The Locus for Hypoglycemic Detection Shifts With the Rate of Fall in Glycemia

The Role of Portal–Superior Mesenteric Vein Glucose Sensing

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OBJECTIVE—To ascertain whether portal glucose sensing extends beyond the portal vein to the superior mesenteric vein and then test whether the role of portal–superior mesenteric glucose sensors varies with the rate of fall in glycemia.

RESEARCH DESIGN AND METHODS—Chronically cannulated rats underwent afferent ablation of the portal vein (PV) or portal and superior mesenteric veins (PMV) or sham operation (control). One week later, animals underwent hyperinsulinemic-hypoglycemic clamps in which the hypoglycemic nadir, 2.48 ± 0.06 mmol/l, was reached at a rate of decline in glucose of −0.09 or −0.21 mmol · l^{-1} · min^{-1} (PMV and control only). Additional PMV and control animals received an intravenous injection of the glucopenic agent 2-deoxyglucose.

RESULTS—Inducing hypoglycemia slowly, at a rate of −0.09 mmol · l^{-1} · min^{-1}, resulted in a 26-fold increase in epinephrine (23.39 ± 0.62 nmol/l) and 12-fold increase in norepinephrine (11.42 ± 0.92 nmol/l) for controls ($P < 0.001$). The epinephrine response to hypoglycemia was suppressed by 91% in PMV (2.09 ± 0.07 nmol/l) vs. 61% in PV (9.05 ± 1.59 nmol/l) ($P < 0.001$). The norepinephrine response to hypoglycemia was suppressed by 94 and 80% in PMV and PV, respectively, compared with that in controls. In contrast, when arterial glucose was lowered to 2.49 ± 0.06 mmol/l within 20 min, no significant differences were observed in the catecholamine responses for PMV and controls over the first 45 min of hypoglycemia (20–65 min). Only at min 105 were catecholamines significantly lower for PMV vs. controls. Injection of 2-deoxyglucose induced a very rapid sympathoadrenal response with no significant differences between PMV and controls.

CONCLUSIONS—The critical locus for hypoglycemic detection shifts away from the portal-mesenteric vein to some other loci (e.g., the brain) when hypoglycemia develops rapidly. ***Diabetes*** **57:1380–1386, 2008**

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CGRP, calcitonin gene–related peptide; CNS, central nervous system.

See accompanying commentary on p. 1158.

Hypoglycemic counterregulation represents a classic negative feedback system in which the fall in blood glucose concentration initiates a series of neurohumoral responses leading to the restoration of euglycemia. The first step in this process, hypoglycemic detection, is the domain of specialized sensory neurons found within both the central nervous system (CNS) and the peripheral vasculature (1,2). Within the CNS, neurons responding to hypoglycemia have been identified both in the forebrain (1,2), particularly the hypothalamus, and the hindbrain, e.g., nucleus of the solitary tract and area postrema (3). Induction of glucopenia either in the ventromedial hypothalamus or hindbrain has been shown to elicit a counterregulatory hormonal response causing hyperglycemia (3–5). Glucose-sensing afferents have also been identified throughout the periphery including the oral cavity (6), gastrointestinal tract (7), portal vein (8,9), and carotid body (10). Lesioning either the portal vein or carotid body has been shown to significantly impair counterregulation to systemic hypoglycemia (11–13). Thus, the body contains a number of glucose sensory loci capable of detecting hypoglycemia and mediating a counterregulatory response.

While a number of glucose-sensing loci important for hypoglycemic detection have been identified, the manner in which information from these different sensory neurons is integrated to yield the appropriate counterregulatory response remains unclear. Studies utilizing the brain clamp technique, in which brain glucose is maintained euglycemic during systemic hypoglycemia, suggest that the glucose-sensing loci of the forebrain and hindbrain may serve in a redundant capacity to protect the brain. That is, clamping only the forebrain or hindbrain at euglycemia during systemic hypoglycemia did not significantly impair counterregulation, which only occurred when the entire brain was maintained euglycemic (14–16). In contrast, normalizing plasma glucose across either the whole brain or portahepatis during systemic hypoglycemia has proven effective in suppressing counterregulation (14,16,17). As such, CNS and peripheral glucose sensory afferents do not appear to reflect simple redundant sensory loci but instead may subserve different functional roles. In our initial studies, we employed a moderately rapid drop in blood glucose, achieving the glycemic nadir within 30 min. Clamping the portahepatis under these conditions resulted in a 40–50% suppression in the sympathoadrenal response (18). Subsequently, we employed a stepped hypoglycemic clamp in which the nadir was not

reached for over 3 hours and observed an 80% suppression in the epinephrine response to a similar level of hypoglycemia (17). Albeit a historical comparison, these findings suggest that the relative contribution of portal glucose sensing toward hypoglycemic detection might be rate sensitive. To test this hypothesis, we compared the effect of portal vein sensory ablation on the sympathoadrenal response to a rapid versus slow induction of hypoglycemia. We further examined the impact of portal vein deafferentation on the response to a large bolus injection of 2-deoxyglucose, a glucopenic agent capable of inducing maximal sympathoadrenal responses within 5–15 min (19). Prior to these experiments, we sought to better define the portal vein glucose-sensing locus. While we had previously excluded the liver (20) and hepatic artery (21), we failed to consider whether portal glucose sensors might extend beyond the portal vein proper to include aspects of the superior mesenteric vein.

