

Hypoglycemic Detection Does Not Occur in the Hepatic Artery or Liver

Findings Consistent With a Portal Vein Glucosensor Locus

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Our laboratory has previously demonstrated that hypoglycemic detection occurs in the portal vein, not the liver. To ascertain whether hypoglycemic detection may also occur in the hepatic artery, normoglycemia was established across the liver via a localized hepatic artery glucose infusion. Male mongrel dogs ($n = 7$) were infused with insulin ($5.0 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) via the jugular vein to induce systemic hypoglycemia. Animals participated in two hyperinsulinemic-hypoglycemic clamp experiments distinguished by the site of glucose infusion. During the liver irrigation protocol, glucose was infused via the hepatic artery (HA protocol) to maintain liver normoglycemia as systemic glucose concentrations were systematically lowered over 260 min (nadir = $2.2 \pm 0.01 \text{ mmol/l}$). During control experiments, glucose was infused peripherally (PER protocol) to control reductions in blood glucose. Arterial glucose concentrations were not significantly different at any time between the two protocols ($P = 0.73$). Hepatic artery and liver glucose concentrations were significantly elevated in the HA versus PER protocol throughout the duration of the progressive hyperinsulinemic-hypoglycemic clamp. During the PER protocol, epinephrine and norepinephrine concentrations increased significantly above basal values (0.53 ± 0.06 and $0.85 \pm 0.2 \text{ nmol/l}$, respectively) to plateaus of 4.4 ± 0.86 ($P = 0.0001$) and $3.6 \pm 0.69 \text{ nmol/l}$ ($P = 0.001$), respectively. There were no significant differences between the two protocols in the epinephrine ($P = 0.81$) and the norepinephrine ($P = 0.68$) response to hypoglycemia. The current findings indicate that glucosensors important to hypoglycemic detection do not reside in the hepatic artery. Furthermore, these data confirm our previous findings that glucosensors important to hypoglycemic detection are not present in the liver, but are in fact localized to the portal vein. *Diabetes* 50:399–403, 2001

The ability to detect the onset of hypoglycemia and initiate appropriate counterregulation is substantially impaired in many individuals with diabetes (1). Such individuals are predisposed to severe episodes of hypoglycemia that require assistance, a situation that has been exacerbated with the advent of more aggressive therapies (2). As a result, hypoglycemia is now recognized as the primary limitation in the effective treatment of type 1 diabetes (3). Efforts to understand the pathology of defective glucose sensing in diabetes has led to renewed interest in elucidating the loci of hypoglycemic detection.

To date, most efforts to elucidate the loci for hypoglycemic detection have focused on the brain and in particular the ventromedial hypothalamus (VMH). Although it is clear that the VMH is critical for hypoglycemic counterregulation, it does not appear to be the exclusive site for glycemic detection. Glucose-receptive neurons have been identified within the hindbrain (4), and appear to be widespread throughout the brain (5) and peripheral tissues (6,7). Although many of the peripheral glucose-sensitive neurons may not be involved in detection of hypoglycemia, portohepatic neurons have been shown to be critical for the counterregulatory response to insulin-induced hypoglycemia. The functional integrity of portohepatic glucosensors has been shown to be essential to engendering a full sympathetic response to hypoglycemia in both the dog and rat (8–10). Examined under conditions of hypoglycemia developed within hours, portohepatic glucosensors appear to be the primary glucosensors modulating the sympathoadrenal response. Possible functional alterations of portohepatic glucosensors in diabetes has yet to be investigated.

Evidence supporting the existence of glucose receptors in the portohepatic region has been functional; that is, irrigation of the portal vein and liver by glucose (8–10) or denervation of the region (11) was shown to markedly suppress the catecholamine response to insulin-induced hypoglycemia. We originally considered that the putative receptors were localized to the liver. However, our most recent experimental studies pointed to the portal vein as the locus of these receptors, at least in rat (10). Furthermore, glucose sensing appears to be mediated by afferents innervating the portal vein (11). Although elucidating the importance of portal glucosensors, these findings did not exclude the existence of other glucosensory sites in the splanchnic region. Of particular interest was the hepatic artery, which like the portal vein is richly innervated and perfuses the liver (12,13).

