Importance of Hepatic Glucoreceptors in Sympathoadrenal Response to Hypoglycemia

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To ascertain whether hepatic glucoreceptors are important to hypoglycemic counterregulation, a localized euglycemic clamp was employed across the liver during general hypoglycemia. Dogs were infused peripherally with insulin (18–21 pmol · kg^{-1} · min^{-1}) for 150 min to induce systemic hypoglycemia. During the liver-clamp (LC) protocol, glucose was infused via the portal vein to maintain euglycemia at the liver. In control experiments, i.e., matched infusion (MI), glucose was infused peripherally at a rate determined to yield similar arterial glycemia levels in the two protocols. Arterial glucose concentrations were not different between protocols during the final hour of insulin infusion (3.26 ± 0.21 and 3.25 ± 0.21 mM during LC and MI, respectively; P = 0.91). Calculated hepatic glucose concentrations during the same period were significantly higher for LC (5.22 ± 0.23 mM) than for MI (3.25 ± 0.21 mM). During MI, both epinephrine and norepinephrine rose significantly from basal values of 562 ± 87 pM and 1.21 ± 0.19 nM to plateaus of 3691 ± 1097 pM (P = 0.0001) and 2.38 ± 0.35 nM (P = 0.0002), respectively. However, during LC, the elevation in epinephrine was suppressed by 42 ± 8% (P = 0.015) relative to MI. Six of seven animals demonstrated a suppression in the norepinephrine response, averaging 32 ± 13% (NS, P = 0.068). The glucagon response to hypoglycemia was unaffected by the level of hepatic glycemia. Hepatic hypoglycemia is essential to produce the full sympathoadrenal response to insulin-induced hypoglycemia. *Diabetes* 40:155–58, 1991

Hypoglycemia constitutes a major threat to both the function and integrity of the central nervous system (CNS) (1). To defend against hypoglycemia, the body uses a host of counterregulatory measures resulting in an elevation of glucose production and reduction in glucose uptake (2). Principal among these responses are elevations in several hormones (e.g., glucagon, epinephrine, norepinephrine, and cortisol) whose specific functions and relative importance in counterregulation have been well characterized (2). In contrast, relatively little is known regarding the regulation and integration of these counterregulatory responses. In particular, how and where changes in blood glucose concentration are detected remains unknown.

Traditionally, the CNS, specifically the ventromedial hypothalamus (VMH), has been viewed as the residence for the glucoreceptors responsible for detecting fluctuations in blood glucose (3,4). This is based primarily on the well-established efferent role the VMH can effect in glucose mobilization (5,6). However, there is little evidence to suggest that the afferents responsible for detecting hypoglycemia actually reside in the VMH. Attempts in vivo have failed to establish an essential role for glucoreceptors residing in this area of the CNS (7,8). There is accumulating evidence that the loci for the critical glucoreceptors reside distal from the hypothalamus in other areas of the CNS (9,10) and/or the periphery (11,12). Glucoreceptors afferently linked to the hypothalamus have been identified in the portohepatic region (13) and implicated in a hepatoglucoregulatory reflex (12,14). Although previously implicated in feeding behavior (15,16), it has yet to be demonstrated that these portohepatic glucoreceptors serve in a glucoregulatory capacity.

To ascertain whether the putative portohepatic glucoreceptors are important for the hypoglycemic counterregulatory response, we employed a localized euglycemic clamp across the liver (LC). Systemic hypoglycemia was induced via insulin infusion, and the elevations in epinephrine, norepinephrine, and glucagon were assessed in the presence and absence of liver euglycemia.

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RESEARCH DESIGN AND METHODS

Experiments were conducted on male mongrel dogs (weighing 28.5 ± 1.4 kg, n = 7) 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 before initiating experiments, animals were chronically cannulated under anesthesia (0.5–1.0% halothane). Cannulas were placed in the portal vein (Silastic, ID = 0.04 inch) for glucose infusion during LC, the carotid artery (Tygon, ID = 0.05 inch) for arterial blood sampling, and the jugular vein (Tygon, ID = 0.05 inch) for insulin infusion. Additionally, the femoral vein was cannulated (Tygon, ID = 0.05 inch) with the tip of the catheter advanced into the inferior vena cava, rostral to the hepatic vein. An inflatable cuff (model VO-4, Rhodes Medical) was surgically implanted around the inferior vena cava just caudal to the hepatic vein. Inflation of the cuff temporarily occludes flow caudal to the cuff, allowing mixed hepatic venous blood to be sampled from the femoral catheter (17). All cannulas and the actuating tubing for the inflatable cuff were tunneled subcutaneously and exteriorized at the back of the neck.