RESEARCH DESIGN AND METHODS

Experiments were conducted on male Wistar rats (weight 250.9 ± 7.9 g; n = 50) in the conscious relaxed state. All surgical and experimental procedures were preapproved by the University of Southern California institutional animal care and use committee. All animals were housed in individual cages, fed ad libitum, and subjected to standard 12-h light-dark cycle. One week before the experiments, animals were chronically cannulated under single-dose anesthesia (3:3:1 ketamine HCL:xylazine:acepromazine maleate; 0.10 ml/100 g body wt) given intramuscularly. Cannulas were placed in the carotid artery (PE-50; Clay Adams,) for arterial blood sampling and in the jugular vein (dual cannula Silastic, 0.025 mm) for peripheral infusions of insulin and glucose or infusion of 2-deoxyglucose. At the time of cannulation, experimental animals also underwent permanent desensitization of capsaicin-sensitive afferents via topical capsaicin application to the portal vein alone (n = 8) or portal and mesenteric veins (n = 16). A laparotomy was performed and the intestines reflected to the right and wrapped with sterile gauze moistened with warm (37°C) saline. The portal and superior mesenteric veins were isolated, and small strips of filter paper were measured to fit the length and width of the vessels. The filter strips were then soaked in a 1% capsaicin solution (10% ethanol and 10% Tween 80 in 0.9% saline) and carefully placed either on the portal vein alone, or on both the portal and superior mesenteric veins. Adjacent tissues were covered with sterile gauze and parafilm to prevent contact with the capsaicin-soaked strips. The filter strips were removed after 15 min and the veins rinsed thoroughly with normal saline to remove any excess capsaicin solution. For control animals (n = 16), all procedures were as described above, except that the vehicle solution applied to the portal and mesenteric veins contained no capsaicin. Cannulas were tunneled subcutaneously and exteriorized at the back of the neck with all wounds closed via individual sutures. Animals were then fitted with a Covance harness (Instech Laboratories, Plymouth Meeting, PA) to protect the cannulas and allowed 5–6 days of recovery to regain body weight. Following recovery, body weights for control (232.8 ± 4.6 g) and capsaicin-treated (259.9 ± 9.6 g) animals were not significantly different.

Slow hyperinsulinemic-hypoglycemic clamp. The day before the experiment, portal vein denervated (PV) (n = 8), portal-superior mesenteric vein denervated (PMV) (n = 6), and control (n = 6) animals had their cannulas connected to a dual channel swivel via a tethering system (Instech Laboratories, Plymouth Meeting, PA). Twenty-four hours before the experiment, all food was removed from the cage. On the day of the experiment, jugular catheter extensions from the dual channel infusion swivel were connected to infusion pumps for insulin and glucose. Animals were then allowed 30 min of rest before initiating sampling (60 to −30 min). Basal arterial samples were drawn via the carotid cannula at −30 and 0 min for analysis of glucose, insulin, and catecholamines. At min 0, insulin (25 mU · kg^{-1} · min^{-1}) and glucose infusions (variable) were initiated and maintained for 105 min. Glucose infusion was decreased slowly over time (−0.09 mmol · l^{-1} · min^{-1}) to achieve a hypoglycemic nadir of 2.47 ± 0.05 mmol/l by min 75 and was adjusted to maintain this level through min 105. Arterial plasma samples were drawn at 60, 75, 90, and 105 min for catecholamine analysis, with an additional sample drawn at min 105 for insulin. Sampled blood was replaced with donor blood over the course of the experiment.

Fast hyperinsulinemic-hypoglycemic clamp. Before initiating the experiments, PMV (n = 6) and control (n = 6) animals were handled as described above for the slow hyperinsulinemic-hypoglycemic clamps. On the day of the experiment, animals were allowed to rest for 30 min (−70 to −40 min). At min −40, insulin (25 mU · kg^{-1}· min^{-1}) and glucose infusions (variable) were initiated and a hyperinsulinemic-euglycemic clamp established for the next 40 min (−40 to 0 min). Arterial blood was sampled every 10 min for glucose with larger samples (250 μl) drawn at −40 and 0 min for catecholamine analysis. At min 0, glucose infusion was reduced so as to achieve the hypoglycemic nadir, 2.49 ± 0.06 mmol/l, by min 20 (−0.21 mmol · l^{-1} · min^{-1}), which was sustained for the remainder of the clamp (20–105 min). Serial sampling for glucose analysis was performed every 5 min between mins 0 and 20 and every 10 min thereafter, with larger samples drawn at mins 20, 35, 50, 65 and 105 for catecholamine analysis. Additional blood samples for insulin analysis were taken at mins 0 (basal) and 105 (deep hypoglycemia).

2-deoxyglucose injections. Before initiating the experiments, PMV (n = 4) and control (n = 4) animals were handled as described above for the slow and fast hyperinsulinimic-hypoglycemic clamps. On the day of the experiment, animals were allowed 30 min to rest (−60 to −30 min) in individual metabolic chambers. Basal samples were drawn at −30 and 0 min for analysis of glucose, catecholamines, and insulin. At min 0, following arterial sampling, a single dose of 2-deoxyglucose (500 mg/kg in 0.5 ml saline) was administered via the jugular cannula. Serial blood samples were drawn from the carotid cannula at mins 5, 15, 30, and 45 and analyzed for glucose, catecholamines, and insulin concentrations.