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ANOVA, analysis of variance; CNS, central nervous system; HA, hepatic artery infusion; HPF, hepatic plasma flow; ICG, indocyanine green dye; MHAG, mean hepatic artery glucose; MHG, mean hepatic glycemia; PER, peripheral infusion; VMH, ventromedial hypothalamus.

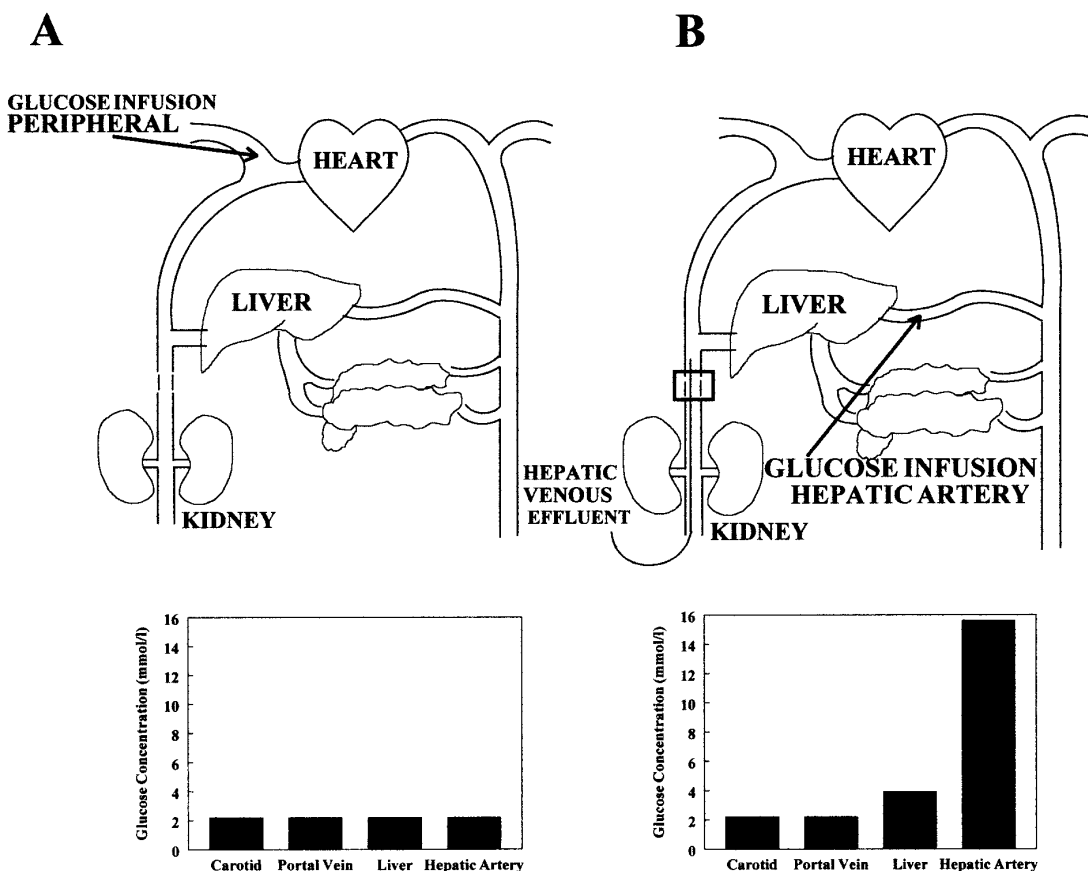


FIG. 1. Schematic diagram of the two glucose infusion conditions. *A*: PER protocol represents the control experiment in which whole-body hypoglycemia was induced. *B*: HA protocol allowed for the elevation of hepatic artery and the normalization of liver glucose concentrations during systemic hypoglycemia. Bar graphs represent the target glycemia for arterial circulation (carotid artery sampling), portal vein, liver, and hepatic artery during both glucose infusion experiments.

