

# Novel Glucosensor for Hypoglycemic Detection Localized to the Portal Vein

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In this investigation, we sought to constrain the locus of essential portohepatic glucosensors and test the hypothesis that they reside strictly in the portal vein and not the liver. Male Wistar rats were chronically cannulated in the carotid artery (sampling), jugular vein (infusion), and portal vein, either adjacent to (POR<sub>ADJ</sub>, 0.6 ± 0.1 cm, *n* = 6) or upstream from (POR<sub>UPS</sub>, 2.7 ± 0.1 cm, *n* = 8) the liver. Animals were exposed to one of three protocols distinguished by the site of glucose infusion: POR<sub>UPS</sub>, POR<sub>ADJ</sub>, or peripheral (PER). Systemic hypoglycemia (2.4 ± 0.1 mmol/l) was induced via jugular vein insulin infusion (50 mU · kg<sup>-1</sup> · min<sup>-1</sup>). Arterial plasma catecholamines were assessed at basal (-30 and 0 min) and during sustained hypoglycemia (60, 75, 90, 105 min). By design, hepatic glucose was significantly elevated during POR<sub>UPS</sub> and POR<sub>ADJ</sub> versus PER (4.3 ± 0.1 vs. 2.4 ± 0.1 mmol/l, respectively; *P* < 0.05). There were no significant differences between protocols in arterial glucose or insulin concentrations (9,372 ± 1,798 pmol/l). When liver and systemic glucose concentrations were allowed to fall concomitantly (PER), epinephrine was elevated 16-fold above basal levels (3.0 ± 0.6 vs. 46.4 ± 4.3 nmol/l, *P* < 0.001). When portohepatic normoglycemia was maintained during POR<sub>UPS</sub>, a 67% suppression in the epinephrine response versus that during PER was observed (*P* < 0.001). However, when the cannula was advanced adjacent to the liver, by comparison with PER, there was no suppression in the sympathoadrenal response (*P* = 0.73). While both POR<sub>UPS</sub> and POR<sub>ADJ</sub> yielded elevated liver glycemia in the face of systemic hypoglycemia, only POR<sub>UPS</sub> yielded an elevated portal vein glucose concentration. That only POR<sub>UPS</sub> resulted in a significant suppression of the sympathoadrenal response is consistent with the localization of the glucosensors to the portal vein. *Diabetes* 46:1521-1525, 1997

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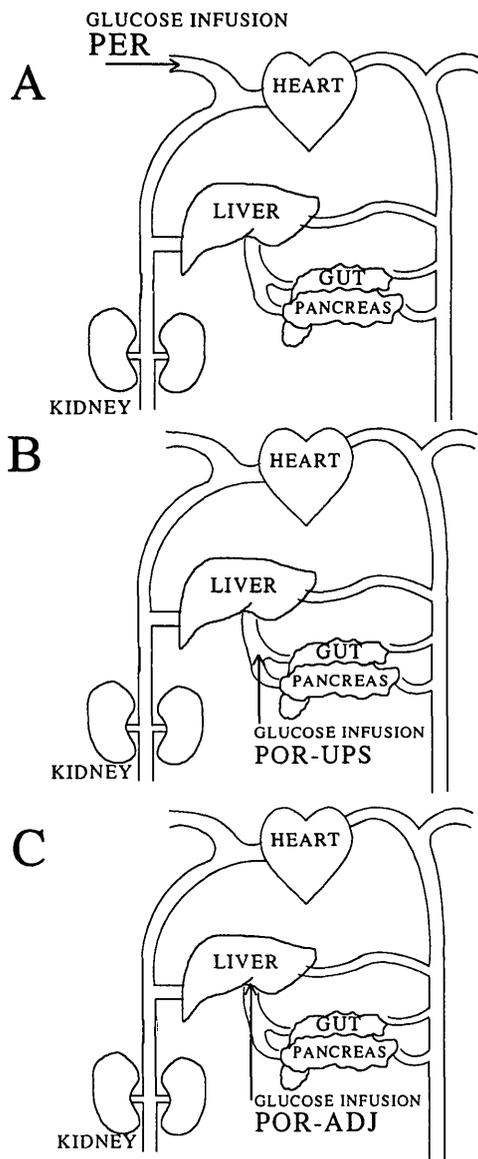
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ALT, alanine aminotransferase; ANOVA, analysis of variance; ID, internal diameter; PER, peripheral infusion; POR<sub>ADJ</sub>, portal infusion adjacent to the liver; POR<sub>UPS</sub>, portal infusion upstream from the liver; RIA, radioimmunoassay.