Immunohistochemical verification. The impact of capsaicin treatment on portal and superior mesenteric vein calcitonin gene–related peptide (CGRP) reactive innervation, a measure of spinal afferent innervation, was assessed as described by Fujita et al. (12). Briefly, a separate group of control (n = 5) and capsaicin-treated (n = 5) animals underwent surgical and denervation procedures identical to those described above for PMV and control animals. Seven days following the surgery, animals were anesthetized (2,2,2-tribromoethanol) and transcardially perfused with paraformaldehyde (4%), and the portal and superior mesenteric veins were removed for immunohistochemical analysis of CGRP reactivity. Whole mount sections (~0.5 cm) of portal and superior mesenteric veins were reacted for 48 h at 4°C with a mouse monoclonal anti-CGRP antibody (no. 4901; CURE Digestive Diseases Research Center Antibody Core) diluted in Tris-buffered saline (with 2% normal donkey serum and 0.25% Triton) to a final concentration of 1:12,000. To amplify the reaction, sections were subsequently reacted for 24 h with a donkey anti-mouse IgG (H+L) conjugated to CY3 (no. 715-165-150; Jackson Immuno Research Labs) diluted to 1:500. Following a final series of washes with Tris-buffered saline, sections were mounted, dehydrated, cleared, and cover-slipped with a mounting medium (Vectashield) for fluorescence microscopy. The immunoreactive sections were then digitally photographed using a camera fitted on a bright field microscope (Nikon Microphot SA Light Microscope fitted with a Spot RT Color Digital Camera; Diagnostics Instruments) using SPOT Image (version 3.5.5 for Mac OS). To quantify the extent of innervation, a grid of five equidistant horizontal lines was overlaid on randomly selected sample field views (92 × 80 um) and the number of CGRP reactive fibers intersecting these lines counted (22).

Analytical procedures. Glucose was assayed by the glucose oxidase method (YSI, Yellow Springs, OH). Epinephrine and norepinephrine concentrations were determined using a single-isotope derivative radioenzymatic assay (23). Insulin samples were assayed via radioimmunoassay utilizing a kit from Linco Research (St. Charles, MO).

Data analysis. Results are expressed as means ± SE. Comparisons of animal characteristics between groups were made using a one-way ANOVA for independent groups. Comparisons between treatments over time were made by repeated-measures ANOVA using Tukey's test for post hoc analysis. Significance was set at $P < 0.05$.

RESULTS

Verification of capsaicin-induced denervation. Immunohistochemical analysis revealed an extensive mesh-like network of CGRP reactive fibers in the portal and superior mesenteric veins taken from control animals (Fig. 1). However, 7 days following the topical application of capsaicin, CGRP reactivity was substantially decreased in both veins. The number of CGRP reactive fibers per sample area declined from 45.8 ± 4.5 to 16.2 ± 2.9 in the portal vein and from 64.0 ± 5.3 to 12.2 ± 2.1 in the superior mesenteric vein following capsaicin treatment ($P < 0.001$).

Slow hyperinsulinemic-hypoglycemic clamp: PV vs. PMV. Insulin infusion, initiated at min 0, increased the plasma insulin concentration from a basal level of 25 ± 9 to 970 ± 110 μU/ml during the hypoglycemic clamp, with

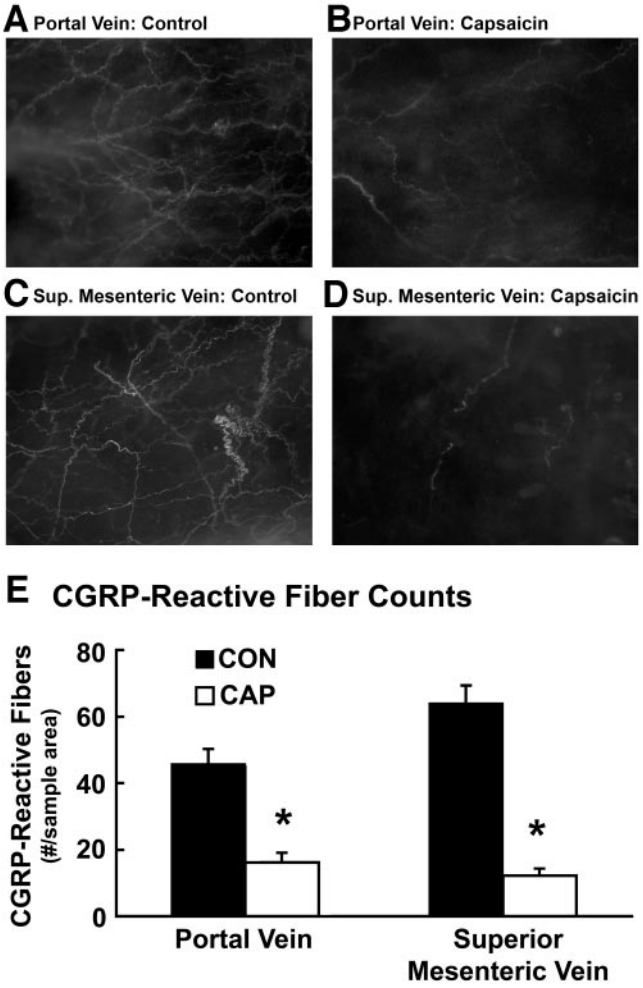

FIG. 1. Photomicrographs of portal vein and superior mesenteric vein sections reacted with CGRP monoclonal antibody from control (*A* and *C*) and capsaicin-treated (*B* and *D*) animals. Number of CGRP reactive fibers per sample area (*E*) are expressed as means ± SE. *Significant difference between control and capsaicin treated ($P < 0.001$).