The current study was undertaken to ascertain whether glucosensors, similar to those found in the portal vein, might also reside in the hepatic artery. Using the local irrigation technique, hepatic artery and liver glycemia were normalized despite systemic hypoglycemia induced by hyperinsulinemia. Because hepatic artery and portal blood mix in the hepatic sinusoids, this approach also allowed us to reevaluate the role of the liver in hypoglycemic detection. Results from liver irrigation experiments were compared with those achieved when equivalent hypoglycemia was allowed to develop in the entire body, including the hepatic artery and liver.

RESEARCH DESIGN AND METHODS

Animals. Experiments were conducted on conscious male mongrel dogs (30.2 ± 1.2 kg; n = 7). Dogs were housed under controlled conditions (12:12-h light:dark) in the university vivarium and were fed once per day with a standard diet (25% protein, 9% fat, 45% carbohydrate; Wayne Dog Chow, Alfred Mills, Chicago). Dogs were used for experiments only if judged to be in good health as determined by body temperature, hematocrit, regularity of food intake, and direct observation. The University of Southern California Institutional Animal Care and Use Committee approved all surgical and experimental procedures.

Surgery. One week before initiating the experiment, animals were chronically cannulated under anesthesia induced by thiamylal sodium (Biotal, Bio-Ceutic Laboratories) and maintained with 0.5–1.0% halothane and nitrous oxide. The common hepatic artery was cannulated directly and secured so flow was not occluded. The tip of the cannula was advanced 1.5 cm past the origin of the left gastric artery, and the gastroduodenal artery was ligated. Cannulas (Tygon; ID = 0.13 cm) were also placed in the carotid artery for sampling and the jugular vein for insulin infusion. In addition, a femoral vein cannula

advanced to the inferior vena cava superior to the hepatic vein was used for sampling mixed hepatic venous blood. An inflatable cuff (Model VO-4; Rhodes Medical) was placed around the inferior vena cava caudal to the hepatic vein (Fig. 1). Cuff inflation temporarily occluded subhepatic vena caval flow, allowing mixed hepatic venous blood to be sampled from the femoral catheter. All cannulas and the actuating tubing for the inflatable cuff were tunneled subcutaneously and exteriorized at the back of the neck. The cannulas were filled with heparinized saline (100 U/ml), coiled and capped, and placed in a small gauze pouch secured to the back of the neck. Catheter placement was confirmed at necropsy.

Experimental design. Each animal participated in two hyperinsulinemic-hypoglycemic clamp experiments distinguished by the site of glucose infusion: peripheral (via the cephalic vein; PER protocol) and hepatic artery (HA protocol) (Fig. 1). Intracatheters (19-gauge; Deseret Medical, Sandy, UT) were acutely placed in the right cephalic vein for infusion of indocyanine green dye (ICG) and in the left cephalic vein for the PER protocol. A constant infusion of ICG (0.13 mg/min) was initiated at –120 min, followed by a 90-min equilibration period. A 30-min basal sampling period (–30 to 0 min) followed, during which serial samples, arterial (glucose, insulin, ICG, epinephrine, and norepinephrine), and hepatic venous (ICG), were taken at 15-min intervals. At min 0, insulin infusion (5.0 mU · kg⁻¹ · min⁻¹) was initiated and maintained for the remaining 260 min of the experimental period. Peripheral glucose infusion was initiated simultaneously in order to clamp arterial glycemia at ~5.55 mmol/l for the subsequent 60 min. Thereafter, the glucose infusion rate was adjusted every 10 min to achieve ~0.55 mmol/l reductions in blood glucose over a 40-min period. This provided a stepwise decrease in arterial glycemia, reaching a nadir of 2.2 mmol/l between 220 and 260 min. Serial blood samples were taken every 10 min for glucose and insulin and every 20 min for ICG during the 260-min experimental period. Additional arterial blood samples were taken every 10 min during the final 20 min (i.e., at 20, 30, and 40 min) of each 40-min stage for measurements of epinephrine and norepinephrine. An identical protocol was used for the HA protocol; however, glucose was infused via the hepatic artery instead of the cephalic vein. By design, the HA

