Hypoglycemic detection at the portal-mesenteric vein (PMV) appears mediated by spinal afferents and is critical for the counter-regulatory response (CRR) to slow-onset, but not rapid-onset, hypoglycemia. Since rapid-onset hypoglycemia induces Fos protein expression in discrete brain regions, we hypothesized that denervation of the PMV or lesioning spinal afferents would suppress Fos expression in the dorsal medulla during slow-onset hypoglycemia, revealing a central nervous system reliance on PMV glucosensors. Rats undergoing PMV deafferentation via capsaicin, celiac-superior mesenteric ganglionectomy (CSMG), or total subdiaphragmatic vagotomy (TSV) were exposed to hyperinsulinemic–hypoglycemic clamps where glycemia was lowered slowly over 60–75 min. In response to hypoglycemia, control animals demonstrated a robust CRR along with marked Fos expression in the area postrema, nucleus of the solitary tract, and dorsal motor nucleus of the vagus. Fos expression was suppressed by 65–92% in capsaicin-treated animals, as was epinephrine (74%), norepinephrine (33%), and glucagon (47%). CSMG also suppressed Fos expression and CRR during slow-onset hypoglycemia, whereas TSV failed to impact either. In contrast, CSMG failed to impact upon Fos expression or the CRR during rapid-onset hypoglycemia. Peripheral glucosensory input from the PMV is therefore required for activation of hindbrain neurons and the full CRR during slow-onset hypoglycemia.

Iatrogenic hypoglycemia has emerged as the primary obstacle for achieving glycemic control in insulin-dependent diabetic patients (1). Since establishing euglycemia holds the potential to ameliorate many of the serious microvascular complications, there has been a renewed interest in understanding the mechanisms underlying hypoglycemic detection. Originally viewed as the domain of the ventromedial hypothalamus, it is now known that glucose-sensing neurons are widely distributed within the central nervous system (CNS) as well as in the periphery (2). In addition to the ventromedial hypothalamus, neurons responding specifically to hypoglycemia have been identified within the paraventricular nucleus, lateral hypothalamic area, arcuate nucleus, area postrema (AP), nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus (DMX). Peripheral glucose sensors responding to hypoglycemia have been identified in the carotid bodies (3) and portal-mesenteric vein (PMV) (4,5). The relative importance of the various glucose-sensing loci is an area of considerable debate and may in part depend upon the rate at which hypoglycemia develops. We have shown that portal-mesenteric glucose sensors are particularly important for the detection of slow-onset hypoglycemia (5). Lesioning portal-mesenteric glucose sensors essentially eliminates sympathoadrenal responses to hypoglycemia and severely constrained glucose counter regulation. In contrast, rapid-onset hypoglycemia elicited a counter-regulatory response (CRR) that appears largely independent of peripheral glucose sensory input.

While peripheral and central glucose sensors may subserve different roles, it has been proposed that these various glucose-sensing loci comprise an integrated neural network for defending the glycemic status of the body.
Animals were housed in individual cages, fed ad libitum,
remains speculative. averted by spinal afferents (17), though any connection with
response to hypoglycemia (5,18,19). Alternatively, evidence
functional neural networks underlying many stress responses,
were re
lary urogenital and suprarenal neural axis were based primarily on
observations of glucose elevations in the portohepatic cir-
culation. Increasing portal vein glucose levels inhibits the
firing of hepatic vagal afferents (9), inhibits glucose-excited
neurons in the lateral hypothalamic area and NTS (10,11),
reduces the adrenal nerve firing rate (6), and suppresses
appetite (12–14). Since sectioning the hepatic vagus elimi-
nates most of these responses, it is generally presumed
that glucose sensory input from the portal vein is conveyed
to the CNS via glucose-sensitive vagal afferents. However,
CRRs to hypoglycemia have repeatedly been shown to be
unaffected by vagotomy (15–17), despite the fact that PMV
glucose sensors play a critical role in the sympathoadrenal
response to hypoglycemia (5,18,19). Alternatively, evidence
suggests hypoglycemic detection at the PMV may be medi-
ated by spinal afferents (17), though any connection with
brain glucose sensory loci remains speculative.

