Portal vein hypoglycemia is essential for full induction of hypoglycemia-associated autonomic failure with slow-onset hypoglycemia

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Matveyenko AV, Bohland MA, Saberi M, Donovan CM. Portal vein hypoglycemia is essential for full induction of hypoglycemia-associated autonomic failure with slow-onset hypoglycemia. Am J Physiol Endocrinol Metab 293: E857–E864, 2007. First published July 17, 2007; doi:10.1152/ajpendo.00283.2007.—Antecedent hypoglycemia leads to impaired counterregulation and hypoglycemic unawareness. To ascertain whether antecedent portal vein hypoglycemia impairs portal vein glucose sensing, thereby inducing counterregulatory failure, we compared the effects of antecedent hypoglycemia, with and without normalization of portal vein glycemia, upon the counterregulatory response to subsequent hypoglycemia. Male Wistar rats were chronically cannulated in the carotid artery (sampling), jugular vein (glucose and insulin infusion), and mesenteric vein (glucose infusion). On day 1, the following three distinct antecedent protocols were employed: 1) HYPO-HYPO: systemic hypoglycemia (2.52 ± 0.11 mM); 2) HYPO-EUG: systemic hypoglycemia (2.70 ± 0.36 mM) with normalization of portal vein glycemia (portal vein glucose = 5.86 ± 0.10 mM); and 3) EUG-EUG: systemic euglycemia (6.33 ± 0.31 mM). On day 2, all groups underwent a hyperinsulinemic-hypoglycemic clamp in which the fall in glycemia was controlled so as to reach the nadir (2.34 ± 0.04 mM) by minute 75. Counterregulatory hormone responses were measured at basal (−30 and 0) and during hypoglycemia (60–105 min). Compared with EUG-EUG, antecedent hypoglycemia (HYPO-HYPO) significantly blunted the peak epinephrine (10.44 ± 1.35 vs. 15.75 ± 1.33 nM; P = 0.01) and glucagon (341 ± 16 vs. 597 ± 82 pg/ml; P = 0.03) responses to next-day hypoglycemia. Normalization of portal glycemia during systemic hypoglycemia on day 1 (HYPO-EUG) prevented blunting of the peak epinephrine (15.59 ± 1.43 vs. 15.75 ± 1.33 nM; P = 0.94) and glucagon (523 ± 169 vs. 597 ± 82 pg/ml; P = 0.66) responses to day 2 hypoglycemia. Consistent with hormonal responses, the glucose infusion rate during day 2 hypoglycemia was substantially elevated in HYPO-HYPO (74 ± 12 vs. 49 ± 4 μmol·kg−1·min−1; P = 0.03) but not HYPO-EUG (39 ± 7 vs. 49 ± 4 μmol·kg−1·min−1; P = 0.36). Antecedent hypoglycemia local to the portal vein is required for the full induction of hypoglycemia-associated counterregulatory failure with slow-onset hypoglycemia.

glucose sensor; sympathoadrenal; counterregulation

INTENSIVE INSULIN REPLACEMENT therapy has proven to be an effective tool in the reduction of mean plasma glycemia and subsequent prevention of microvascular and macrovascular complications in patients with type 1 diabetes (T1DM) and late-stage type 2 diabetes (14, 34). However, intensive glucose management is associated with increased frequency of iatrogenic hypoglycemia (14), which results in increased morbidity and has been described as the major obstacle in the management of the disease (6). In diabetes, hypoglycemia results from imperfect insulin replacement combined with an impaired sympathoadrenal response, absent glucagon secretion, and hypoglycemia unawareness (10). The defective counterregulatory response and hypoglycemia unawareness are a direct consequence of the exposure to hypoglycemia, a syndrome termed hypoglycemia-associated autonomic failure (HAAF). Exposure to antecedent hypoglycemia significantly reduces the counterregulatory response to next-day hypoglycemia in healthy (22) and diabetic (39) humans as well as laboratory animals (38). Furthermore, T1DM patients that are able to carefully avoid exposure to hypoglycemia restore their hypoglycemic counterregulatory response and hypoglycemic awareness in as little as two weeks (19). Although the exact mechanism responsible of HAAF is still unknown, there is increased recognition that hypoglycemia-associated counterregulatory failure is a direct consequence of a defective hypoglycemic sensing. There is growing evidence that portal vein glucose sensing is essential for initiating the counterregulatory response to hypoglycemia and consequent hypoglycemia awareness, especially during gradual falls in glycemia (23, 24, 46). This view is supported by studies showing that normalizing portal vein glycemia (23) or inducing portal vein hyperlactatemia (31) suppresses the sympathoadrenal response to systemic hypoglycemia. Furthermore, ablation of portal vein afferents, either via total surgical hepatic denervation (29) or by the topical application of phenol (24), resulted in a blunting of the sympathoadrenal response to hypoglycemia comparable to that achieved via portal glucose infusion. More recently, we reported that sectioning the celiac ganglion through which spinal afferents innervating the portahepatis pass results in a similar suppression of the sympathoadrenal response to hypoglycemia (21). In the current study, we sought to ascertain whether antecedent hypoglycemia impairs glucose sensing at the portal vein, thereby contributing to the induction of HAAF. Specifically, we tested whether selectively normalizing portal vein glycemia during an antecedent bout of hypoglycemia would preserve the counterregulatory response to a subsequent bout of slowly developing hypoglycemia.