**H**ypoglycemia currently constitutes a significant limitation in the treatment of IDDM. This arises from the current attempts to more aggressively control blood glucose levels in diabetic patients. While the efficacy of such treatment in ameliorating long-term complications has been demonstrated (1), individuals undergoing such therapy are exposed to a threefold greater risk for severe hypoglycemic episodes (2). The susceptibility of IDDM patients to hypoglycemia can in part be attributed to their defective counterregulatory hormonal response, which fails to stimulate adequate glucose production (3,4). This defect has been shown to be specific for glucose, as the hormonal response to other stimuli is apparently normal in diabetic subjects (4). Such specificity suggests a defect in the afferent limb of glucose counterregulation, i.e., glucose sensing.

Efforts to elucidate the nature of this defect have in part been constrained by our lack of understanding regarding the locus for glycemic detection. Traditionally attributed to the central nervous system, glucose sensing by the portohepatic region is now known to play an important role in modulating the response to hypoglycemia. That glucosensors reside in the splanchnic region has been known for some time (5-10), and the requisite neural circuitry (i.e., afferent and efferent limbs) for a portohepatic-sympathetic loop has been identified (8,9). Our own work suggests that the portohepatic glucosensors are important mediators of the sympathetic response to hypoglycemia (10,11). Further, under conditions of progressive hypoglycemia, as is seen in many clinical situations, these portohepatic glucosensors may be the most important regulators of this sympathetic response (11).

The portohepatic glucosensors have generally been presumed to reside within the liver (5,7,9,10,12). Afferent nerve terminals have been located in the proximity of the hepatocytes (12,13), and efferent innervation of the liver has been well established (9,12). However, fine varicose terminals of the hepatic vagus nerve have been observed in the adventitia of the portal vein (14-16), raising the possibility of a portal locus for the glucosensors. To ascertain whether those glucosensors essential to modulation of the sympathetic response in hypoglycemia reside in the portal vein, as opposed to the liver, we used a variation of the local glucose irrigation technique in which liver glycemia is normalized in the presence of general systemic hypoglycemia (10,11). In this variation, placement of the portal vein cannula was adjusted so as to normalize either liver glycemia alone or both portal vein and liver glycemia. Results indicated that portal vein, not liver, glucose concentration determines the sym-



**FIG. 1.** Schematic diagram of the three experimental conditions distinguished by the site of glucose infusion: peripheral glucose infusion via the jugular vein, portal vein glucose infusion upstream from the liver, and portal vein glucose infusion adjacent to the liver. **A:** peripheral glucose infusion (PER) was considered the control condition, in which whole-body hypoglycemia was induced. **B:** glucose infusion via a portal cannula positioned upstream from the liver (POR<sub>UPS</sub>) allowed for the normalization of liver and portal vein glycemia during systemic hypoglycemia. **C:** glucose infusion via a portal cannula placed adjacent to the liver (POR<sub>ADJ</sub>) provided normalization of liver glycemia only. Because of the cannula position, portal vein glucose concentration was assumed equal to the arterial glucose concentration ( $\sim 2.4$  mmol/l) in POR<sub>ADJ</sub>.

pathoadrenal response, a finding consistent with a portal vein glucosensor locus.

## RESEARCH DESIGN AND METHODS

**Animals and surgical procedures.** Experiments were conducted on male Wistar rats (weight  $284 \pm 10$  g,  $n = 22$ ) 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, animals were chronically cannulated under single-dose anesthesia (3:3:1 ketamine HCl, xylazine, acepromazine maleate;  $0.10$  cm<sup>3</sup>/100 g body wt, given intramuscularly). Cannulas were placed in the portal vein (silastic, 0.03 cm internal diameter [ID]) for glucose infusion during liver irrigation, in the carotid artery (Clay Adams, PE-50) for arterial blood sampling, and in

the jugular vein (dual cannula silastic, 0.012 cm ID) for peripheral infusion (PER) of insulin and glucose. The portal vein cannula was placed either adjacent to (POR<sub>ADJ</sub>,  $0.6 \pm 0.1$  cm) or upstream from (POR<sub>UPS</sub>,  $2.7 \pm 0.1$  cm) the liver. All cannulas were tunneled subcutaneously and exteriorized at the back of the neck and encased in silastic tubing (0.18 cm ID) sutured to the skin.