The early gene product Fos is widely used to map
functional neural networks underlying many stress responses,
including hypoglycemia (20). Utilizing this approach, inves-
tigators have identified two primary levels of the CNS acti-
vated by hypoglycemia. In the hindbrain, Fos is observed
primarily in the AP, NTS, and DMX, while activation is
also observed in a number of forebrain regions, including
the hypothalamus (13,21,22). However, these studies have
relied on injections of insulin or 2-deoxyglucose, both of
which induce hypoglycemia or glucopenia rapidly and may
obviate peripheral glucose sensory input (5). Here we test the
hypothesis that activation of the CNS during slow-onset hy-
poglycemia is dependent upon PMV glucose sensory input
via spinal afferents, but not during rapid-onset hypoglycemia.

RESEARCH DESIGN AND METHODS

Animals

Three experimental studies used conscious unrestrained
male Wistar rats (weight = 254.4 ± 2.87 g; total n = 43).
Animals were housed in individual cages, fed ad libitum,
and on 12-h light–dark cycles. All surgical and experimen-
tal procedures were preapproved by the University of
Southern California Institutional Animal Care and Use
Committee.

Surgical Procedures

One week before experiments, all animals were chronic-
ally cannulated under single-dose anesthesia (3:3:1 keta-
mine HCl, xylazine, acepromazine malate; 0.1 mL/0.1 kg
body weight) administered intramuscularly. Cannulas
were inserted into the left jugular vein (dual cannula,
Silastic ID = 0.025 cm) for peripheral administration of
glucose and insulin and the right carotid artery (Clay
Adams PE-50) for arterial blood sampling. At that time,
a laparotomy was performed, and the abdominal contents
were reflected and covered with sterile gauze soaked in
warm saline (37°C). Animals then underwent one of three
separate denervation procedures, with control animals
(n = 16) undergoing an analogous sham operation.

Experiment 1

Portal-mesenteric sensory denervation (capsaicin; n = 5)
was effected via topical application of a 1% capsaicin so-
lution (vehicle solution 10% ethanol and 10% Tween80 in
0.9% saline) as previously described (5,19). Briefly, the
portal and superior mesenteric veins were isolated, and
small strips of filter paper were measured to fit the length
and width of the vessels then soaked in a 1% capsaicin
solution and placed on the veins for 15 min with care taken
to shield surrounding tissues from exposure to the capsaicin.
Strips were removed thereafter, and the veins were rinsed
thoroughly with saline. Control animals (n = 5) underwent
a sham operation where only vehicle solution was applied.

Experiment 2

To eliminate spinal afferent innervation of the portal and
superior mesenteric veins, a celiac-superior mesenteric
gangliectomy (CSMG; n = 5) was performed. Following
the laparotomy, the celiac and superior mesenteric ganglia
were located on the ventral aspect of the descending
aorta, caudal to the celiac artery, and rostral to the su-
perior mesenteric artery. The ganglia were then gently re-
moved by blunt dissection, severing all visible connections
(17). To eliminate vagal afferent innervation of the PMV,
a total subdiaphragmatic vagotomy (TSV; n = 5) was per-
formed involving bilateral sectioning of the anterior and
posterior trunks of the vagus nerve located along the
esophagus just caudal to the diaphragm (17). Control ani-
mals (n = 5) underwent a sham operation in which the
subdiaphragmatic branches of the vagus and CSMG were
exposed but not severed.

Experiment 3

A CSMG was performed as above (n = 6) with control
animals (n = 6) undergoing a sham operation.

Following denervation procedures, the stomach and
intestines were returned to the abdominal cavity and the
abdominal musculature sutured to close the cavity. Ven-
tral skin incisions were closed separately with in-
dividual sutures, reinforced with suture glue (Nexaband)
and swabbed with an antibacterial agent (Betadine).
Cannulas were tunneled subcutaneously, exteriorized at
the back of the neck, and incased in an infusion harness
(Instech Laboratories). Animals were allowed 6 days to
recover from surgery and to regain their original body
weight. No significant differences in body weight were
observed between experimental groups on the day of
experiments. Sixteen hours prior to experiments, all
access to food (but not water) was removed.

