and control animals (NS). Basal norepinephrine concentrations were not significantly different between the groups (1.24 ± 0.29 and 1.55 ± 0.67 nmol/l for CSMG and control) and increased significantly after 2-DG injection, reaching peak values of 10.09 ± 0.10 vs. 10.03 ± 1.54 nmol/l for CSMG and control animals (Fig. 6*B*). As with epinephrine, the norepinephrine response to 2-DG was not significantly different between CSMG and control animals.

Acute hepatic vagotomy. Under anesthesia, adrenalectomized, overnight-fasted rats maintained a basal arterial insulin concentration of 25.59 ± 1.58 μU/ml. Acute sectioning of the hepatic vagus induced a 43% increase in the arterial insulin concentration to 36.62 ± 2.39 μU/ml ($P > 0.05$) within 30 min. A similar response was observed for portal vein insulin concentrations, which were observed to increase from a basal value of 39.95 ± 2.21 to 63.81 ± 4.09

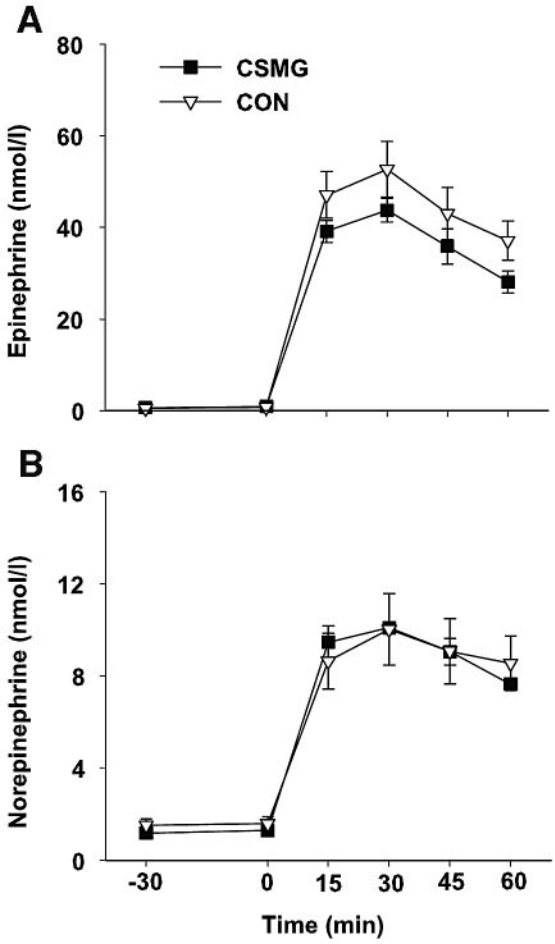

FIG. 6. Epinephrine (*A*) and norepinephrine (*B*) concentrations at basal and in response to 2-DG injection for control (CON) and CSMG groups. Data are means ± SE.

μU/ml, a 60% increase ($P < 0.05$). These values are in agreement with those of previous reports (28–31), confirming a successful HV procedure.

DISCUSSION

We have previously demonstrated that intact innervation of the portal vein is essential for eliciting the full sympathoadrenal response to hypoglycemia (11). Until recently, it had been presumed that these glucose-sensitive afferents were vagal in origin, with substantial evidence supporting the existence of glucose-sensitive vagal afferents innervating the portohepatis amassing over the years (13–15,18). Although there is no direct evidence linking these vagal afferents to any counterregulatory response, a functional role in the induction of satiety has been demonstrated (15,16). However, more recent observations have cast doubt on the role of vagal afferents in hypoglycemic detection. Jackson and colleagues (19,20) demonstrated that the interruption of vagal firing at the neck level in dogs had no effect on the counterregulatory response to hypoglycemia. Although glucose-sensitive vagal afferents have not been described for the dog, our current results extend this observation to the rat, the animal model from which most of our understanding of glucose-sensitive hepatic vagal afferents is derived (17,18). In the current study, neither HV nor TSV had any significant impact on the sympathoadrenal response to deep hypoglycemia (Fig. 2*A* and *B*). In contrast, removal of the celiac and superior mesenteric ganglia resulted in a 50% decrease in the epinephrine and norepinephrine responses to insulin-induced hypoglycemia (Fig. 4*A* and *B*). Although CSMG may cut off some vagal input, the fact that the TSV had no impact on the sympathoadrenal response suggests that the primary impact of CSMG is to interrupt spinal afferents. Thus, our findings are consistent with the existence of glucose-sensitive spinal afferents that mediate hypoglycemic detection at the portal vein.

