

## Rapid Publication

# Portal Vein Afferents Are Critical for the Sympathoadrenal Response to Hypoglycemia

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We sought to elucidate the role of the portal vein afferents in the sympathetic response to hypoglycemia. Laparotomy was performed on 27 male Wistar rats. Portal veins were painted with either 90% phenol (denervation group [PDN]) or 0.9% saline solution (sham-operated group [SHAM]). Rats were chronically cannulated in the carotid artery (sampling), jugular vein (infusion), and portal vein (infusion). After a recovery period of 5 days, animals were exposed to a hyperinsulinemic-hypoglycemic clamp, with glucose infused either portally (POR) or peripherally (PER). In all animals, systemic hypoglycemia ( $2.48 \pm 0.09$  mmol/l) was induced via jugular vein insulin infusion ( $50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). Arterial plasma catecholamines were assessed at basal ( $-30$  and  $0$  min) and during sustained hypoglycemia ( $60$ ,  $75$ ,  $90$ , and  $105$  min). By design, portal vein glucose concentrations were significantly elevated during POR versus PER ( $4.4 \pm 0.14$  vs.  $2.5 \pm 0.07$  mmol/l;  $P < 0.01$ , respectively) for both PDN and SHAM. There were no significant differences in arterial glucose or insulin concentration between the four experimental conditions at any point in time. When portal glycemia and systemic glycemia fell concomitantly (SHAM-PER), epinephrine increased 12-fold above basal ( $3.75 \pm 0.34$  and  $44.56 \pm 6.1$  nmol/l;  $P < 0.001$ ). However, maintenance of portal normoglycemia (SHAM-POR) caused a 50% suppression of the epinephrine response, despite cerebral hypoglycemia ( $22.2 \pm 3.1$  nmol/l,  $P < 0.001$ ). Portal denervation resulted in a significant blunting of the sympathoadrenal response to whole-body hypoglycemia (PDN-PER  $27.6 \pm 3.8$  nmol/l vs. SHAM-PER;  $P < 0.002$ ). In contrast to the sham experiments, there was no further suppression in arterial epinephrine concentrations observed during PDN-POR versus PDN-PER ( $P = 0.8$ ). These findings indicate that por-

tal vein afferent innervation is critical for hypoglycemic detection and normal sympathoadrenal counterregulation. *Diabetes* 49:8–12, 2000

Catecholamines constitute the primary defense against hypoglycemia for individuals with type 1 diabetes, in which the ability to suppress insulin is absent and the capacity to secrete glucagon is diminished. The catecholamine response has been well characterized for both normal and diabetic individuals (1–4). Under normal conditions, this sympathetic response appears sufficient to compensate for the loss of other counterregulatory measures (1,2). Unfortunately, over time, the catecholamine response for individuals with type 1 diabetes diminishes relative to the fall in blood glucose (1,2). The diminished catecholamine response appears to have been further exacerbated by increasingly aggressive attempts to control blood glucose levels in type 1 diabetic patients (3,4).

Although iatrogenic hypoglycemia has been invoked as a partial explanation, in general, the pathology of the diminished sympathetic response in patients with type 1 diabetes remains poorly understood. In part, this can be attributed to the lack of knowledge concerning the locus for hypoglycemic detection. Although there is little doubt that the brain plays a central role in coordinating the response to hypoglycemia, the exclusivity of its role in the detection of hypoglycemia has been brought into question. Our investigative efforts of the portohepatic region have consistently demonstrated that portohepatic hypoglycemia is requisite to engendering a full sympathoadrenal response. When the portal vein glucose concentration is normalized during general systemic hypoglycemia, a marked suppression in the sympathoadrenal response is observed (5–8). Under conditions of progressive hypoglycemia, as is seen in many clinical situations, portohepatic glucosensors appear to be the critical regulators of sympathoadrenal response, i.e., they account for 60–100% of catecholamine response. It should be noted that this suppression in counterregulation occurs despite deep cerebral hypoglycemia ( $2.2$ – $2.5$  mmol/l).