On the day of the experiments, intracatheters (19 gauge, Deseret, Sandy, UT) were placed in the cephalic vein, as needed, for indocyanine green dye (ICG) and peripheral glucose infusion during the matched systemic infusion (MI). At −120 min, infusion of ICG (0.15 mg/min) was initiated and allowed 90 min to stabilize. A 30-min basal collection period (−30 to 0 min) followed, during which serial blood samples, arterial and hepatic venous, were taken every 10 min for glucose, insulin, and ICG. At min 0, insulin infusion (18–21 pmol · kg^{-1} · min^{-1}) was initiated and maintained for the remaining 150 min of the experimental period. Serial sampling for glucose and insulin continued every 10 min, whereas sampling for ICG was reduced to every 20 min between 0 and 150 min. Beginning at −30 min, additional arterial blood samples were taken every 30 min for analysis of epinephrine, norepinephrine, and glucagon.

During LC, the portal glucose infusion rate was adjusted every 10 min to maintain total mean hepatic (i.e., portal venous and hepatic arterial) glycemia at basal arterial values. The rate of portal glucose infusion required to maintain hepatic euglycemia was calculated as

$$GINF_p = HPF \times (G_b - G_a)$$

where $GINF_p$ is portal glucose infusion (mmol/min), HPF is hepatic plasma flow (L/min) as determined via ICG (17), G_b is basal arterial glucose concentration (mM) measured at −30 to 0 min, and G_a is ambient systemic arterial concentration during insulin infusion (mM). The control MI involved peripheral glucose infusion at a rate calculated to match arterial glycemia in the two conditions. The rate of peripheral glucose infusion during MI was adjusted every 10 min with the formula

$$GINF_s = nHGO_{LC} - HGB_{MI}$$

where $GINF_s$ is peripheral systemic glucose infusion (mmol/min), $nHGO_{LC}$ is net hepatic glucose output during LC (mmol/min), and HGB_{MI} is hepatic glucose balance during MI (mmol/min). Each animal was studied under both protocols, i.e., LC (systemic hypoglycemia with hepatic euglycemia) and MI (systemic and hepatic hypoglycemia). Because the peripheral glucose infusion during MI was established to match the glycemia in LC, protocols could not be randomized, and the LC protocol always preceded the MI protocol by 7–9 days.

During MI, the mean hepatic glucose concentration was assumed equal to the ambient arterial glucose concentration. For LC, the mean hepatic glucose concentration was calculated as

$$G_h = G_a + (GINF_p/HPF)$$

where G_h is mean hepatic glucose (mM).

Glucose was assayed with the glucose oxidase method (YSI, Yellow Springs, OH). ICG concentration was determined spectrophotometrically at 805 nm. Radioimmunoassays were used to determine insulin (18) and glucagon (Novo-Nordisk kit 32, antisera K-5563, Copenhagen). Epinephrine and norepinephrine concentrations were assayed with a single-isotope radioenzymatic approach (19).

Comparisons between protocols were made with a nonparametric analysis of variance.

RESULTS

Insulin infusion, initiated at min 0, led to a rapid increase in plasma insulin followed by a sustained plateau (1147 ± 217 and 1038 ± 135 pM for LC and MI, respectively; Fig. 1). Subsequent to the elevation in insulin, arterial glucose fell from a basal value of 5.22 ± 0.23 mM to a nadir of 3.26 ± 0.21 mM, which was sustained for the remainder of the LC. The efficacy of the MI was such that no significant differences in arterial glucose concentrations were observed between protocols over the course of the experiment. In contrast, calculated hepatic glucose concentrations (3.25 ± 0.21 and 5.28 ± 0.22 mM for MI and LC, respectively) were markedly different over the same period. Hepatic venous glucose levels were significantly elevated between 30 and 110 min of LC compared with values for MI (Fig. 1). However, hepatic venous values during LC were lower than the calculated hepatic glucose concentrations, with an average arteriovenous difference of 1.22 ± 0.25 mM between 90 and 150 min. Thus, despite systemic hypoglycemia, we observed apparent hepatic glucose uptake during LC.

General systemic hypoglycemia led to a significant elevation in catecholamine concentrations, which peaked at 60–90 min and were sustained for the remainder of the experiment. Epinephrine rose from a basal value of 562 ± 87 pM to a plateau of 3691 ± 1097 pM between 90 and 150 min during MI. A similar response was observed for norepinephrine, in which values rose from a basal value of 1.21 ± 0.19 nM to a plateau of 2.38 ± 0.35 nM. Clamping the liver at euglycemia during systemic hypoglycemia resulted in a suppression of the epinephrine response for all seven animals compared with the MI (Fig. 2). This suppression averaged 42 ± 8% (P = 0.015). Six of the seven animals demonstrated a similar response for norepinephrine, i.e., a 41 ± 10% suppression during LC. However, because one animal demonstrated suppression of epinephrine but not of norepinephrine in response to LC, the mean suppression for