no significant differences between the three groups either at basal or during hypoglycemia ($P = 0.65$). Arterial glucose decreased from a peak value of 8.12 ± 0.54 mmol/l to a nadir of 2.47 ± 0.05 mmol/l at a rate of -0.09 mmol · l^{-1} · min^{-1} with no significant difference between groups over the course of the experiment (Fig. 2). Glucose infusion rates at the onset of the hyperinsulinemic-hypoglycemic clamp were not significantly different between the three groups (19.0 ± 0.3, 18.7 ± 0.3, and 19.2 ± 0.2 mg · kg^{-1} · min^{-1} for control, portal vein, and PMV, respectively; $P = 0.9$) (Fig. 3). However, over the final 30 min, the glucose infusion for PMV, 9.7 ± 0.8 mg · kg^{-1} · min^{-1}, was significantly elevated above that for portal vein (5.8 ± 1.4 mg · kg^{-1} · min^{-1}) and control (3.4 ± 0.8 mg · kg^{-1} · min^{-1}) ($P < 0.01$). The glucose infusion for portal vein was also significantly elevated above that for control ($P < 0.05$).

Basal epinephrine (0.97 ± 0.24 nmol/l) and norepinephrine (1.18 ± 0.26 nmol/l) values were not significantly different between groups ($P = 0.65$ and 0.30, respectively) (Fig. 4*A*). In response to whole-body hypoglycemia, epinephrine values for control animals increased throughout the experiment, reaching a peak value of 23.39 ± 0.62 nmol/l by 105 min. With portal vein denervation alone, the peak epinephrine response to hypoglycemia was suppressed by 61% (9.05 ± 1.59 nmol/l) ($P < 0.001$) compared with that for control. Denervating both the portal and superior mesenteric veins resulted in further suppression of the peak epinephrine response to 2.09 ± 0.07 nmol/l ($P < 0.001$), i.e., <10% of the control response. Norepinephrine concentrations demonstrated a similar response, with control demonstrating an 11-fold increase above basal (10.45 ± 1.02 nmol/l) by min 105 (Fig. 4*B*). The norepinephrine responses for PV and PMV animals were suppressed by 80 and 94%, respectively, compared with those for control ($P < 0.001$). For PMV animals, norepinephrine concentrations during hypoglycemia were not significantly different from basal, i.e., 0.55 ± 0.28 nmol/l at min 105 vs. 0.80 ± 0.18 nmol/l at basal.

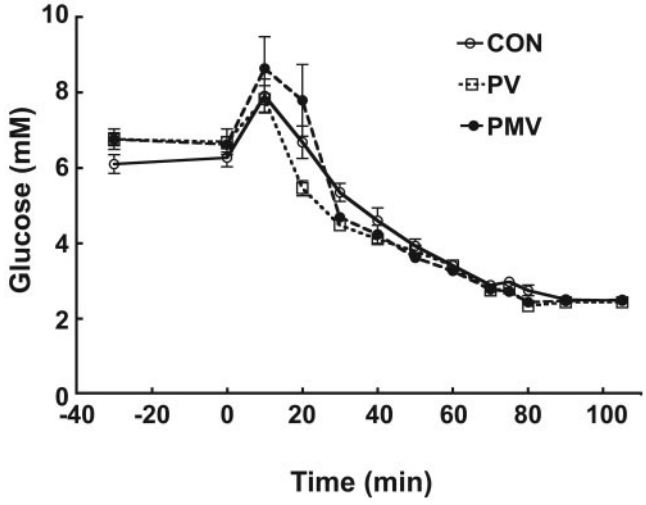

FIG. 2. Data are expressed as means ± SE for arterial glucose concentration at basal (−30 to 0) and during the hyperinsulinemic-hypoglycemic clamp (0–105) for control (CON), PV, and PMV animals.

Fast hyperinsulinemic-hypoglycemic clamp. Insulin infusion, initiated at −40 min, increased the plasma insulin concentration from 24 ± 6 μU/ml to a hyperinsulinemic value of 985 ± 213 μU/ml, with no significant differences either at basal or during hyperinsulinemia between control and PMV. Arterial glucose fell from a peak value of 6.82 ± 0.31 mmol/l to a nadir of 2.53 ± 0.06 mmol/l by min 20

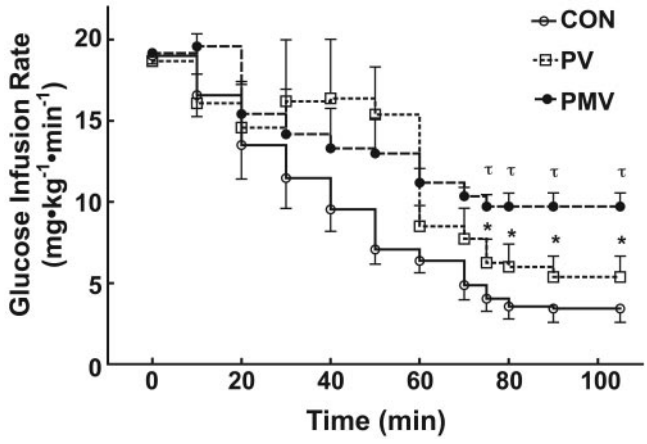

FIG. 3. Data are expressed as means ± SE for glucose infusion rates at basal (−30–0) and during the hyperinsulinemic-hypoglycemic clamp (0–105) for control (CON), PV, and PMV animals. *Significant difference between portal vein and control ($P < 0.05$). τSignificant difference between PMV and the other two groups (control and portal vein; $P < 0.01$).