**Study design.** Animals were allowed 5 days' recovery from surgery to regain body weight. Three days after surgery, 300  $\mu$ l of blood was drawn and analyzed for plasma alanine aminotransferase (ALT) activity, a sensitive measure of hepatocellular integrity. There were no significant differences in ALT activity between animals portally cannulated (PER, POR<sub>ADJ</sub>, POR<sub>UPS</sub>) and those littermates randomly assigned to the blood donor group with only a carotid cannula ( $18.9 \pm 3.0$  vs.  $16.5 \pm 2.4$  U/l, respectively). Twenty-four hours before the experiment, all food was removed from the cage.

**Hyperinsulinemic-hypoglycemic clamp.** All animals were exposed to the same general protocol for the induction of hypoglycemia. Before the experiment, animals were placed in a modified metabolic chamber and allowed to rest for 30 min ( $-60$  to  $-30$  min). Basal samples were drawn at  $-30$  and 0 min for analysis of glucose and catecholamines. At 0 min, after arterial sampling, insulin ( $50$  mU  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) and glucose infusions (variable) were initiated and maintained for 105 min of the hypoglycemic clamp. Serial sampling for glucose was performed at 10-min intervals until deep hypoglycemia was attained at 60 min, so as to maintain the integrity of the hypoglycemic clamp and control the rate of fall in blood glucose. Deep hypoglycemia ( $\sim 2.5$  mmol/l) was achieved by 60 min and was sustained for the remaining 45 min of the experiment (60–105 min). Arterial catecholamine and glucose samples were taken at 60, 75, 90, and 105 min of deep hypoglycemia.

**Experimental design.** Each animal participated in one of three specific experimental conditions distinguished by the site of glucose infusion (Fig. 1). Figure 1A represents peripheral glucose infusion (PER) via the jugular vein. This protocol effectively served as the experimental control group in which whole-body hypoglycemia was induced. Figure 1B represents the portal vein upstream (POR<sub>UPS</sub>) experimental condition whereby portal and liver glucose concentrations were normalized during systemic hypoglycemia. Figure 1C represents glucose infusion via the portal vein adjacent to the liver (POR<sub>ADJ</sub>) whereby the cannula was advanced to the liver, thus normalizing only the liver glucose concentrations while the portal vein and periphery remained hypoglycemic. According to the working hypothesis, if the sensors reside exclusively in the portal vein, then portal vein concentrations will dictate the sympathoadrenal response irrespective of liver glycemia.

**Calculations.** The estimated liver glucose concentration ( $G_L$ ) was calculated as  $G_L = G_A + (\text{GINF}_{\text{POR}}/\text{HPF})$ , where  $G_A$  is the arterial glucose concentration (in micromoles per milliliter),  $\text{GINF}_{\text{POR}}$  is the portal glucose infusion rate (in micromoles per minute), and  $\text{HPF}$  is the hepatic plasma flow rate (in milliliters per minute). The estimated portal vein glucose concentration ( $G_{\text{PV}}$ ) was calculated as  $G_{\text{PV}} = G_A + (\text{GINF}_{\text{PORUPS}}/\text{PVPF})$ , where  $\text{GINF}_{\text{PORUPS}}$  is the upstream portal glucose infusion rate (in micromoles per minute) and  $\text{PVPF}$  is the portal vein plasma flow rate (in milliliters per minute). Hepatic plasma flow was estimated to be  $1.3$  ml  $\cdot$  g<sup>-1</sup> liver  $\cdot$  min<sup>-1</sup>  $\cdot$  liver wt (g), and the portal vein flow rate was assumed to be 80% of the hepatic plasma flow rate (17,18). Because experiments were preceded by a 24-h fast, portal vein and hepatic glycemia were assumed equal to the arterial glucose concentration in the absence of any portal glucose infusion. Due to cannula placement during POR<sub>ADJ</sub> (advanced to the liver), portal glycemia was assumed to be equal to arterial glucose concentration.