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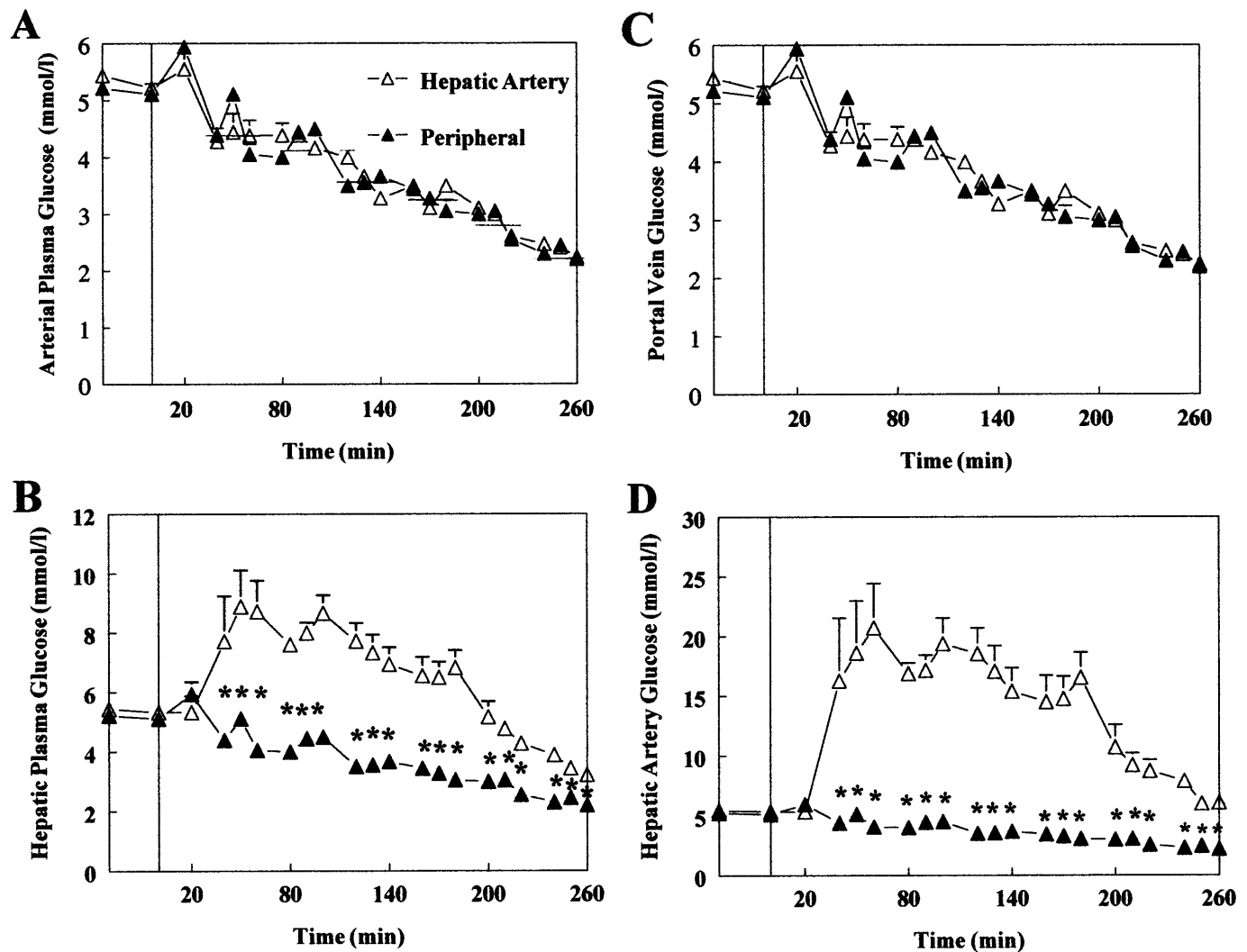


FIG. 2. Data are means \pm SE for arterial plasma glucose (A), hepatic plasma glucose (B), portal vein plasma glucose (C), and hepatic artery glucose (D) at basal and during the hyperinsulinemic-hypoglycemic clamp. Δ , HA protocol; \blacktriangle , PER protocol. * $P < 0.05$ between infusion protocols.

protocol allowed hepatic artery and liver glycemia to remain markedly elevated above arterial concentrations. Each animal was used for both the PER and HA protocols, with a 1-week interval between experiments. The experimental order of protocols was randomized to avoid an order effect.