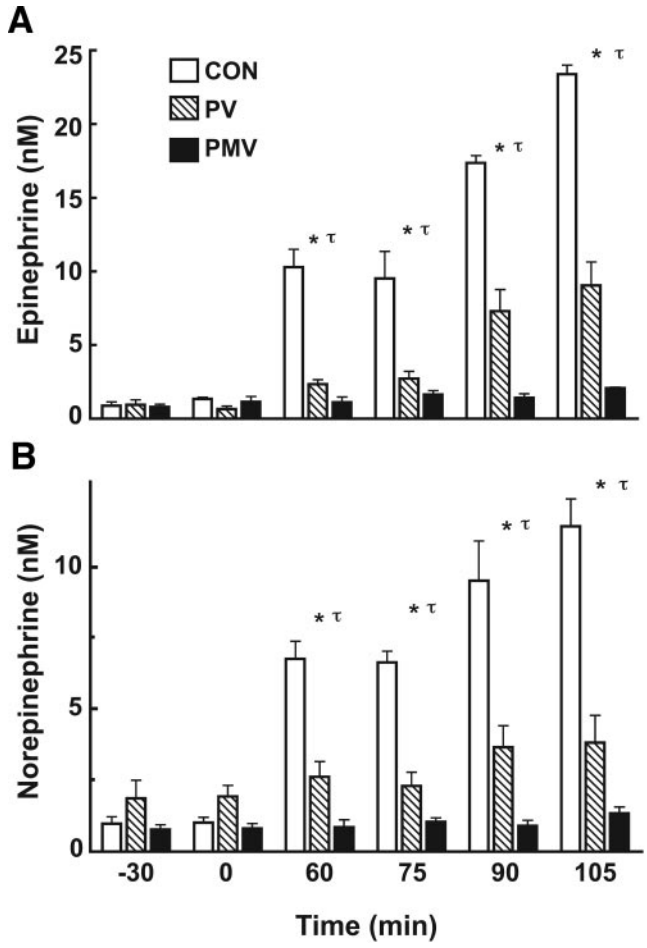

FIG. 4. Epinephrine (*A*) and norepinephrine (*B*) concentrations at basal (−30–0) and during sustained hypoglycemia (60–105) for control (CON), PV, and PMV animals, expressed as means ± SE. *Significant difference between portal vein and control ($P < 0.05$). τSignificant difference between PMV and the other two groups (control and portal vein; $P < 0.05$).

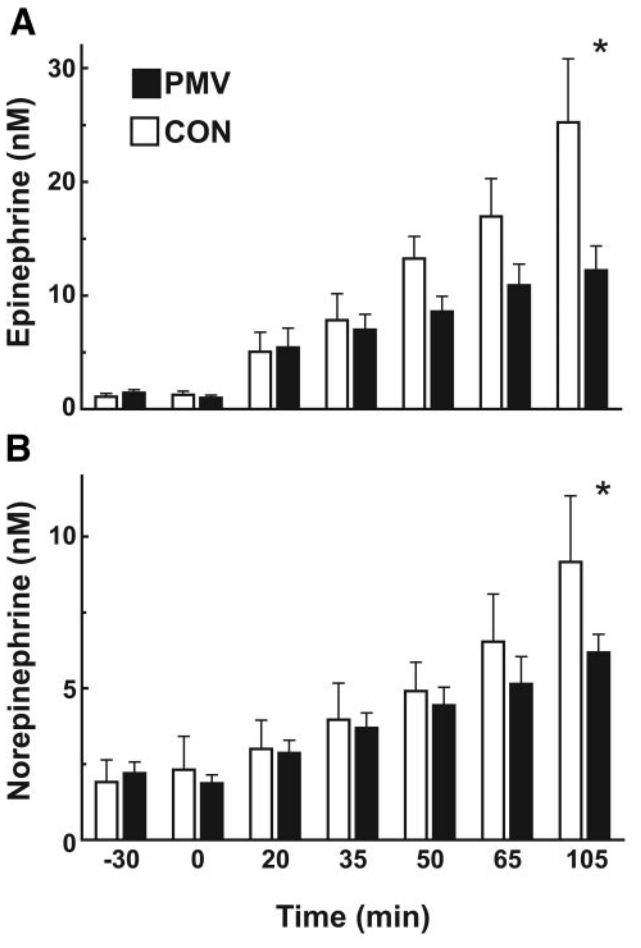

FIG. 6. Epinephrine (*A*) and norepinephrine (*B*) concentrations during euglycemia (−30–0) and during rapidly induced sustained hypoglycemia (20–105) for control (control-FAST), and PMV (PMV-FAST) animals, expressed as means ± SE. *Significant difference between PMV-FAST and control-FAST $P < 0.05$.