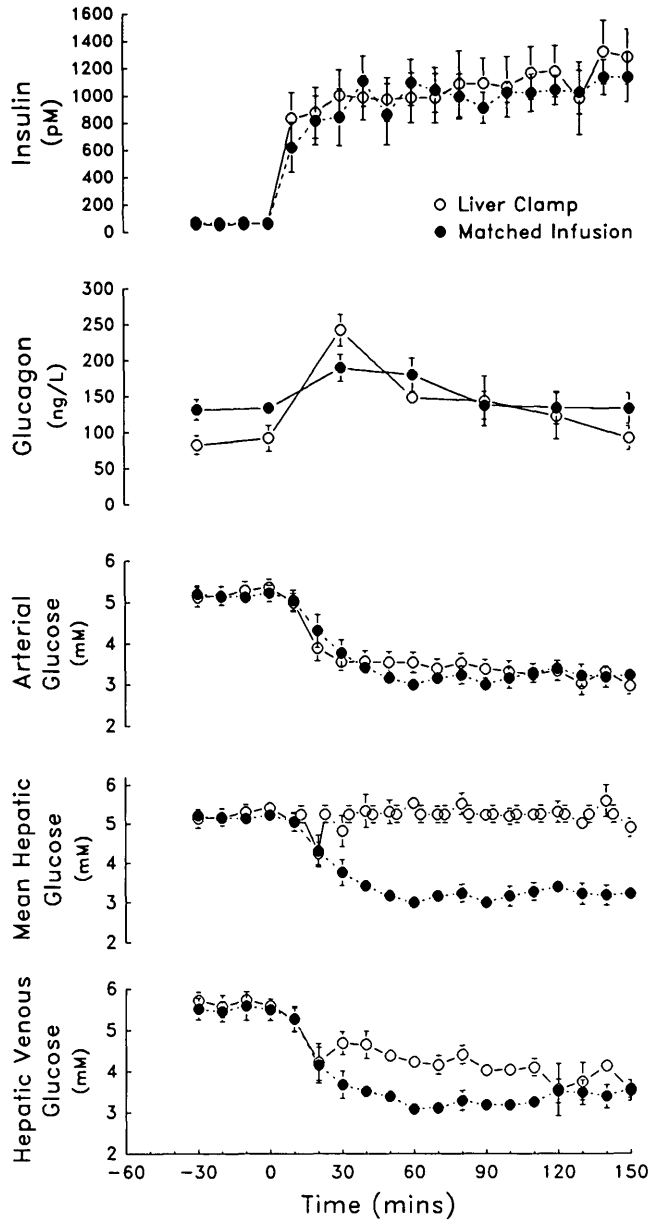

FIG. 1. Plasma insulin, glucagon, systemic (arterial) glucose, calculated mean hepatic (portal venous and hepatic arterial) glucose, and hepatic venous glucose as function of time during constant insulin infusion (mean ± SE). Insulin (pork) infusion was initiated at 0 min and maintained for 150 min. Arterial blood samples were taken every 10 min, and portal glucose infusion ($GINF_p$) was adjusted within 3 min to return hepatic glucose concentration to basal. Adjustment in $GINF_p$ at 3 min after sampling is reflected in calculated hepatic glucose values for liver clamp. Significant differences between liver clamp and matched infusion ($P < 0.05$) were observed for hepatic glucose at 30–150 min, hepatic venous glucose at 30–110 min, and glucagon at 0 min.

norepinephrine in all animals was 32 ± 13% (NS at $P < 0.05$, $P = 0.068$).

LC failed to significantly impact on either peak glucagon values (246 ± 22 and 214 ± 24 ng/L for LC and MI, respectively) or glucagon values observed at any time point during hypoglycemia (Fig. 1).

DISCUSSION

Hepatic hypoglycemia is essential to elicit the full sympathoadrenal response to hypoglycemia. When systemic arterial hypoglycemia was matched at 3.25 mM, establishing euglycemia across the liver suppressed the rise in epinephrine levels by >40% (Fig. 2). A similar mean degree of suppression was observed for norepinephrine in six of seven animals (Fig. 2). Despite the discrepant response for one animal, epinephrine and norepinephrine values during hypoglycemia for both MI and LC were highly correlated ($r = 0.97$). The tight coupling of epinephrine and norepinephrine responses is indicative of a common hepatosympathoadrenal reflex in hypoglycemia. This may reflect a common source for catecholamines in hypoglycemia (i.e., the adrenal medulla) or a coordinated sympathetic activation of the adrenals and postganglionic neurons.