**Analytical procedures.** Glucose was assayed by the glucose oxidase method (YSI, Yellow Springs, OH). Epinephrine and norepinephrine concentrations were assayed using a single-isotope radioenzymatic approach (19). Basal insulin samples were assayed via a radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO), and steady-state hyperinsulinemic samples were assayed using an RIA according to Herbert et al. (20). ALT was assayed spectrophotometrically (21).

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

## RESULTS

For all experimental protocols, arterial insulin concentrations increased from a mean basal value of  $61 \pm 6$  pmol/l to a hyperinsulinemic plateau of  $9,372 \pm 1,798$  pmol/l. No significant differences were observed between protocols with respect to basal or elevated insulin concentrations. Basal arterial glucose concentrations ( $7.2 \pm 0.3$  mmol/l) were not significantly different between protocols. By design, there were no significant differences in arterial glucose concentrations during deep sustained hypoglycemia ( $2.4 \pm 0.1$

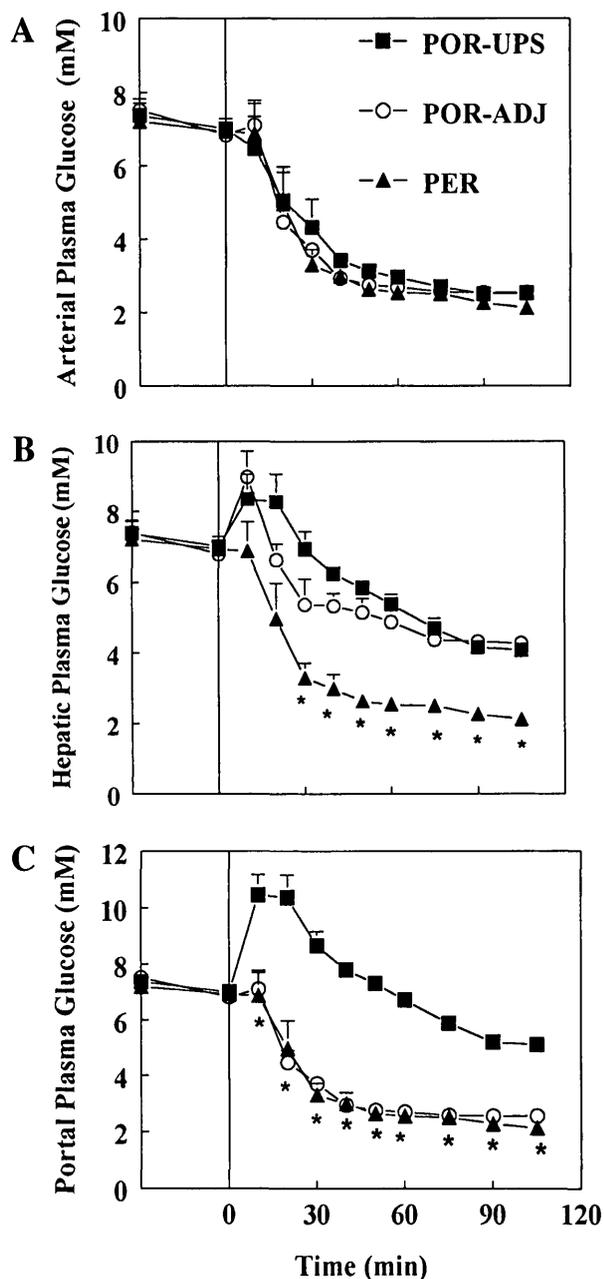


FIG. 2. Data are expressed as means  $\pm$  SE for arterial glucose concentration (A), estimated hepatic glucose concentration (B), and estimated portal vein glucose concentration (C) at basal and during the hyperinsulinemic-hypoglycemic clamp.  $\blacktriangle$ , PER experiment;  $\blacksquare$ , POR<sub>UPS</sub> experiment;  $\circ$ , POR<sub>ADJ</sub> experiment. \*Significance between infusion protocols ( $P < 0.05$ ).