Assays. Arterial plasma was assayed on-line for glucose by means of the glucose oxidase method (YSI, Yellow Springs, OH). Arterial and hepatic venous plasma ICG concentrations were determined spectrophotometrically at 805 nm. Insulin was measured in duplicate via radioimmunoassays (Linco Research, St. Charles, MO). Arterial blood samples for catecholamines (2 ml) were collected in chilled culture tubes containing heparin and glutathione and centrifuged; plasma was maintained at -60°C for subsequent analysis. Epinephrine and norepinephrine concentrations were assayed using a single-isotope radioenzymatic approach (14).

Calculations. Hepatic plasma flow (HPF) in dl/min was calculated as $\text{HPF} = I_{\text{ICG}} / (\text{ICG}_{\text{A}} - \text{ICG}_{\text{HV}})$, where I_{ICG} is the ICG infusion rate (0.13 mg/min), ICG_{A} is the arterial ICG concentration, and ICG_{HV} is the hepatic-venous ICG concentration. The mean hepatic glycemia (MHG) in $\mu\text{mol/ml}$ was calculated as $\text{MHG} = G_{\text{A}} + (\text{GINF}_{\text{HA}} / \text{HPF})$, where G_{A} is arterial glucose ($\mu\text{mol/ml}$), GINF_{HA} is the hepatic artery glucose infusion rate ($\mu\text{mol/min}$), and HPF is the hepatic plasma flow (ml/min). The mean hepatic artery glucose concentration (MHAG) in $\mu\text{mol/ml}$ was calculated as $\text{MHAG} = G_{\text{A}} + (\text{GINF}_{\text{HA}} / [\text{HPF} \times 0.25])$.

Statistical analyses. Comparisons between treatments over time were assessed using repeated measures analysis of variance (ANOVA) with Tukey's test for post hoc comparisons. In additional, one-way ANOVA was used where appropriate for within animal pre- and posttreatment comparisons. Statistical significance was set at $P < 0.05$.

RESULTS

During the 260-min insulin infusion, arterial insulin concentrations increased significantly from basal to a hyperinsulinemic plateau that was not significantly different between protocols (1560 ± 170 [PER] vs. 1615 ± 145 pmol/l [HA]). Basal arterial glucose concentrations (5.3 ± 0.1 and 5.4 ± 0.2 mmol/l for PER and HA, respectively) as well as arterial glucose concentrations at the various stages of progressive hypoglycemia were not significantly different between infusion protocols (Fig. 2A). Hepatic artery and liver glucose levels declined at a rate identical to arterial glycemia in the PER protocol. A mean hypoglycemic nadir of 2.2 ± 0.05 mmol/l was attained for PER experiments (Fig. 2B and D). In contrast, when glucose was infused directly into the hepatic artery, hepatic artery and calculated liver glucose concentrations were significantly elevated above arterial glucose, from min 40 to termination of the experiment at 260 min ($P < 0.001$). During both PER and HA protocols, the portal vein glucose concentration was lowered to hypoglycemic levels identical to systemic concentrations; thus there was no difference in portal glucose concentration between the two experimental protocols (Fig. 2C).