Our findings support and extend the previously described hepatosympathetic reflex hypothesis (12,14). In this concept, hepatic glucoreceptors are viewed as providing input concerning visceral glucose levels to the CNS, where the information is integrated and the appropriate efferent response initiated. This hypothesis was based primarily on the following observations: *1*) identification of glucose-responsive hepatic vagal afferents, characterized by firing rates inversely proportional to the portal glucose concentration (13); *2*) evidence for functional links between the hepatic glucose-responsive afferents and glucose-sensitive neurons of the nuclear tractus solitarius (12) and the lateral hypothalamus (20); and *3*) the extensively characterized efferent links between the hypothalamus and the liver, pancreas, and adrenal medulla (5,6). However, data directly implicating this putative reflex in a physiological response were lacking. We now provide in vivo evidence for a functional link between the portohepatic glucoreceptors and a recognized counterregulatory response to hypoglycemia.

In contrast to the elevation in catecholamines, the glucagon response tended to be transient, peaking between

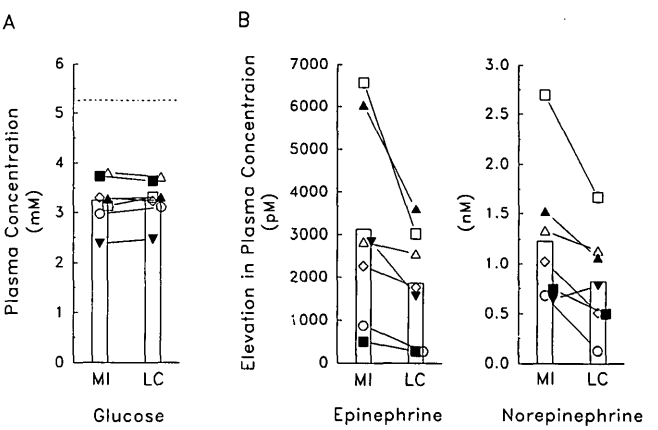

FIG. 2. *A*: average plasma glucose concentrations during final 60 min (90–150 min) of liver clamp (LC) and matched systemic infusion (MI). Mean values were 3.26 ± 0.21 and 3.25 ± 0.21 mM for LC and MI, respectively. *Dashed line*, mean basal arterial glucose concentration before insulin infusion; *bars*, mean values between 90 and 150 min. No significant differences were observed between conditions. *B*: average elevation above basal for plasma epinephrine and norepinephrine concentrations for min 90, 120, and 150 of LC and MI. *Bars*, mean values between 90 and 150 min. Epinephrine values for LC were significantly lower than for MI ($P = 0.015$). Norepinephrine values for LC were not significantly lower than for MI at $P < 0.05$ ($P = 0.068$). Basal values for epinephrine (562 ± 87 and 579 ± 71 pM for MI and LC, respectively) and norepinephrine (1.21 ± 0.19 and 1.22 ± 0.13 nM) were not significantly different. *Symbols* represent different dogs.

30 and 90 min and receding thereafter toward basal levels. The transient rise in glucagon is a consistent observation and suggests a response to the initial rapid drop in glycemia (7,8). Therefore, the current design, in which hepatic glycemia was adjusted every 10 min and only after an initial drop in blood glucose was observed (Fig. 1), may have precluded resolving any differences between experimental conditions. Alternatively, the glucagon response to moderate hypoglycemia may be either nonneurally mediated (21–23) or dissociated from the neural control of the catecholamine response (14).

The observation that the liver demonstrated apparent net glucose uptake with portal glucose infusion during LC, despite general systemic hypoglycemia, is surprising because glucagon, epinephrine, and norepinephrine were all significantly elevated. Because the liver is directly innervated by efferents, this may reflect an overriding influence of the purported hepatohepatic reflex (13,14). Alternatively, glucose infusion into the portal vein during LC resulted in a large portal-arterial gradient in the liver, and this gradient has been implicated in hepatic glucose uptake (24). The influence of the portal-arterial gradient may in fact be a manifestation of the hepatohepatic reflex (25).

Current results demonstrate a significant role for the portohepatic glucoreceptors in modulating the sympathoadrenal response to hypoglycemia. The quantitative contribution of these glucoreceptors relative to other regulatory mechanisms (e.g., other essential glucoreceptors, autoregulation) remains to be elucidated. Our design may have led to an underestimation of their relative importance. Prior episodes of hypoglycemia have been reported to suppress subsequent counterregulatory responses; therefore, the order of protocols (i.e., LC before MI) would have tended to minimize the impact of the LC. Furthermore, the role for catecholamines in counterregulation may not be fully manifest until "deep" hypoglycemia is achieved (2). Only a modest level of hypoglycemia (3.25 mM) was achieved in our study. As such, the hepatic glucoreceptors may be of even greater importance in mediating the sympathoadrenal response to hypoglycemia than currently reported.

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