(-0.21 mmol · l^{-1} · min^{-1}), with no significant differences between groups at any point during the clamp (Fig. 5). In response to whole-body hypoglycemia, epinephrine values for controls increased throughout the experiment, reaching 25.25 ± 5.56 nmol/l by min 105 (Fig. 6). The epinephrine response to hypoglycemia for PMV was not significantly different from that of control over the first 45 min of deep hypoglycemia, i.e., mins 20–65 ($P = 0.64$). However, by min 105 PMV epinephrine values were significantly lower than control, i.e., 12.21 ± 2.13 vs. 25.25 ± 5.56 nmol/l ($P < 0.001$; Fig. 6*A*). The norepinephrine concentration for control animals submitted to the fast hyperinsulinemic-hypoinsulinemic clamp increased to 7.04 ± 1.77 nmol/l by min 105 (Fig. 6*B*). As with epinephrine, the norepinephrine response remained intact for PMV between mins 20 and 65, with the only significant difference between control and PMV occurring at min 105. At min 105, norepinephrine was suppressed by ~41% when compared with control (4.14 ± 0.39 nmol/l vs. 7.04 ± 1.77 nmol/l) ($P < 0.001$). Consistent with the hormonal responses, glucose infusions over the final 65 min were not significantly different between groups during rapidly induced hypoglycemia ($P = 0.48$) (Table 1).

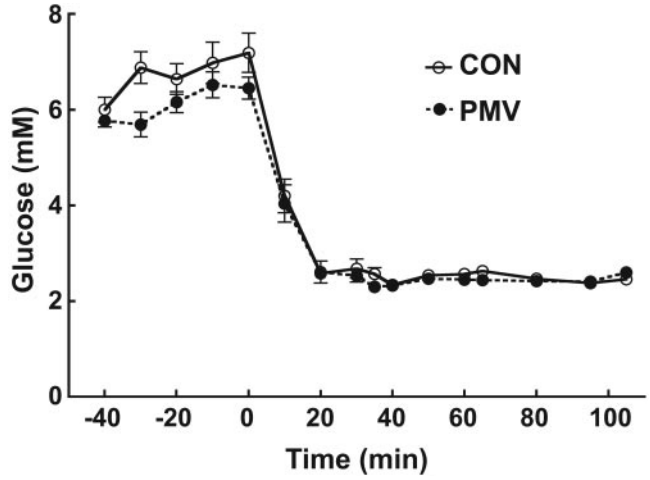

FIG. 5. Data are expressed as means ± SE for arterial glucose concentration during a hyperinsulinemic-euglycemic clamp (−40–0) and during a rapidly induced hyperinsulinemic-hypoglycemic clamp (0–105) for control (control-FAST) and PMV (PMV-FAST) animals.

2-deoxyglucose–induced glucopenia. The basal glucose concentration, 7.47 ± 0.54 mmol/l, was not significantly different between control and PMV (Fig. 7). In response to the 2-deoxyglucose injection, glucose increased rapidly over the first 5 min and rose progressively throughout the remainder of the experiment, reaching a peak value of 17.6 ± 0.87 mmol/l by min 45. No significant differences in

TABLE 1
Glucose infusion rates (mg · kg^{-1} · min^{-1}) during the final 65 minutes of the hyperinsulinemic-hypoglycemic clamp following rapid induction of hypoglycemia for control and PMV deafferented animals

Group	30–40 min	40–50 min	50–60 min	60–105 min
Control	2.26 ± 0.47	2.53 ± 0.46	2.32 ± 0.53	2.69 ± 0.65
PMV	3.61 ± 0.54	3.92 ± 0.45	4.00 ± 0.48	4.13 ± 0.50

Data are means ± SE.

glucose concentration were observed between PMV and control at any time during the experiment ($P = 0.86$). Basal insulin concentrations were similar between groups, averaging 26 ± 7 μU/ml. The 2-deoxyglucose injection did not significantly affect plasma insulin concentrations for either group (22 ± 9 vs. 27 ± 4 μU/ml for PMV and control, respectively).

Basal epinephrine concentrations for control (1.54 ± 0.49 nmol/l) and PMV (0.97 ± 0.20 nmol/l) were not significantly different ($P = 0.98$). In response to the 2-deoxyglucose injection, the epinephrine concentration for both groups rose rapidly, increasing 15-fold above basal within 5 min and attaining peak values within 15 min, i.e., 27.53 ± 4.98 and 26.25 ± 2.92 nmol/l for PMV and control, respectively (Fig. 8). The epinephrine response to 2-deoxyglucose was not observed to be different between groups at any time point during the experiment. As with epinephrine, basal norepinephrine concentrations were not significantly different between groups (3.47 ± 0.51 vs. 2.32 ± 0.20 nmol/l for PMV and control, respectively). Plasma norepinephrine also increased rapidly following 2-deoxyglucose injection for both groups, reaching peak values 8.26 ± 0.64 and 10.28 ± 0.30 mmol/l for PMV and control, respectively, within 15–30 min. The norepinephrine response to 2-deoxyglucose injection was similar for both groups, with no significant difference observed at any time point (Fig. 8*B*).