In Vivo Clamps

Slow-Onset Hyperinsulinemic–Hypoglycemic Clamps
(Experiments 1 and 2)

On the day of the experiment, all animals were exposed to
the same protocol to induce hypoglycemia. Animals were
placed in an infusion chamber and their jugular catheters
attached to extensions from a dual-channel infusion
swivel connected to infusion pumps for insulin and glucose infusions. Animals were allowed to rest for 60 min (−90 to −30 min) before sampling was initiated. Basal arterial samples were drawn at −30 and 0 min for analysis of glucose, insulin, glucagon, and catecholamines. At minute 0, whole-body hypoglycemia was induced slowly via insulin infusion (25 mU·kg⁻¹·min⁻¹) and variable exogenous glucose infusion (20% dextrose). Glucose infusion was slowly decreased to achieve deep hypoglycemia, 2.6 mmol/L, within 60–75 min. During this time, additional glucose samples were drawn every 10 min to control the rate of glycemic decline. Glucose infusions were adjusted thereafter to sustain hypoglycemia at 2.6 mmol/L until minute 105. Arterial plasma samples for glucose and catecholamines were taken at minutes 60, 75, 90, and 105 of the clamp. The volume of blood sampled was replaced with an equal volume of whole blood from donor animals. An additional sample was taken at minute 105 for the determination of glucagon and insulin concentrations.

**Hyperinsulinemic–Euglycemic Clamps (Experiment 1 Only)**

To ascertain the potential impact of hyperinsulinemia versus hypoglycemia on brain Fos expression, hyperinsulinemic–euglycemic clamps were performed on a separate group of sham control animals (n = 6). One week later, they underwent identical clamp and sampling protocols to those described above except that euglycemia (~6.5 mmol/L) was maintained throughout the entire 105-min experiment utilizing variable glucose (20%) infusions.

**Rapid-Onset Hyperinsulinemic–Hypoglycemic Clamps (Experiment 3)**

Animals were placed in an infusion chamber, and catheters were connected as described above. Animals were allowed 55 min to rest (−100 to −45 min) before insulin (25 mU·kg⁻¹·min⁻¹) and glucose infusions (variable) were initiated and a hyperinsulinemic–euglycemic clamp established from minute −45 to 0. At minute 0, the glucose infusion was reduced to achieve the hypoglycemic nadir, 2.5 mmol/L, by minute 20, which was sustained for the remainder of the clamp (20–110 min). Sampling for glucose analysis was performed every 5–15 min between minutes −45 and 110. Larger arterial samples (250 μL) were drawn at minutes −45, 0, 20, 40, 60, 90, and 110 for catecholamine analysis, with insulin and glucagon samples taken at minutes 0 (basal) and 110 (deep hypoglycemia).

**Tissue Processing and Immunocytochemistry**

At the terminus of the clamp, rats were killed (sodium pentobarbital, overdose) and rapidly decapitated; brains were collected and fixed in paraformaldehyde, sectioned, and then processed as previously described (23). In brief, 30 μm coronal hindbrain sections were cut between levels 68–71 of the Swanson rat brain atlas (24). Sections were reacted for 72 h at 4°C with a rabbit polyclonal Fos antibody (Ab-5; 1:50 K, Chemicon) followed by a biotinylated goat anti-rabbit IgG. The immunocomplexes were then conjugated to streptavidin-peroxidase (Vector) and developed with a 0.5 mg/mL solution of 3,3-diaminobenzidine tetrahydrochloride containing 0.1 μL/mL hydrogen peroxide followed by 0.5% cobalt acetate (Sigma-Aldrich) for 10 min. Sections were then mounted on gelatin-coated slides, air-dried overnight, dehydrated in alcohols, cleared in xylenes, and cover slipped. To limit the impact of possible processing variations on the results, all sections from a single experiment were processed together. Under these circumstances, immunocytochemistry is a semiquantitative method, so comparing the numbers of Fos between separate experiments is not possible.

Sections were photographed using bright-field illumination with a Nikon Microphot SA microscope and a SPOT RT Digital Camera (Diagnostics Instruments Inc.) using SPOT Image (version 3.5.5, Mac OS). Brightness and contrast of the photomicrographs were matched across sections using the Photoshop CS3 (www.adobe.com) Curves and Brightness/Contrast tools. For each animal, the section corresponding to level 70 of Swanson (24) was identified, and the AP, NTS, and DMX were demarcated using local cytoarchitectural features in adjacent thionin-stained sections. Fos-ir nuclei in these three regions on both sides of the brain were counted manually.