Recently, we have determined that these “portohepatic” glucosensors are specifically localized to the portal vein, rather than the liver (8). Neurophysiologic evidence suggests that glucose sensing may be mediated via afferents innervating the portal vein. It has been known for some time that an inverse relationship exists between the firing rate of certain hepatic

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ALT, alanine aminotransferase; ANOVA, analysis of variance; HPF, hepatic plasma flow rate; ID, internal diameter; PDN, 90% phenol portal vein denervation group; PDN-PER, portal denervation peripheral glucose infusion; PDN-POR, portal denervation portal glucose infusion; PER, peripheral infusion of glucose; POR, portal infusion of glucose; SHAM, 0.9% saline solution sham-operated group; SHAM-PER, sham-operated peripheral glucose infusion; SHAM-POR, sham-operated portal glucose infusion.

vagal afferents and the portal vein glucose concentration (9–12). A similar relationship between firing rate and portal glucose concentration has been observed for sympathetic neurons innervating the adrenals (13). More recently, investigators have confirmed the existence of fine varicose afferent terminals in the adventitia of the portal vein (14,15).

The purpose of the current investigation was to directly ascertain whether innervation of the portal vein is critical for the detection of hypoglycemia and modulation of the catecholamine response. This was accomplished by assessing the impact of portal vein denervation upon the sympathoadrenal response to general hypoglycemia. We further sought to determine whether portal vein innervation is essential to the suppression in the sympathoadrenal response observed when portal vein glucose is normalized during systemic hypoglycemia. Toward that end, we assessed the catecholamine response in both portally denervated and control animals during normalization of portal vein glucose via local irrigation. Results indicate that portal vein afferents are critical for hypoglycemic detection and modulation of the subsequent catecholamine response.

## RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Experiments were conducted on 27 male Wistar rats (weight  $272.7 \pm 5.75$  g) in the conscious relaxed state. All surgical and experimental procedures were preapproved by the University of Southern California Institutional Animal Care and Use Committee.

One week prior to experiments, the animals were chronically cannulated under single-dose anesthesia (ketamine HCl, xylazine, acepromazine maleate;  $0.10 \text{ cm}^3/100$  g body weight) given intramuscularly. After a midline abdominal incision and intestinal reflection, portal vein denervation (PDN) was effected in half of the animals. Phenol (90% wt/vol) was applied to a 1.5-cm region of the portal vein proximal to the liver. Portal vein extravascular tissue necrosis, denoted by vessel discoloration, was observed in all phenol-treated animals (17). The same physical procedure was performed on the remaining control animals (sham-operated group [SHAM]) with the exception that 0.9% NaCl was substituted for 90% phenol. A cannula was placed in the portal vein (Silastic, 0.03-cm internal diameter [ID]; Dow Corning, Midland, MI) for glucose infusion during portal infusion of glucose (POR), the carotid artery (PE-50; Clay Adams, Becton Dickinson, Sparks, MD) for arterial blood sampling, and in the jugular vein (dual cannula Silastic, 0.03-cm ID) for insulin and peripheral glucose infusion (PER). All cannulas were tunneled subcutaneously, exteriorized at the back of the neck, and encased in Silastic tubing (0.18-cm ID) sutured to the skin. Animals were allowed 5 days to recover from surgery to restore body weight. Three days after surgery, 300  $\mu\text{l}$  of arterial blood was drawn and analyzed for plasma alanine aminotransferase (ALT) activity, a measure of hepatocellular integrity. Comparisons in ALT activity were made between all animals portally cannulated (SHAM vs. PDN) and those littermates randomly assigned to the blood donor group possessing a carotid cannula exclusively (18). Animals were fasted 24 h before the clamp experiment.

Experimental design. Each animal was used for only one experimental condition distinguished by the surgical procedure, SHAM or PDN, and the site of glucose infusion, PER or POR. To ascertain whether afferents innervating the portal vein are critical for hypoglycemic detection, the catecholamine response to whole-body hypoglycemia was compared between control (SHAM-PER,  $n = 6$ ) and portally denervated (PDN-PER,  $n = 8$ ) animals. The relative attenuation in the catecholamine response to systemic hypoglycemia during portal glucose normalization was compared between SHAM (SHAM-POR,  $n = 7$ ) and denervated (PDN-POR,  $n = 6$ ) animals to determine whether the glucosensors responsible for detecting hypoglycemia are the same glucosensors responsible for the detection of elevations in glycemia as well.