RESEARCH DESIGN AND METHODS

Animal care and surgical procedure. Male Wistar rats (weight 258 ± 3 g, n = 21) in the conscious relaxed state were used in all experimental procedures. All animals were housed in individual cages, fed ad libitum, and subjected to a standard 12:12-h light-dark
cycle. The University of Southern California Institutional Animal Care and Use Committee approved surgical and experimental procedures. Before the study (1 wk), rats were anesthetized (3:1 ketamine hydrochloride-xylazine-acepromizine maleate; 0.1 ml/0.1 kg body wt im), and indwelling catheters were inserted in the right internal jugular vein, left carotid artery, and the mesenteric vein as previously described in detail (23). Catheters were placed in the superior mesenteric vein [Silastic tubing, 0.051-cm (ID) cannula with 0.03-cm (ID) tip] for glucose infusion in the portal vein, in the carotid artery [polyethylene tubing, 0.058 cm (ID)] for arterial blood sampling, and in the jugular vein [dual Silastic cannula, 0.03-cm (ID) tip] for insulin, glucose, and saline infusions. Catheter placement was confirmed at the autopsy. All cannulas were tunneled subcutaneously, exteriorized on the back of the neck, and incased in an infusion harness (Instech). Catheters were flushed daily with 100 U/ml heparin/saline solution except on the day of clamp experiments when catheters were flushed with saline only to avoid heparin “spill over” in systemic circulation. All subjects were allowed at least 1 wk to recover from surgical procedures to regain their original body weight, and all maintained normal hematocrit (>40%). Animals were fasted overnight before day 1 and day 2 experimental procedures.

Study design (day 1). Animals were randomly assigned in one of the three experimental protocols (HYPO-HYPO, HYPO-EUG, and EUG-EUG) based on day 1 systemic and portal vein glycemia. Schematic representation of all three protocols is shown in Fig. 1. Animals in the first protocol (HYPO-HYPO, n = 6) were exposed to a single bout (0–75 min) of a hyperinsulinemic-hypoglycemic clamp induced via constant jugular vein insulin (Novolin; Novo Nordisk, Princeton, NJ) infusion (25 mU·kg⁻¹·min⁻¹) and variable 50% dextrose infusions. By design, deep systemic hypoglycemia (~2.5 mmol/l) was achieved by minute 45 and maintained until the end of the insulin infusion at minute 75. Immediately thereafter exogenous dextrose infusion rates were increased to reestablished euglycemia. Arterial plasma glucose levels were measured every 15–20 min to maintain the integrity of the clamp. Animals in the second protocol (HYPO-EUG, n = 5) were exposed to an identical 75-min bout of a hyperinsulinemic-hypoglycemic clamp except exogenous dextrose was delivered in the superior mesenteric vein to selectively normalize local portal vein glycemia in the midst of the systemic hypoglycemia. Control animals (EUG-EUG) underwent either a hyperinsulinemic-euglycemic clamp (n = 6) induced by jugular vein insulin infusion (25 mU·kg⁻¹·min⁻¹) and variable 50% dextrose infusions or received comparable infusions of saline (n = 4). Because, day 1 and 2 glucose and catecholamine levels in euglycemic clamp or saline-infused animals were virtually identical, we combined them into one control group (EUG-EUG) to increase the statistical power of our analysis. At the end of the day 1 procedure, all rats were allowed to rest and had free access to food and water.