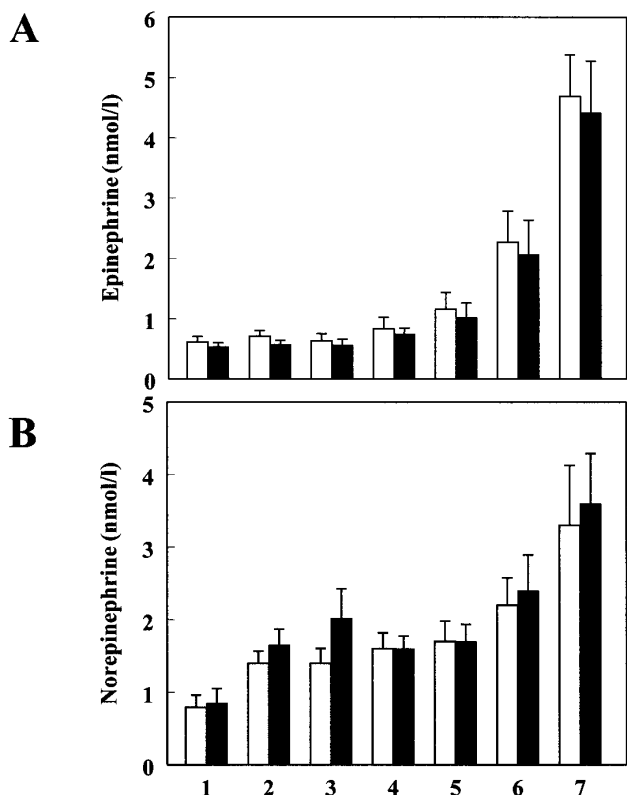


FIG. 3. Average values (means \pm SE) for epinephrine (A) and norepinephrine (B) concentrations at basal (stage 1) and during progressive hypoglycemic stages (stages 2–7). Stages are 40-min periods. Average values were determined from three samples taken 10 min apart during each sampling stage. □, HA protocol; ■, PER protocol. No significant differences were observed between infusion protocols.

As expected, whole body hypoglycemia in the PER protocol elicited a robust sympathetic response. Arterial epinephrine concentrations increased eightfold above basal, from 0.54 ± 0.06 to a mean of 4.4 ± 0.86 nmol/ml by the final 40-min stage of hypoglycemia ($P = 0.0001$) (Fig. 3A). Prevention of hepatic arterial and liver hypoglycemia by direct hepatic artery glucose infusion had no effect on the sympathetic response to systemic hypoglycemia; that is, increases in plasma epinephrine after systemic hypoglycemia were identical with or without hepatic arterial hyperglycemia ($P = 0.74$). A similar pattern was observed between protocols for norepinephrine. In response to whole-body hypoglycemia in the PER protocol, norepinephrine increased fourfold to 3.6 ± 0.69 nmol/l (Fig. 3B). Normalizing hepatic artery and liver glycemia in the HA protocol had no impact on the norepinephrine response at any time point when compared with the response during whole-body hypoglycemia in the PER protocol (Fig. 3B).

DISCUSSION

The regulation of blood glucose concentration to guarantee adequate energy supply for the central nervous system (CNS) is a fundamental physiological control system critical for organisms exposed to the vicissitudes of feeding and fasting. When challenged by the onset of hypoglycemia, a series of well-defined hormonal responses (i.e., counterregulation)

are evoked to restore and support blood glucose levels (2,3). In contrast to the efferent limb, considerably less is known about the afferent aspect in which hypoglycemia is sensed and information transmitted to the CNS. The traditional concept that the CNS, the VMH in particular, is the exclusive site for hypoglycemic detection has been challenged by recent data. There is now substantial evidence that peripheral cells sense hypoglycemia and transmit this information to the CNS (8–10,16,19).

Our laboratory has consistently demonstrated a suppression of the sympathoadrenal response to systemic hypoglycemia of 3.3 ± 0.2 mmol/l or less during portal glucose infusion (i.e., portohepatic normoglycemia) (8–11). When hypoglycemia was allowed to develop slowly, these portohepatic glucosensors were shown to be the primary (70–100%) regulators of the sympathoadrenal response. This has been observed only when animals were cannulated in the portal vein upstream from the liver so that both the portal vein and liver glycemia were normalized (8,9). Subsequent refinement of the liver irrigation technique revealed that normalization of the liver glucose alone, in the absence of normalizing portal vein glucose, was insufficient to blunt the sympathoadrenal response to systemic hypoglycemia (10). These results were the first to reveal a portal vein, but not a liver, locus for glucosensors essential to hypoglycemic detection.