All animals were exposed to the same general protocol for the induction of hypoglycemia. Before the experiment, the animals were placed in a modified metabolic chamber and allowed to rest for 30 min ( $-60$  to  $-30$ ). Basal samples were drawn at  $-30$  and 0 min for glucose and catecholamine analysis. At 0 min, following arterial sampling, insulin ( $50 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and peripheral glucose infusions (variable) were initiated and maintained for 105 min of the hypoglycemic clamp. Serial sampling for glucose was performed at 10-min intervals to control the rate of fall in blood glucose until deep hypoglycemia was achieved (60 min). Arterial plasma catecholamine and glucose samples were taken at 60, 75, 90, and 105 min of sustained deep hypoglycemia.

Calculations. The estimated liver glucose concentration ( $G_L$ ) was calculated as follows:  $G_L = G_A + (\text{GINF}_{\text{POR}} / \text{HPF})$ , where  $G_A$  = arterial glucose concentration (micromoles per milliliter),  $\text{GINF}_{\text{POR}}$  = portal glucose infusion rate (micromoles per minute), and  $\text{HPF}$  = hepatic plasma flow rate (milliliters per minute). The estimated portal vein glucose concentration ( $G_{\text{PV}}$ ) was calculated as  $G_{\text{PV}} = G_A + (\text{GINF} / \text{PVPF})$ , where  $\text{GINF}_{\text{POR}}$  = portal vein glucose infusion rate (micromoles per minute), and  $\text{PVPF}$  = portal vein plasma flow rate (milliliters per minute). Hepatic plasma flow was assumed to be  $1.3 \text{ ml} \cdot \text{g}^{-1} \text{ liver} \cdot \text{min}^{-1} \times \text{liver weight (g)}$ , with portal vein flow rate assumed to be 80% of the  $\text{HPF}$  (19,20).

Analytical procedures. Glucose was assayed online with the glucose oxidase method (YSI, Yellow Springs, OH). Epinephrine and norepinephrine concentrations were assayed with use of a single-isotope radioenzymatic approach (21). Basal rat insulin and hyperinsulinemic porcine samples were assayed via radioimmunoassay (Linco Research, St. Charles, MO). ALT was assayed spectrophotometrically (18).

Data analysis. The results are expressed as means  $\pm$  SE. Comparisons of animal characteristics between groups were made by means of one-way analysis of variance (ANOVA) for independent groups. Comparisons between treatments over time were made by repeated-measures ANOVA with Tukeys test for post hoc comparisons. Significance was set at  $P < 0.05$ .

## RESULTS

No significant differences were observed between portally denervated and SHAM-operated animals for body weight ( $270 \pm 5.48$  vs.  $275.3 \pm 6.02$  g), liver weight ( $9.56 \pm 0.5$  vs.  $9.17 \pm 0.34$  g), ALT ( $13.4 \pm 1.39$  vs.  $14.1 \pm 1.22$  U/l), or hematocrit ( $46.48 \pm 1.10$  vs.  $45.54 \pm 0.69\%$ ), respectively.

Arterial insulin concentrations increased from a mean basal value of  $0.164 \pm 0.03$  nmol/l ( $P = 0.48$  between groups) to a hyperinsulinemic plateau of  $16.6 \pm 1.0$  nmol/l ( $P = 0.37$  between groups), with no significant differences between groups. Basal glucose concentration,  $7.05 \pm 0.22$  mmol/l, was not significantly different between groups ( $P = 0.69$ ), nor were the arterial glucose concentrations during the fall in glycemia or at the hypoglycemic nadir ( $2.48 \pm 0.09$  mmol/l,  $P = 0.89$ ; Fig. 1A). During PER, portal vein and liver glycemia were assumed equal to the arterial glycemia (Fig. 1B). By design, both portal vein ( $5.0 \pm 0.21$  mmol/l) and liver ( $3.94 \pm 0.2$  mmol/l) glycemia were significantly elevated above arterial glycemia during portal glucose infusion experiments, for both groups ( $P < 0.01$ ). When comparing SHAM versus denervated animals for a given glucose infusion protocol, i.e., POR or PER, there were no significant differences in either portal vein or liver glycemia.