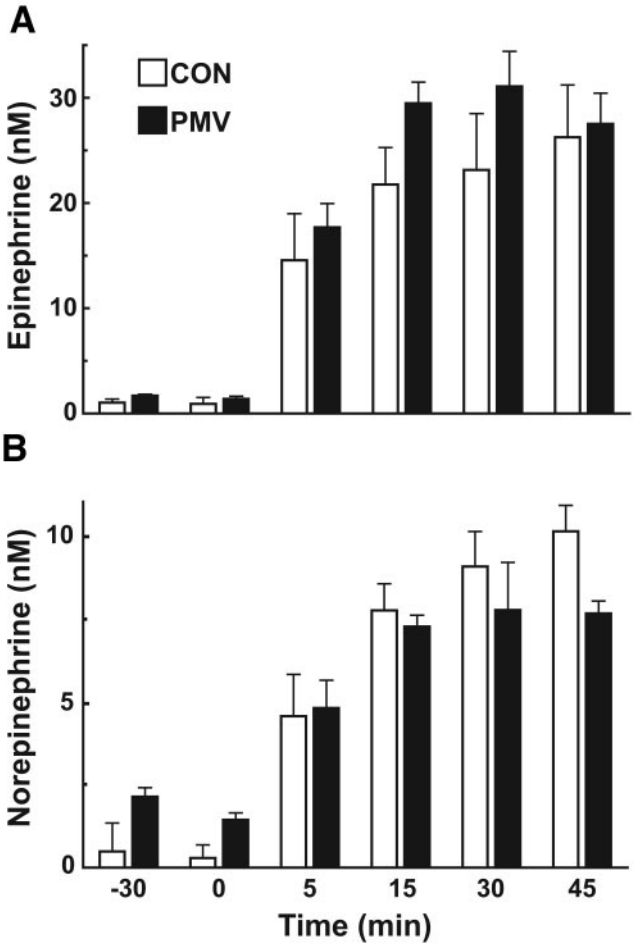

FIG. 8. Epinephrine (*A*) and norepinephrine (*B*) concentrations at basal (−30–0) and in response to a 2-deoxyglucose injection (0–45) for control (CON) and PMV animals.

DISCUSSION

The current findings suggest that the roles for CNS and portal-mesenteric glucose sensors are functionally distinct. When hypoglycemia developed slowly, over 70 min, the input from portal-mesenteric vein glucose sensors was essential for engendering the sympathoadrenal response. Eliminating the input from these sensors suppressed the catecholamine response by over 90% despite the fact that the CNS and other glucose-sensing loci (e.g., carotid body) were exposed to deep hypoglycemia (Fig. 4). Concomitant with the reduced sympathoadrenal output, the requisite glucose infusion rate for PMV was approximately threefold greater than that for control during deep hypoglycemia (Fig. 3). However, when deep hypoglycemia ensued quickly, within 20 min, portal-mesenteric glucose sensory input appeared much less important for initiating the counterregulatory response. Over the first 45 min of rapidly induced hypoglycemia, ablation of the portal-mesenteric glucose sensors had no significant impact on the sympathoadrenal response (Fig. 6). Only at min 105, 85 min after the induction of hypoglycemia, was PMV glucose sensory ablation seen to have any significant impact and then only a 50% suppression. While the glucose infusion tended to be higher in PMV animals with rapidly induced hypoglycemia, this failed to achieve statistical significance. That the sympathoadrenal response was not significantly different until the very later stages of our protocol may have precluded any observed effect on hepatic glucose production. Though it is impossible to assess the degree of glucopenia induced by the 2-deoxyglucose injection, the plasma glucose and catecholamine responses suggest that detection must have occurred even sooner

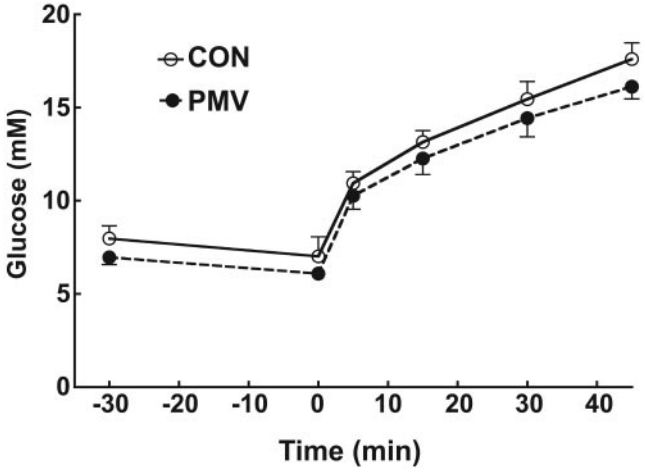

FIG. 7. Data are expressed as means ± SE for arterial glucose concentrations at basal (−30–0) and in response to a 2-deoxyglucose injection (0–45) for control (CON) and PMV animals.

than occurred for rapidly induced hypoglycemia (Figs. 7 and 8). Consistent with what was observed for the rapid induction of hypoglycemia via insulin, ablating the portal-mesenteric vein glucose sensors had no impact on the sympathoadrenal and glycemic responses to the 2-deoxy-glucose injection. Thus, under conditions that characterize the majority of clinical hypoglycemic events, i.e., a slow prolonged fall in glucose (24–26), the CNS appears relatively insensitive in the absence of peripheral glucose-sensory input from the portal-mesenteric vein. Alternatively, under extreme conditions, where blood glucose falls precipitously, portal-mesenteric input no longer appears essential presumably because CNS glucose sensors (or some alternative loci) are activated.