mmol/l;  $P = 0.80$ ) among the three glucose infusion protocols. Hepatic glycemia was also allowed to decrease to a deep hypoglycemic level of  $2.3 \pm 0.2$  mmol/l during PER (Fig. 2). In contrast, hepatic glycemia remained elevated in the normal range during both liver irrigation protocols, POR<sub>ADJ</sub> and POR<sub>UPS</sub> (Fig. 2). Between 60 and 105 min, estimated liver glycemia for POR<sub>UPS</sub> and POR<sub>ADJ</sub> were  $4.4 \pm 0.3$  mmol/l. Estimated hepatic glucose concentrations were not significantly different between POR<sub>ADJ</sub> and POR<sub>UPS</sub> ( $P > 0.05$ ). Under both PER and POR<sub>ADJ</sub>, the portal vein was allowed to become hypoglycemic ( $2.4 \pm 0.2$  mmol/l). Only during POR<sub>UPS</sub> was the portal vein maintained normoglycemic, averaging  $5.5 \pm 0.3$

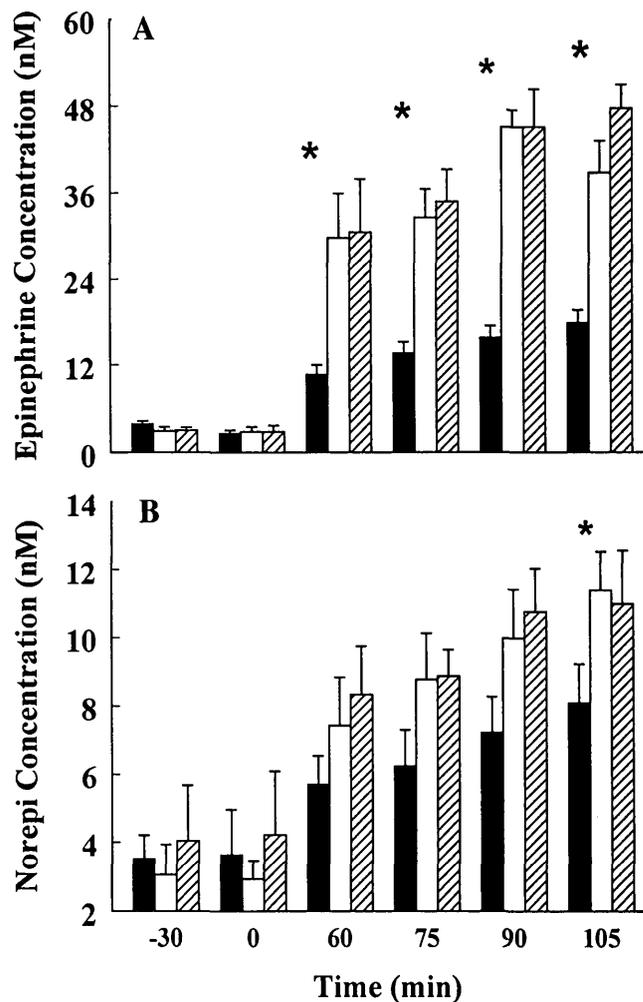


FIG. 3. Epinephrine (A) and norepinephrine (B) concentrations at basal and during sustained deep hypoglycemia, expressed as mean values  $\pm$  SE.  $\blacksquare$ , POR<sub>UPS</sub> experiment;  $\square$ , POR<sub>ADJ</sub> experiment;  $\hatched$ , PER experiment. \*Significance between POR<sub>UPS</sub> and other infusion protocols ( $P < 0.05$ ).

mmol/l between 60 and 105 min of the hypoglycemic clamp.

In response to whole-body hypoglycemia (PER), arterial epinephrine concentrations increased 16-fold, from basal  $3.0 \pm 0.6$  nmol/l to a mean of  $46.4 \pm 4.3$  nmol/l by 105 min (Fig. 3). Infusing glucose into the liver only, reestablishing hepatic euglycemia had no effect on the maximal epinephrine response ( $42.0 \pm 3.4$  nmol/l;  $P = 0.73$ ). However, during POR<sub>UPS</sub>, when both the portal vein and liver were maintained normoglycemic, there was a 67% suppression in the epinephrine response when compared with either PER or POR<sub>ADJ</sub> ( $P < 0.001$  for both).