In response to whole-body hypoglycemia, arterial epinephrine concentrations increased 12-fold in SHAM, from  $3.75 \pm 0.3$  nmol/l at basal to  $45.3 \pm 5.4$  nmol/l, by 75 min of hypoglycemia (Fig. 2A). Portal denervation resulted in a 47% suppression in the epinephrine response,  $24.2 \pm 3.3$  nmol/l ( $P < 0.002$ ) to whole-body hypoglycemia (Fig. 2A). The norepinephrine response to whole-body hypoglycemia was similar, as plasma norepinephrine increased from  $3.7 \pm 0.54$  to  $10.5 \pm 1.2$  nmol/l in SHAM (Fig. 2B; SHAM-PER). Similar to the epinephrine response, portal denervation led to a significant attenuation (36%) in norepinephrine concentrations during whole-body hypoglycemia ( $6.7 \pm 1.0$  nmol/l,  $P < 0.05$ ; Fig. 2B). When expressed as the response above basal, the norepinephrine response to hypoglycemia was suppressed by 55% with portal vein denervation.

Normalization of portal vein glucose concentration in SHAM during systemic hypoglycemia effected a 50% suppression in the epinephrine response when compared with peripherally infused animals ( $22 \pm 3.1$  vs.  $44.6 \pm 6.1$  nmol/l, respectively,  $P < 0.01$ ; Fig. 3A). A similar degree of suppression was observed in the norepinephrine response for control

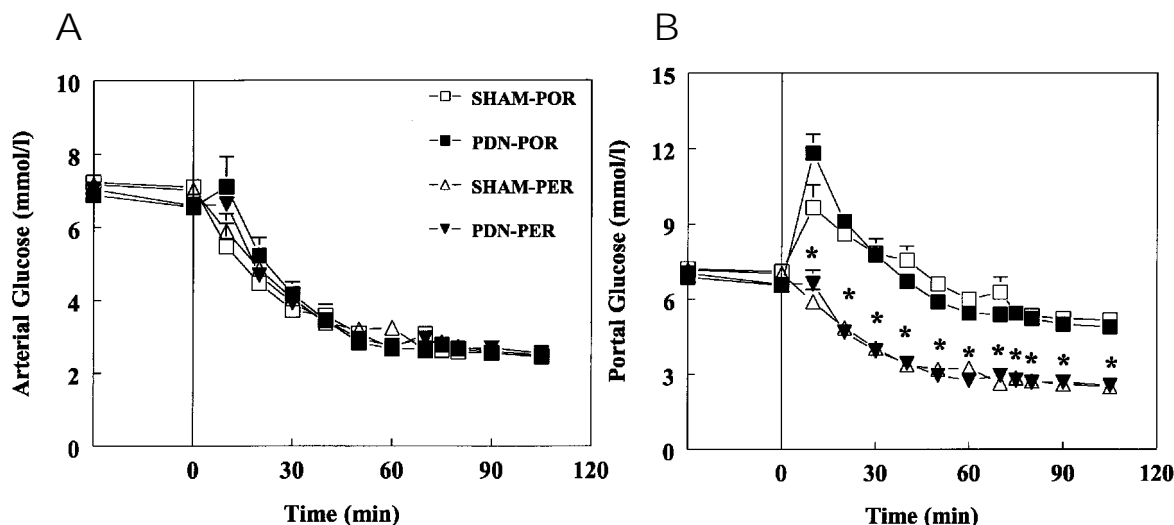


FIG. 1. Data are expressed as means  $\pm$  SE for arterial glucose concentration (A) and estimated portal vein glucose concentration (B) at basal and during the hyperinsulinemic-hypoglycemic clamp.  $\square$ , The portal vein glucose infusion experiment in SHAM-operated rats;  $\blacksquare$ , the portal vein glucose infusion experiment in portally denervated animals;  $\triangle$ , the peripheral glucose infusion experiment in SHAM-operated rats;  $\blacktriangledown$ , the peripheral glucose infusion experiment in portally denervated rats. \*Significance between infusion protocols ( $P < 0.05$ ).

animals (SHAM-POR =  $5.9 \pm 1.2$  vs. SHAM-PER =  $9.73 \pm 0.9$ ,  $P < 0.05$ ; Fig. 3B). In sharp contrast, portal vein glucose infusion had no impact on the sympathoadrenal response to systemic hypoglycemia in portally denervated animals. That is, in portally denervated animals, normalization of portal vein glucose during systemic hypoglycemia yielded no suppression in the catecholamine response to systemic hypoglycemia, unlike that observed for SHAM ( $P = 0.90$ ).