The portohepatis of the rat is extensively innervated by sensory fibers originating from dorsal root ganglia at the T7–T13 level of the thoracic spine (21). The pattern of spinal afferent innervation in the rat, as indicated by calcitonin gene-related peptide (CGRP) immunohistochemistry, is very dense for the portal vein while being virtually absent from the liver parenchyma (36,37). This distribution is consistent with our earlier observations constraining the locus of hypoglycemic detection to the portal vein and not the liver (10). It has been shown that CSMG leads to a 66% reduction in CGRP-immunoreactive fibers in the portal vein of the rat (36). In the same study, TSV was observed to have no significant impact on CGRP immunoreactivity. Although spinal afferents innervating the abdominal viscera and vasculature have generally been implicated in nociception, they are now known to support a variety of functions, including chemosensing (38,39). It has been suggested that input from portohepatic spinal afferents relevant to metabolic status may ascend via the spinosolitary and spinomesencephalic tracts to integrative areas of the brain, including the nucleus tractus solitarius, parabrachial nucleus, and paraventricular nucleus (17).

Although it was not the intent of the current study to necessarily account for all portal vein glucose sensory input, the quantitative suppression observed with CSMG was intriguing in light of our previous observations. As noted above, we observed a 50% suppression in the epinephrine and norepinephrine response to hypoglycemia after the celiac-superior mesenteric ganglion complex was sectioned. We have previously elucidated the portal vein glucose sensor by selective normalization of portal vein glycemia during systemic hypoglycemia (7,12). Using local glucose infusion to mask the portal vein glucose sensors under similar hypoglycemic conditions, the sympathoadrenal response could be attenuated by 50–67% (10). In addition, with complete portal vein denervation via topical phenol, we observed a 50% suppression in both the epinephrine and norepinephrine responses during whole-body hypoglycemia (11). The finding that all of these procedures yield similar degrees of suppression in the sympathoadrenal response suggests that hypoglycemic detection at the portal vein is mediated largely by sensory afferents traversing the celiac-superior mesenteric ganglia. When this suggestion is considered along with the negative findings for TSV, it would appear that hypoglycemic detection at the portal vein occurs primarily via spinal afferents ascending by the splanchnic nerve.

Our observation that neither HV nor TSV had any demonstrable effect on the sympathoadrenal response to hypoglycemia in the current study should not be interpreted as suggesting the lack of any glucose-sensitive vagal afferents. The existence of portal glucose receptors was first proposed by Russek (40) based on the observation that portal glucose infusions induced satiety. Niijima (13,14) then demonstrated that the firing rate for select afferents in the hepatic vagus was suppressed by portal

glucose infusions and was inversely related to the portal glucose concentration. Since that time, a substantial amount of evidence has accumulated for the existence of glucose-sensitive afferents within the hepatic vagus that play a significant role in mediating feeding responses to changes in portal glucose concentrations. Anterograde and retrograde tracing studies from the nodose ganglia reveal extensive innervation of the portal vein, hepatic artery, and bile ducts by vagal afferents (36,41–43), with little or no innervation of the liver parenchyma (36,42). Sectioning the celiac branch of the vagus nerve blocks the anorexic response induced by intraperitoneal injections of epinephrine (44). TSV eliminates the feeding response induced by portal injection of 2-DG (45) as well as the anorexic response induced by intraduodenal glucose infusion (16). Consistent with Niijima's earlier findings, portal injections of 2-DG have been shown to increase the firing rate for those same glucose-sensitive vagal afferents (46). Thus, the existence of glucose-sensitive vagal afferents innervating the portal vein is not in question; rather, the current findings suggest a second type of portal glucose sensor: a glucose-sensitive spinal afferent. The two portal glucose sensors appear to be functionally distinct, with vagal afferents mediating satiety and spinal afferents mediating counterregulatory responses.