The relative importance of portal glucose sensing has previously been questioned based on the apparent inability of the portal glucose clamp or portal denervation to fully suppress hypoglycemic counterregulation. The current findings make it clear that this observation derives from the inability of earlier attempts to fully account for the glucose sensing in the superior mesenteric vein. We have previously shown that capsaicin, when applied topically to the portal vein, effectively eliminates CGRP reactive nerve fibers as well as portal vein glucose sensing (12). In the current study, when capsaicin was applied to the portal vein alone, it resulted in a 61 and 80% suppression of the epinephrine and norepinephrine responses, respectively, to slowly developing hypoglycemia. The degree of suppression in the sympathoadrenal response was consistent with our previous results for denervation of the portal vein via capsaicin (12) or phenol (11) and comparable with clamping the portal vein at normoglycemia (20). However, applying capsaicin to both the portal vein proper and superior mesenteric vein led to an almost complete suppression of the epinephrine and norepinephrine responses, i.e., a 91 and 94% suppression, respectively (Fig. 4). For norepinephrine, the topical application of capsaicin to both the portal and superior mesenteric veins effectively eliminated the response to slowly developing hypoglycemia; i.e., hypoglycemic values were not significantly different from basal (Fig. 4*B*). Concomitant with the suppression in the sympathoadrenal response, PMV animals required a significantly greater glucose infusion during deep hypoglycemia compared with portal vein animals (Fig. 3). That these glucose sensors extend beyond the portal vein proper into the superior mesenteric vein is perhaps not surprising. The superior mesenteric and portal veins lie in series and are distinguished only by the entry of the gastrosplenic (ileal) vein, which joins perpendicular to the superior-mesenteric vein (27–29). The other major tributary, the pancreaticoduodenal (pyloric) vein, enters the portal vein more proximal to the liver also at a right angle. While the portal vein is a useful anatomical and surgical construct, little distinguishes it from the superior mesenteric vein beyond these tributary inputs. Both the portal and superior mesenteric veins demonstrate an extensive network of CGRP reactive nerve fibers (Fig. 1*A* and *C*) associated with their ability to detect blood glucose. Thus, when both the portal and superior mesenteric veins were denervated, as evidenced by a depletion of CGRP reactive fibers (Fig. 1*B* and *D*), the degree of suppression in the sympathoadrenal output was equal to or greater than that seen in previous studies involving the brain glucose clamp (i.e., normalizing CNS glycemia during systemic hypoglycemia) (14,16).

Given that the brain contains glucose-sensing neurons that respond to changes in glucose within the physiological range (1,30), it is unclear why peripheral glucose sensory input from the portal-mesenteric vein is essential for detecting slow, but not rapid, drops in blood glucose. It is possible that the firing rate for all glucose sensing neurons (central and peripheral) is proportional to the rate of fall in glucose; i.e., the slower the fall, the lower the firing rate at any given glucose concentration. If the efferent response to hypoglycemia reflects the summation of all glucose sensory inputs, the elimination of any single source might be sufficient to impair counterregulation during slow-onset hypoglycemia. This would explain why masking glucose sensing at either the brain (14,16) or portal vein (17,20) can suppress the counterregulation to systemic hypoglycemia. Alternatively, the latter observations might be explained by a fortuitous choice of experimental protocols. The effectiveness of either ventromedial hypothalamus glucose perfusion or clamping brain plasma glucose has only been demonstrated under conditions in which systemic hypoglycemia was induced rapidly (14,16,31). Thus, it is possible that CNS glucose sensors only respond to catastrophic drops in glycemia and normally rely on peripheral input for glycemic detection. Consistent with this idea, Fanelli et al. (32) observed that cognitive function was impaired to a greater extent when hypoglycemia ensued rapidly as opposed to a slow fall. It is now recognized that glucose-sensing neurons in the CNS respond to a variety of metabolic and hormonal inputs relevant to overall energy homeostasis (2,33). As metabolic sensors with an integrative role, they may not be optimally designed to detect changes in any single metabolite. This would be particularly true in vivo where alternative substrates, e.g., lactate produced by astrocytes, circulating ketones, or stored glycogen may be available to initially buffer changes in glycolytic flux. A relative insensitivity to glucose alone might also explain the existence of CNS glucose-sensing neurons in regions of the brain with very different glucose concentrations, e.g., ~1.5 mmol/l in the ventromedial nucleus of the hypothalamus (34) versus the arcuate nucleus at perhaps ≥3 mmol/l (2). Finally, input from the portal-mesenteric glucose-sensitive afferents may serve to sensitize CNS glucose-sensing neurons to their own ambient glucose concentrations. In the absence of peripheral input, CNS glucose sensors might then be unable to detect slow or small changes in their own ambient glucose concentration.

Though portal vein glucose sensing has been observed in a variety of species (17,20,35), including man (36), some caution should be employed when extending animal-based findings to humans. For example, as with most rat studies, we employed a substantially higher insulin infusion rate than that used in most human studies. As a result, plasma insulin levels are elevated in rats relative to those in humans during hyperinsulinemic-hypoglycemic clamps. While not a universal observation (37,38), exaggerated catecholamine responses to hypoglycemia have been reported when insulin levels were increased to ~3,300 pmol/l in both dogs (39) and humans (40). Though this has not been examined in rats, the elevated insulin levels observed during hyperinsulinemic-hyperglycemic clamps on rodents may serve a cautionary note when translating these findings to humans. While unlikely to explain the almost complete blunting of the sympathoadrenal response observed for PMV animals, to the extent that elevated insulin levels might increase the catecholamine

response for controls, this would impact on the determined magnitude of suppression.

In summary, we have demonstrated that portal vein glucose sensors, responsible for hypoglycemic detection, extend beyond the portal vein proper into the superior mesenteric vein. Further, when both the portal and superior mesenteric veins are denervated via capsaicin, the sympathoadrenal response to slow-onset hypoglycemia is effectively lost. This, however, is not the case for rapid-onset hypoglycemia in which the sympathoadrenal response is substantially less impaired by the loss of portal–superior mesenteric vein glucose sensing. Thus, while the rate of fall in hypoglycemia does not impact on the magnitude of the sympathoadrenal response to hypoglycemia (Figs. 4 and 6), as noted by previous investigators (41), it does shift the critical locus for hypoglycemic detection.

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