DISCUSSION

The current findings demonstrate that intact portal vein afferents are essential for the full sympathoadrenal response to hypoglycemia. The induction of whole-body hypoglycemia resulted in a 12-fold increase in epinephrine and a 3-fold increase in norepinephrine for control animals with intact portal vein nerves. In contrast, when portal vein afferents were destroyed and whole-body hypoglycemia was induced, the epinephrine and norepinephrine responses above basal were blunted by 51 and 55%, respectively. These results are consistent with our previous observation that the glucosensors critical for hypoglycemic detection reside in the portal vein (8). In that earlier study, the portal vein locus was elucidated via normalization of portal vein glycemia during systemic hypoglycemia. During portal vein glucose infusion, we previously observed a 67 and 50% attenuation in the epinephrine and norepinephrine responses, respectively. Quantitatively, the similar suppression of the sympathoadrenal response by either denervation or normalization of portal vein glycemia suggests that the two experimental approaches elucidate the same population of glucosensors. This was confirmed in the current study when portal vein denervation eliminated the attenuation in the catecholamine response typically observed with normalization of portal vein glycemia during systemic hypoglycemia. Thus, elimination of portal vein afferents was shown to compromise detection of both normoglycemia and hypoglycemia.

The existence of a portohepatic-glucoregulatory neural axis had been previously postulated, on the basis of several lines of evidence (11–13). The influence of efferent output

from the ventromedial hypothalamus upon glucose metabolism has been well characterized (13). Histological studies have revealed extensive innervation of the portohepatic region, with afferent fibers composing a significant portion of those neurons (14–16). Although the extent and significance

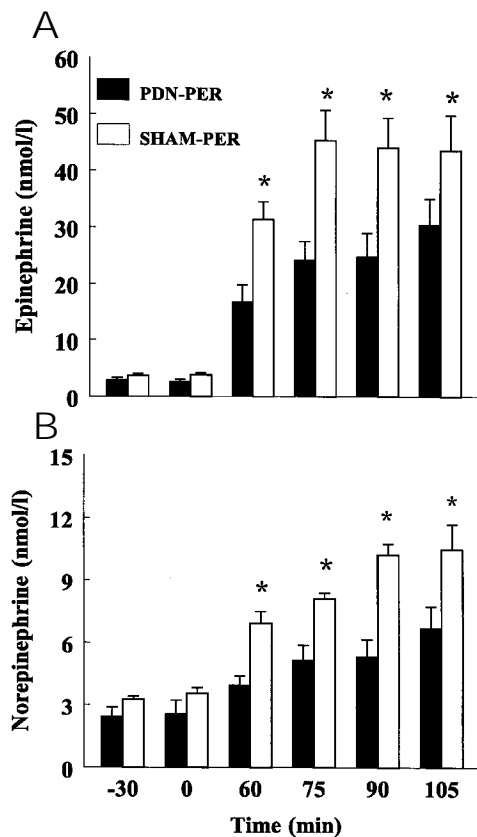


FIG. 2. Epinephrine (A) and norepinephrine (B) concentrations at basal and during sustained hypoglycemia. Data are expressed as means  $\pm$  SE for peripheral glucose infusion experiments in portally denervated ( $\blacksquare$ ) and SHAM-operated ( $\square$ ) animals. \*Significant difference between PDN and SHAM ( $P < 0.05$ ).