These earlier results did not exclude the possibility of hepatic artery glucose sensors, and suggested some importance for the level of glycemia entering the liver. However, the current findings demonstrate that the magnitude of the sympathoadrenal response to hypoglycemia is unaffected by the blood glucose concentration present in the hepatic artery. If glucosensors critical for detection of hypoglycemia were located in the hepatic artery, normalization of hepatic artery glycemia during systemic hypoglycemia should have led to a suppression of the sympathoadrenal response. Despite a substantial elevation in hepatic artery glucose concentration during the HA protocol, the catecholamine response to systemic hypoglycemia was virtually identical to the response observed during whole-body hypoglycemia (i.e., without hepatic artery glucose infusion). In addition, because in the current study the rate of glucose infusion via the hepatic artery was sufficient to normalize total liver glycemia, these results confirm our previous observation regarding the lack of any essential liver glucosensors (10). When considered in the context of our previous findings (8–11), the current results serve to further constrain the portohepatic site for hypoglycemic detection to the portal vein.

Much of the nerve supply to the liver ramifies around the portal vein (12), and studies have clearly demonstrated terminal vagal afferents in the adventitia of the portal vein (15). In situ studies have provided evidence of an inverse relationship between portal vein glucose concentration and the rate of vagal afferent firing (7,16). Glucose-sensitive neurons of the lateral hypothalamus and nucleus solitarius have been shown to be responsive to changes in portal vein glucose concentration (17,18). In addition, the firing of the adrenal nerve has been shown to be inversely proportional to portal vein glucose concentration (16). This has led to the proposal of a portohepatic–adrenal neural reflex for the regulation of glucose homeostasis (19,20).

The importance of portal vein afferent innervation for hypoglycemic detection has recently been demonstrated by our laboratory (11). Denervation of the portal vein alone was shown to have a blunting effect on the sympathoadrenal response to hypoglycemia quantitatively similar to that observed with normalization of portal vein glucose; that is, in the presence of whole-body hypoglycemia, animals with denervated portal veins demonstrated a sympathoadrenal response that was only 50% of normal. Furthermore, portally denervated animals were unable to respond to glucose normalization of the portal vein; that is, no suppression of the sympathetic response was observed during portal vein glucose infusion when compared with whole-body hypoglycemia. These data suggest that those afferents responding to portal glucose infusion are the same as those that detect portal vein hypoglycemia. Consistent with the critical nature of the portal vein glucose-sensitive afferents, studies involving denervation of the liver alone have demonstrated no impact on the counterregulatory responses to hypoglycemia (21). However, when the portal vein was clearly denervated along with the liver, there was an observed suppression in the catecholamine response to hypoglycemia (22).

In the current study, as with most studies of hypoglycemia, we induced a fall in blood glucose by elevating the circulating insulin concentration. It has been reported that pharmacological insulin levels result in exaggerated catecholamine responses to hypoglycemia (23,24). Although this has not been a universal observation (25,26), the potential impact of hyperinsulinemia on our own findings remains unclear. It should be noted that Davis et al. (27) subsequently attributed the impact of hyperinsulinemia on hypoglycemic counterregulation to its impact on the CNS. Also, our data across several studies (8–11) have demonstrated a quantitatively similar impact of portal glucose infusion on the catecholamine response to hypoglycemia over a wide range of insulin levels. However, because our lowest insulin levels remain substantially above normal physiological values, we cannot exclude hyperinsulinemia as a possible confounding variable.

The current findings exclude the hepatic artery and liver as loci for hypoglycemic detection, and are consistent with previous findings of a portal vein locus. Although not excluding other glucosensor loci (e.g., the brain), we have consistently demonstrated the importance of the portal vein in hypoglycemic detection and sympathoadrenal counterregulation in both the rat and dog. That there are no essential glucosensors in the liver has now been confirmed in both of these species. Consistent with these observations, it has recently been shown that humans undergoing liver transplantation, in which the portal vein innervation is clearly severed, demonstrate a significantly impaired sympathoadrenal response to hypoglycemia (28). These observations suggest that portal glucose sensing is a critical mechanism for hypoglycemic detection that is conserved across mammalian species.

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