Study design (day 2). On the 2nd day, all animals were exposed to identical hyperinsulinemic-hypoglycemic clamps, and plasma catecholamines were assayed at basal and during hypoglycemia. Preceding the experiment, rats were placed in a metabolic chamber and allowed to rest for at least 60 min (~90 to ~30 min). At minute 0, whole body hypoglycemia was induced via jugular vein insulin infusion (25 mU·kg⁻¹·min⁻¹) and variable exogenous dextrose infusions (50% dextrose). By design, deep systemic hypoglycemia (2.34 ± 0.04 mmol/l) was achieved by minute 75 and maintained until the end of the insulin infusion. Arterial plasma catecholamines were assayed at basal (~30 and 0 min) and during sustained hypoglycemia (60, 75, 90, and 105 min). Basal arterial glucose was measured at ~30 and 0 min, and subsequent serial sampling for glucose was performed at 10-min intervals throughout the duration of the clamp. Additional...
arterial plasma samples were drawn during the basal period (30 min) and deep hypoglycemia (105 min) for the measurement of insulin, glucagon, and corticosterone.

Calculations. The estimated portal vein glucose concentration (GPV) was calculated as GPV = GA + (GINFPOR/PVPF), where GA is arterial glucose concentration in micromoles per milliliter, GINFPOR is the portal vein glucose infusion rate in micromoles per minute, and PVPF is portal vein plasma flow rate in milliliters per minute. Portal vein plasma flow rate was assumed to be 80% of hepatic plasma flow rate, where hepatic plasma flow rate was assumed to be 1.3 ml/g liver−1·min−1 (26). Portal vein and arterial glucose concentrations were assumed equal in the absence of any portal vein glucose infusion.

Analytical procedures. Glucose was assayed by the glucose oxidase method (YSI, Yellow Springs, OH). Catecholamines were analyzed using a single-isotope radioenzymatic method (37). Insulin and glucagon samples were assayed using RIA kits (Linco Research, St. Charles, MO), and corticosterone was measured by an RIA kit purchased from MP Biomedicals (Orangeburg, NY).

Data analysis. Experimental results were expressed as means ± SE. Data were analyzed using standard one-way ANOVA with repeated measures where appropriate. Fisher’s post hoc analysis was used to determine significant differences among experimental groups. Significance was set at P < 0.05.

RESULTS

Day 1 (arterial and portal vein glucose). On day 1, there were no significant differences in mean basal arterial glucose levels (6.06 ± 0.17 mmol/l; Fig. 2) among the three experimental protocols. By design, during day 1 hypoglycemia (40–75 min), mean arterial glucose concentrations in the HYPO-HYPO and HYPO-EUG were decreased significantly compared with the EUG-EUG group (2.60 ± 0.07 vs. 6.33 ± 0.31 mmol/l; P = 0.001; Fig. 2). Day 1 portal vein plasma glucose levels in HYPO-EUG and EUG-EUG groups remained within basal levels (6.51 ± 0.21 mmol/l, Fig. 2), whereas portal vein glycemia was allowed to decrease to deep hypoglycemia (2.52 ± 0.11 mmol/l; P = 0.001 vs. HYPO-EUG and EUG-EUG) during 40–75 min in the HYPO-HYPO group.

Day 2 (arterial, portal vein glucose, and insulin levels). On day 2, there were no significant differences between all treatments with respect to arterial and portal vein glucose concentrations at basal (6.31 ± 0.11, P = 0.55 between groups, Fig. 3), during the fall in glycemia, and during sustained deep hypoglycemia (2.34 ± 0.04, P = 0.10 between groups, Figure 3). Arterial plasma insulin increased from an average basal value of 6 ± 1 μU/ml to a hyperinsulinemic plateau of 915 ± 44 μU/ml (P = 0.61, between groups, Fig. 4).

Day 2 (counterregulatory hormonal response). In response to day 2 insulin-induced hypoglycemia, EUG-EUG animals demonstrated a robust sympathoadrenal response where plasma epinephrine concentrations increased ~16-fold from 0.94 ± 0.1 nmol/l at basal to a peak value of 15.75 ± 1.33 nmol/l by 105 min (Fig. 5). In contrast, a single episode of antecedent hypoglycemia in the HYPO-HYPO group resulted in a significantly delayed and quantitatively diminished (34%
suppression, \( P = 0.01 \) epinephrine response to next-day hypoglycemia compared with the EUG-EUG group (Fig. 5). Normalization of portal vein glycemia during day 1 hypoglycemia in the HYPO-EUG group failed to augment the epinephrine response compared with the HYPO-HYPO group (Fig. 5). Additionally, antecedent hypoglycemia with or without portal vein glucose normalization failed to impact upon the corticosterone response to next-day hypoglycemia (Fig. 6).