Although the norepinephrine response was less dramatic than the epinephrine response, 2.7- vs. 16-fold above basal for PER, a similar pattern was observed between protocols. In response to whole-body hypoglycemia (PER), norepinephrine increased from  $4.0 \pm 1.8$  to  $10.9 \pm 1.4$  nmol/l (Fig. 3). During POR<sub>ADJ</sub>, norepinephrine levels increased to  $10.3 \pm 1.3$  nmol/l, not significantly different from those during PER ( $P = 0.84$ ). By comparison, there was a significant suppression in the norepinephrine concentration for POR<sub>UPS</sub> ( $7.6 \pm 1.0$  nmol/l) when compared with PER and POR<sub>ADJ</sub> during the final sampling interval ( $P = 0.035$ ).

## DISCUSSION

The current findings demonstrate that the magnitude of the sympathoadrenal response to hypoglycemia is dictated by the blood glucose concentration present in the portal vein, not by that in the liver. Clamping arterial blood glucose concentrations ensured that all tissues, except the portohepatis, were exposed to identical levels of glucose during the three experimental protocols: PER,  $POR_{UPS}$ , and  $POR_{ADJ}$ . When the liver and portal vein were allowed to become hypoglycemic along with the rest of the body, we observed a full-blown sympathetic response that included a 16-fold increase in epinephrine and ~3-fold increase in norepinephrine. A similar full-blown sympathetic response was observed when liver glycemia, but not portal glycemia, was normalized ( $POR_{ADJ}$ ). In sharp contrast, a significant suppression of the sympathoadrenal response to hypoglycemia (67% for epinephrine and 50% for norepinephrine) was observed when the blood glucose concentrations for both the portal vein and the liver were normalized ( $POR_{UPS}$ ). Because the only difference between  $POR_{UPS}$  and  $POR_{ADJ}$  was the concentration of glucose in the portal vein, our findings demonstrate a portal vein locus for those glucosensors that mediate the sympathetic response to hypoglycemia.

There is substantial neurophysiological data supporting a portal vein locus for the glucosensors associated with glucose regulation. The portohepatic region is known to be innervated by afferent fibers sensitive to changes in metabolite concentrations, pressure, and osmolarity (8,9,22). Using fluorescent staining techniques, it has been determined that hepatic vagal afferents, believed to be associated with these portal glucosensors, terminate predominantly in the portal vein (16). Glucose-sensitive afferents, localized to the portal region, have been found to possess firing rates inversely proportional to the portal vein glucose concentration (5,7). Glucose injections into the portal vein have also been shown to influence neuronal activity in both the ventromedial and the lateral hypothalamus (6,23). Shimizu et al. (23) further demonstrated that the majority of identifiable glucose-sensitive neurons within the lateral hypothalamus were responsive to changes in portal blood glucose concentration. Similar observations have been forthcoming for the nuclear tractus solitarius, where the glucose-sensitive neurons were shown to be sensitive to elevations in portal glycemia (24). That these portal glucose-sensitive afferents are part of a portal-sympathetic glucoregulatory reflex has been proposed by several investigators (9,22,24). Sympathetic output from the ventromedial hypothalamus and its influence on glucose metabolism has been well characterized (9). Additionally, the discharge rate from the adrenal and splanchnic efferents has been shown to be depressed by elevations in portal glucose concentration (7). The current findings provide in vivo evidence in support of a functional portal-sympathetic glucoregulatory reflex.

While our previous reports have focused on the importance of the hepatic glucosensors, those findings are in fact consistent with current observations. In those previous studies on dogs, portal cannulation was effected upstream from the liver. As such, both portal and liver glycemia were normalized during portal glucose infusions. While we had presumed the glucosensors to reside in the liver, our protocols did not exclude the possibility of a portal vein locus. That is, our previous protocols had only restricted critical glucosensors to the

portohepatic region. Given that our current study was conducted on a different species, rat versus dog, the findings are surprisingly consistent with our previous observations and may be viewed as general. The magnitudes of the epinephrine (16-fold increase) and norepinephrine (3-fold increase) responses under uniform deep hypoglycemia (PER) were comparable to those observed for dogs under similar conditions (11,25). Also, the degree of suppression in the sympathetic response to hypoglycemia achieved with portal normoglycemia (~50–70%) appears comparable for both species (11,25).

In summary, the current study demonstrates a portal vein, not a liver, locus for those splanchnic glucosensors critical to modulating the sympathoadrenal response to hypoglycemia. While these observations do not exclude other potential glucosensing loci (e.g., the brain), they do suggest a predominant role for the portal glucosensors in controlling sympathetic output. In this respect, our current findings in rats are quantitatively consistent with our previous findings in dogs. That similar observations have been forthcoming for two different mammalian species greatly increases the probability that portal glucosensors are critical for glucose regulation in other species, including humans.