Removal of the celiac-superior mesenteric ganglion complex raises two concerns: *1*) the inadvertent elimination of some vagal afferents and *2*) the potential impact on efferent innervation of the adrenal nerve. It has been shown that few, if any, vagal nerves appear to be in functional contact with celiac or superior mesenteric ganglia (25). However, it is probable that the surgical procedure eliminates vagal afferents from the portohepatis, which reach the dorsal trunk via the celiac artery. Yet we observed that TSV had no significant impact on the sympathoadrenal response to hypoglycemia. Because sectioning of the celiac-superior mesenteric ganglion complex occurs caudal to that for the TSV, it is unlikely that those vagal afferents eliminated by CSMG have any role in hypoglycemic detection. As for the second concern, potential adrenal denervation, it is unlikely that CSMG will lead to any effective denervation of the adrenal medulla. Retrograde tracer studies of the adrenal medulla have failed to demonstrate any labeling of cells within the celiac ganglia (32). However, we wanted to perform a functional test to verify that our ganglionectomy procedure did not impair the efferent aspect of the sympathoadrenal response and thereby compromise our results. To test this, we induced glycopenia via a single bolus injection of 2-DG. This procedure, believed to induce rapid central glucopenia, is known to induce a sympathoadrenal response of equal or greater magnitude than that observed for insulin-induced hypoglycemia (47,48). Further, it has been known for some time that the epinephrine response to hypoglycemia and 2-DG–induced glycopenia requires intact adrenal innervation (33–35). The observation that the peak sympathoadrenal response to 2-DG injection in both CSMG and control animals was similar and approximately twofold greater than the maximal response to insulin-induced hypoglycemia in control animals indicated that CSMG rats were not limited by their capacity for maximal sympathoadrenal response. Thus the observed differences in sympathoadrenal response during insulin-induced progressive hypoglycemia between CSMG and control animals must be attributable to the impairment of the afferent aspect after CSMG.

The observation that the sympathoadrenal response to 2-DG was not impaired in CSMG animals was not surprising. We previously observed that portal glucose sensing appears to be dominant in hypoglycemic detection when the fall in glycemia develops slowly. For example, in our previous studies, the magnitude of suppression in the sympathoadrenal response by selectively normalizing portal glucose was more pronounced when systemic hypoglycemia (2.5 ± 0.1 mmol/l) was induced over 200 min (8) compared with that observed for the same level of hypoglycemia (2.6 ± 0.09 mmol/l) induced within 30 min (12). We hypothesize that when the fall in glycemia is rapid, hypoglycemic detection shifts away from the portal vein to other glucose sensing loci, most likely the brain. Because the response to 2-DG in the current study was very rapid (i.e., within 15 min), it is likely that this led to a rapid central glucopenia that either superceded or overrode input from the periphery.

In conclusion, sectioning the celiac-superior mesenteric ganglion complex led to a substantial impairment of the sympathoadrenal response to hypoglycemia. The magnitude of the suppression was comparable with that previously observed for complete denervation of the portal vein and when portal glucose concentration is normalized during systemic hypoglycemia. By contrast, neither HV nor TSV had any significant impact on the sympathoadrenal response to hypoglycemia. Taken together, these results suggest that the portal vein glucose concentration is monitored by glucose-sensitive afferents traversing the celiac-superior mesenteric ganglion complex that likely originate from the dorsal root ganglia.

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