**DISCUSSION**

In the current study, we sought to test the hypothesis that exposure to antecedent portal vein hypoglycemia results in impaired hypoglycemic detection at the portal vein and a consequent diminished counterregulatory response to next-day hypoglycemia. We therefore compared effects of a single bout of antecedent hypoglycemia with or without selective normalization of portal vein glycemia on epinephrine, norepinephrine, glucagon, and corticosterone responses to next-day slow-onset hypoglycemia. As expected, exposing animals to a single episode of acute antecedent hypoglycemia (HYPO-HYPO) suppressed the overall epinephrine and norepinephrine response to next-day hypoglycemia (Fig. 5). The peak norepinephrine response in HYPO-HYPO was blunted by 32%, although not significantly \( (P = 0.06) \) compared with the EUG-EUG group. Normalization of portal vein glycemia during day 1 hypoglycemia in the HYPO-EUG group failed to augment the norepinephrine response compared with the HYPO-HYPO group (Fig. 5). Additionally, antecedent hypoglycemia with or without portal vein glucose normalization failed to impact upon the corticosterone response to next-day hypoglycemia (Fig. 6).
sensing and the subsequent activation of the sympathoadrenal axis as it pertains to secretion of corticosterone. Furthermore, these data establish that portal vein glucose sensing and the subsequent activation of the sympathoadrenal and glucagon response to hypoglycemia are mediated independent of the HPA axis.

Although the etiology of hypoglycemia-associated counterregulatory failure remains to be established, a number of potential mechanisms have been proposed, including antecedent cortisol exposure (12, 13), increased brain glucose uptake (3, 4), and antecedent exposure to corticotrophin-releasing hormone (20, 32). Although the mechanism of HAAF is diverse and most likely very complex, our current findings demonstrate that hypoglycemia-induced suppression in the epinephrine and glucagon response to next-day hypoglycemia is consistent with previous reports in human (22) and animal studies (18). Furthermore, the percentage suppression of the sympathoadrenal (34%) and glucagon (42%) response is quantitatively similar to previous reports using a similar hypoglycemic insult (18, 22). In contrast to significantly suppressed epinephrine and glucagon responses, antecedent hypoglycemia failed to blunt the corticosterone response to next-day hypoglycemia, which was further unaffected by normalization of day 1 portal vein glycemia. The observation that antecedent hypoglycemia fails to decrease the corticosterone response to next-day hypoglycemia agrees with a number of previous studies (18, 20), suggesting that the acute antecedent hypoglycemia fails to negatively affect the activation of the hypothalomo-pituitary adrenal (HPA) axis as it pertains to secretion of corticosterone.

Inappropriately low epinephrine and glucagon responses to hypoglycemia have been identified as the critical pathophysiological events in T1DM, because in the absence of glucagon secretion, epinephrine constitutes a primary defense against hypoglycemia (6, 8). Therefore, patients that demonstrate abnormalities in both epinephrine and glucagon secretion are several times more likely to suffer from recurrent bouts of hypoglycemia (50). Additionally, impaired counterregulation in T1DM has been shown to be a threshold-related abnormality (1) and specific to the stimulus of hypoglycemia (39), indicating that blunted counterregulatory hormonal response may be a consequence of failed hypoglycemia sensing mechanism(s). Our observations that a single 30-min episode of antecedent hypoglycemia significantly diminishes peak epinephrine and glucagon secretion to subsequent hypoglycemia is consistent with previous reports in human (22) and animal studies (18). Furthermore, the percentage suppression of the sympathoadrenal (34%) and glucagon (42%) response is quantitatively similar to previous reports using a similar hypoglycemic insult (18, 22). In contrast to significantly suppressed epinephrine and glucagon responses, antecedent hypoglycemia failed to blunt the corticosterone response to next-day hypoglycemia, which was further unaffected by normalization of day 1 portal vein glycemia. The observation that antecedent hypoglycemia fails to decrease the corticosterone response to next-day hypoglycemia agrees with a number of previous studies (18, 20), suggesting that the acute antecedent hypoglycemia fails to negatively affect the activation of the hypothalomo-pituitary adrenal (HPA) axis as it pertains to secretion of corticosterone. Furthermore, these data establish that portal vein glucose sensing and the subsequent activation of the sympathoadrenal and glucagon response to hypoglycemia are mediated independent of the HPA axis.