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## REFERENCES

1. The DCCT Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. The DCCT Research Group: Epidemiology of severe hypoglycemia in the Diabetes Control and Complications Trial. *Am J Med* 90:450–459, 1991
3. Cryer PE, Binder C, Bolli GB, Cherrington AD, Gale EAM, Gerich JE, Sherwin RS: Hypoglycemia in IDDM: conference summary. *Diabetes* 38:1193–1199, 1989
4. Gerich JE, Campbell PE: Overview of counterregulation and its abnormalities in diabetes mellitus. *Diabetes Metab Rev* 4:93–111, 1988
5. Nijijima A: Afferent impulse discharges from glucoreceptors in the liver of the guinea pig. *Ann NY Acad Sci* 157:690–700, 1969
6. Schmitt M: Influence of hepatic portal receptors on hypothalamic feeding and satiety centers. *Am J Physiol* 225:1089–1095, 1973
7. Nijijima A: Glucose sensitive afferent nerve fibers in the liver and regulation of blood glucose. *Brain Res Bull* 5 (Suppl. 4):175–179, 1980
8. Nijijima A: Visceral afferents and metabolic functions. *Diabetologia* 20:325–330, 1981
9. Shimazu T: Neuronal regulation of hepatic glucose metabolism in mammals. *Diabetes Metab Rev* 3:185–206, 1987
10. Donovan CM, Halter JB, Bergman RN: Importance of hepatic glucoreceptors in sympathoadrenal response to hypoglycemia. *Diabetes* 40:155–158, 1991
11. Donovan CM, Hamilton-Wessler M, Halter JB, Bergman RN: Primacy of liver glucosensors in the sympathetic response to progressive hypoglycemia. *Proc Natl Acad Sci USA* 91:2863–2867, 1994
12. Forssmann WG, Ito S: Hepatocyte innervation in primates. *J Cell Biol* 74:299–313, 1977
13. Carobi C, Della TG, Magni F: Differential distribution of vagal afferent neurons from the rat liver. *Neurosci Lett* 62:255–260, 1985
14. Barja F, Mathison R: Sensory innervation of the rat portal vein and the hepatic artery. *J Auton Nerv Syst* 10:117–125, 1984
15. Berthoud HR, Kressel M, Neuhuber WL: An anterograde tracing study of the vagal innervation of rat liver, portal vein and biliary system. *Anat Embryol* 186:186–431, 1992
16. Berthoud HR, Kressel M, Neuhuber WL: Vagal afferent innervation of rat abdominal paraganglia as revealed by anterograde Dil-tracing and confocal

- microscopy. *Acta Anat* 152:127-132, 1995
17. Ishise S, Pegram BL, Yamamoto J, Kitamura Y, Frolich ED: Reference sample microsphere method: cardiac output and blood flows in conscious rat. *Am J Physiol* 239:H443-H449, 1980
  18. Yasuhito S, Wagner HN Jr: Measurement of the distribution of cardiac output in unanesthetized rats. *J Appl Physiol* 30:879-884, 1971
  19. Pueler JD, Johnson GA: Simultaneous single isotope derivative radioenzymatic assay for plasma norepinephrine, epinephrine, and dopamine. *Life Sciences* 21:625-636, 1977
  20. Herbert V, Law K, Gottlieb C, Bleicher S: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375-1384, 1965
  21. Reitman S, Frankel S: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 28:56-63, 1957
  22. Lutt WW: Afferent and efferent neural roles in liver function. *Prog Neurobiol* 21:323-348, 1983
  23. Shimizu N, Oomura Y, Novin D, Grijalva CV, Cooper PH: Functional correlation between lateral hypothalamic glucose-sensitive neurons and hepatic portal glucose-sensitive units in rats. *Brain Res* 265:49-54, 1985
  24. Nijima A: Glucose sensors in viscera and control of blood glucose level. *News Physiol Sci* 2:164-167, 1987
  25. Hamilton-Wessler M, Bergman RN, Halter JB, Watanabe RM, Donovan CM: The role of liver glucosensors in the integrated sympathetic response induced by deep hypoglycemia in dogs. *Diabetes* 43:1052-1060, 1994