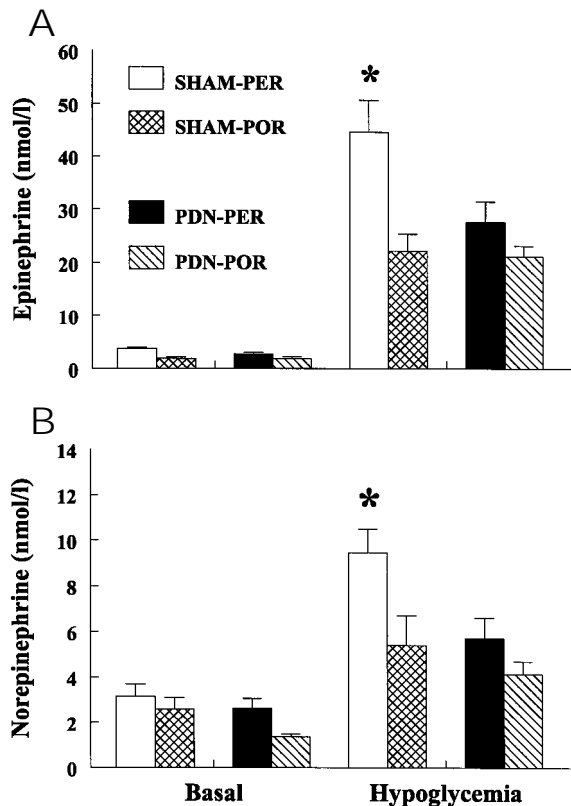


FIG. 3. Data are expressed as means  $\pm$  SE for epinephrine (A) and norepinephrine (B) concentrations at basal and the final two sampling periods (90 and 105 min) of sustained hypoglycemia. □, Peripheral glucose infusion experiments in SHAM-operated animals; ▤, portal vein glucose infusion experiments in SHAM-operated animals; ■, peripheral glucose infusion experiments in portal vein denervated animals; ▨, portal vein glucose infusion experiments in portal vein denervated animals. \*Significant difference between PER and POR protocols ( $P < 0.05$ ).

of afferent innervation of the liver remains in question, recent fluorescent staining techniques have revealed extensive afferent innervation of the portal vein (15). Glucose-sensitive vagal afferents have been found to possess firing rates inversely proportional to the portal vein glucose concentration (9). Fluctuations in portal vein glucose concentration have also been shown to impact upon firing rates of glucose-sensitive neurons located in the lateral hypothalamus and nuclear tractus solitarius (23). Additionally, adrenal and splanchnic efferent discharge rates have been shown to be depressed by elevations in portal glucose concentration (13). Although a recent study has cast some doubt on the relevance of vagal afferents for hypoglycemic detection (27), substantial afferent innervation of the portohepatis ascends via the sympathetic nervous system (14,15,24,25). Our own work has consistently demonstrated the importance of the portohepatis in hypoglycemic detection (5–8), and the current study implicates the afferents innervating the portal vein.

Previous attempts to assess the importance of portohepatic innervation in hypoglycemic detection were met with mixed results. These studies in general have focused on the liver as the source of the afferent signal, rather than the portal vein. Two studies that used complete liver denervation by surgical stripping or cutting found no effect on counterregulation during insulin-induced hypoglycemia (17,26). In contrast, an

investigation in which the liver was denervated by the application of phenol to the hepatic vasculature yielded similar findings to the current investigation (28). This group demonstrated 90 and 82% attenuation in the maximal net adrenal response to hypoglycemia for epinephrine and norepinephrine, respectively. More recently, human subjects who had undergone liver transplant surgery were shown to demonstrate a 57% suppression in the epinephrine response to hypoglycemia (29). A possible explanation for the apparent discrepancy in these observations is that those studies demonstrating no impact of liver denervation failed to adequately denervate the portal vein. Current and previous results (8) indicate a critical portal vein glucosensor locus in the rat approximately 1–3 cm from the liver. The locus for the portal vein glucosensors might be expected to be proportionally more distant in larger species, e.g., dog. Procedures that failed to adequately denervate this area would be expected to have little or no impact on subsequent sympathoadrenal response to hypoglycemia.

Our earlier studies involving hypoglycemic detection by the portohepatis have recently been criticized as an artifact of the methods used (27). That is, the normalization of portohepatic glycemia in the face of systemic hypoglycemia was viewed as inducing a negative arterial-portal glucose gradient. It was suggested that this gradient signal was responsible for the attenuation of the sympathoadrenal response, not the lack of hypoglycemic detection (27). The current findings demonstrate that this is not the case, because denervation of the portal vein compromised the ability to detect hypoglycemia. Furthermore, as noted above, those sensors eliminated by the phenol denervation procedure appear to be the same glucosensors responding to portal vein glucose normalization during systemic hypoglycemia. That the arterial-portal gradient plays no role in our observations is not surprising, because there is no reason to believe it should. The importance of the former has been established for hepatic glucose uptake under euglycemic and hyperglycemic conditions. Alternatively, our observations have been based on the sympathoadrenal response to hypoglycemia. Given the current results, it would appear that the two constitute distinct physiological responses with discrete underlying mechanisms.

In conclusion, afferent innervation of the portal vein is critical for hypoglycemic detection. In the absence of portal vein nerves, animals are unable to generate a full sympathoadrenal response to systemic hypoglycemia. Whereas normally innervated animals demonstrate an attenuation in the catecholamine response to systemic hypoglycemia with portal glucose infusion, this response is lost after portal vein denervation. Thus, portal vein glucosensors responsible for hypoglycemic detection appear to respond to a range of portal glucose concentrations from euglycemia to deep hypoglycemia.

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