Although the etiology of hypoglycemia-associated counterregulatory failure remains to be established, a number of potential mechanisms have been proposed, including antecedent cortisol exposure (12, 13), increased brain glucose uptake (3, 4), and antecedent exposure to corticotrophin-releasing hormone (20, 32). Although the mechanism of HAAF is diverse and most likely very complex, our current findings demonstrate that hypoglycemia-induced suppression in the epinephrine and glucagon response may be in part attributed to defective hypoglycemic sensing at the portal vein. Interestingly, there is evidence that antecedent exposure to low ambient glucose levels impairs intracellular glucose sensing in other known glucose sensors, such as hypothalamic glucose-sensing neurons (17, 48). Glucose sensing by both glucose-excited and glucose-inhibited hypothalamic glucosensors primarily depends on phosphorylation of glucose by the pancreatic form of glucokinase (GK), which has been shown to be the rate-limiting step in hypothalamic glucose sensing (17). Subsequently, glucose-excited neurons utilize ATP-sensitive K+ channels, whereas glucose-inhibited neurons appear to employ chloride channels as final mediators of intracellular glucose sensing (30, 47). Neurons expressing GK are primarily found in areas of the brain known to mediate glucose detection, and pharmacological inhibition of GK activity abolishes the ability of hypothalamic glucosensing neurons to detect changes in glycemia (30). More importantly, recent studies suggest that antecedent exposure to hypoglycemia impairs brain glucose sensing by significantly augmenting GK mRNA expression, thus leading...
to diminished glucose sensitivity in both glucose-excited and glucose-inhibited neurons (17, 40, 48). We have previously demonstrated that the underlying mechanism for portal vein glycemic detection appears mechanistically analogous to that of the hypothalamic glucose sensors, i.e., cellular glycemic detection is mediated by events subsequent to uptake and oxidation of glucose (31). As with hypothalamic glucose sensors, portal vein sensors respond to alternative metabolic substrates such as lactate (31). Moreover, others have reported that cells in the portal area express both GLUT-2 transporter protein (5) and GK (27). Thus antecedent portal vein hypoglycemia may blunt the ability of portal vein glucose sensors to detect ambient changes in plasma glucose levels via similar augmentations in GK activity, leading to an impaired counterregulatory response to subsequent hypoglycemia. However, elucidation of the molecular mechanisms underlying the pathogenesis of impaired portal vein glucose sensing will require identification of the precise cells involved, since it is unlikely that all cells in the portal vein are involved in glucose sensing.

In the current study, normalization of portal vein glycemia during day 1 hypoglycemia HYPO-EUG restored the peak epinephrine and glucagon response but failed to impact upon the apparent glycemic threshold for sympathoadrenal activation. One plausible explanation for this occurrence is that day 1 hypoglycemia impacts not only upon portal vein glucose sensors but those neurons of the brain responsible for integrating peripheral glucose sensory input as well. Thus, despite intact glucose sensing at the portal vein in HYPO-EUG, peripheral input may be improperly processed, resulting in a lowered glycemic threshold or delay in the activation of epinephrine secretion. However, given sufficiently deep and/or sustained hypoglycemia, the presence of intact portal glucose sensors may sustain the absolute magnitude of the epinephrine response. Alternatively, when both portal vein and brain glucose sensors are exposed to antecedent hypoglycemia HYPO-HYPO, the glycemic threshold and the absolute magnitude of the sympathoadrenal response is clearly impaired. If true, then alterations in both portal vein and brain glucose sensors may be required for the full induction of hypoglycemia-associated counterregulatory failure. Functional connections between portal vein glucose sensors and areas of the central nervous system (CNS) purported to mediate the counterregulatory response have been proposed previously (44, 45). More recently we have demonstrated that portal vein glucose sensing and the subsequent sympathoadrenal response are mediated via spinal sympathetic afferents traversing the celiac-superior mesenteric ganglion (21). Eliminating spinal sensory input from the portal vein via celiac-superior mesenteric ganglionectomy also leads to greatly diminished neuronal activation in areas of hindbrain known to respond to hypoglycemia (2). Interestingly, repeated systemic glucose deprivation has been shown to diminish Fos immunoreactivity in this same subset of hindbrain neurons (42).

In this study, the antecedent hypoglycemic insult was apparently insufficient to significantly suppress peak norepinephrine values (Fig. 5), and thus we were unable to fully assess the role of portal vein hypoglycemia per se. That peak norepinephrine was not impacted by antecedent hypoglycemia is consistent with other reports for both rats and humans (11, 28, 36, 43). However, there was a significant delay in the onset of the norepinephrine response, similar to that observed for epinephrine. As with epinephrine, normalizing portal vein glycemia on day 1 did not significantly impact upon these earlier time points, indicative of a lowered glycemic threshold or delay in processing sensory input relevant to the norepinephrine response. These findings are consistent with the idea that the adrenal medulla is the primary source of norepinephrine secretion in insulin-stimulated hypoglycemia (35). The dissociation of sympathetic nerve activity and adrenomedullary responses has been well documented, suggesting they are controlled by distinct neuronal networks (51). Even adrenomedullary responses may be heterogeneous, since epinephrine- and norepinephrine-releasing chromaffin cells within the adrenal medulla appear to be innervated by functionally distinct preganglionic neurons (33, 49). Whether the diminished norepinephrine response previously reported for antecedent hypoglycemia can be attributed to defective glucose sensing at the portal vein or other glucose-sensing loci (e.g., CNS) remains to be elucidated.

In the current study, we induced hypoglycemia slowly over a period of 70 min. We have previously reported that portal vein glucose sensors appear predominant for hypoglycemic detection during gradual falls in glycemia (16) but appear less important during more rapid declines (15). These findings suggest that the critical locus for hypoglycemic detection shifts away from the portal sensors, presumably to the brain, when hypoglycemia develops rapidly. If antecedent hypoglycemia local to the critical glucose sensors is important for the induction of HAAF, then the design of the current study may have favored of the portal vein glucose sensors. In contrast, most studies of recurrent hypoglycemia have employed repeated injections of insulin that induce much more rapid declines in glucose that are sustained for substantially longer periods of time, i.e., ~2 h (20, 38). Under such conditions, it is quite possible that the critical glucose sensors exposed to antecedent hypoglycemia actually reside in the brain, e.g., ventromedial hypothalamic nucleus or hindbrain. This would suggest that, beyond the depth and duration of antecedent hypoglycemia, the rate at which hypoglycemia develops is critical for the underlying pathology of HAAF. Given that the rate of glycemic decline can be affected by a multitude of factors, e.g., the insulin regimen, type of insulin, exercise regimen, meal composition, and the site and timing of insulin injections, both glucose sensory loci and their potential impairment hold clinical significance (7, 9).

With one exception (36), all groups studying antecedent hypoglycemia in the rat have employed protocols involving multiple hypoglycemic insults before testing (18, 20, 25, 28, 38, 41, 43, 48). This has ranged from as few as two episodes the day before (41, 43) to as much as 3–4 wk of daily insulin-induced hypoglycemia (20, 38). The duration of antecedent hypoglycemic exposure in these previous reports appears to have been at least 2 h/episode (18, 20, 25, 28, 38, 41, 43, 48). Thus the single 30-min antecedent hypoglycemic episode (45 min if one includes the time between the hypoglycemic threshold and nadir) employed in the current study appears to be the shortest antecedent exposure to date in a study involving rodents. Whether this limited exposure may have favored the impairment of portal glucose sensors over those of the CNS, i.e., brain glucose sensors require longer and/or more episodes of hypoglycemia to become impaired, is unknown. However, these findings do suggest that impairment
of the portal vein glucose sensors is among the earlier events in the development of HAAF. To our knowledge, only one study has employed a shorter antecedent exposure time, and that was accomplished via two episodes the day before in humans (11). Notably, these authors did not find any significant differences between two 5-min vs. two 30-min antecedent hypoglycemic episodes in the suppression of neuroendocrine and metabolic counterregulatory responses to a subsequent bout of hypoglycemia.

In summary, impaired glucose sensing at the portal vein plays a significant role in mediating hypoglycemia-associated counterregulatory failure. Normalizing portal vein glycemia during an antecedent bout of systemic hypoglycemia (HYPO-EUG) served to preserve the peak epinephrine and glucagon response to a subsequent bout of slow-onset hypoglycemia. However, HYPO-EUG failed to preserve the apparent glycemic threshold for the epinephrine response. Together these observations suggest additional hypoglycemia-susceptible loci intermediate to portal glucose sensory input and adrenal output, e.g., glucose-sensing neurons of the CNS. The norepinephrine response to antecedent portal vein hypoglycemia could not be fully assessed with the current design, but, as with epinephrine, normalizing portal vein glycemia had no impact on the suppression of the apparent glycemic threshold for norepinephrine release. These findings suggest that glycemic detection at the portal vein and its subsequent integration within CNS are important components in the pathogenesis of HAAF.

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