**Analytical Procedures**

Glucose was assayed by the glucose oxidase method (YSI). Catecholamines were analyzed utilizing a single-isotope radioenzymatic method (25). Insulin and glucagon samples were assayed using commercially available radioimmunoassay kits (Linco Research and MP Biomedicals).

**Data Analysis**

Results were expressed as mean ± SEM. Comparisons among treatments over time were made utilizing repeated-measures ANOVA with post hoc analysis via Bonferroni test for multiple comparisons (Prism, GraphPad). For comparisons between groups under basal and hypoglycemic conditions or hypoglycemia alone, two-way ANOVA or one-way ANOVAs were used, respectively, with Bonferroni post hoc comparisons where appropriate.

**RESULTS**

**Effect of PMV Deafferentation on Hindbrain Fos Expression and CRR Hormone Responses to Slow-Onset Hypoglycemia**

Insulin infusion led to an increase in the arterial plasma insulin concentration, 4,930 ± 699 pmol/L that was not significantly different between groups (P > 0.10) (Fig. 1). Basal glucose concentrations were 6.23 ± 0.43, 6.08 ± 0.13, and 5.63 ± 0.18 mmol/L for euglycemia, control, and capsaicin, respectively (not significant; P > 0.10). By design, euglycemic animals were clamped at glucose concentrations not significantly different from basal, while glucose was allowed to fall in a controlled manner over the next 60 min for both control and capsaicin, reaching
hypoglycemic nadirs of 2.52 ± 0.15 and 2.66 ± 0.37 mmol/L, respectively (not significant for control versus capsaicin; P > 0.10).

As expected, euglycemic animals demonstrated epinephrine, norepinephrine, and glucagon values that were not significantly different from basal over the course of the experiment. In response to insulin-induced hypoglycemia, control animals demonstrated a robust sympathoadrenal response, where plasma epinephrine concentrations increased from 0.44 ± 0.10 at basal to 19.73 ± 3.35 nmol/L by minute 105. In contrast, ablation of PMV capsaicin-sensitive afferents led to a 74% suppression of the epinephrine response (5.11 ± 0.62 vs. 19.73 ± 3.35 nmol/L; P < 0.001) by minute 105. Norepinephrine responses to hypoglycemia in control were significantly elevated above euglycemia (P < 0.001) but failed to rise significantly in capsaicin animals (P > 0.05). Basal glucagon values averaged 88.8 ± 12.4 pg/mL and were not significantly different between euglycemia, control, and capsaicin (P > 0.10).

In response to hypoglycemia, control animals demonstrated a fourfold increase in glucagon that was significantly greater than capsaicin or euglycemia (P < 0.05). Deafferentation of the PMV via capsaicin prevented the hypoglycemia-induced rise in glucagon, i.e., values for capsaicin at hypoglycemia were not significantly different from euglycemia (P > 0.10).

Euglycemia failed to induce significant Fos expression in the hindbrain, while hypoglycemia in nerve-intact animals (control) led to a 11-, 22-, and 23-fold increase in Fos expression in the NTS (P < 0.001), DMX (P < 0.001), and AP (P < 0.01), respectively. As with the hormonal responses above, deafferentation of the PMV led to an 86, 65, and 92% suppression in Fos-labeled nuclei within the AP (P < 0.01), DMX (P < 0.01), and NTS (P < 0.001) (Fig. 2), respectively.

Effect of Subdiaphragmatic Vagotomy Versus CSMG on Hindbrain Fos Expression in Response to Slow-Onset Hypoglycemia

Insulin infusion increased plasma insulin values for control, CSMG, and TSV animals to 4,673 ± 437, 5,367 ± 54, and 4,615.17 ± 337 pmol/L, respectively (not significant; P > 0.10). Plasma glucose was matched in all groups during the clamp and slowly reduced from a mean value of 6.01 ± 0.10 mmol/L to a hypoglycemic nadir of 2.49 ± 0.05 mmol/L by minute 75 and maintained until minute 105 (not significant between groups; P > 0.10) (Fig. 3).

Whole-body hypoglycemia increased arterial epinephrine concentrations 25-fold in control animals from a basal value of 1.00 ± 0.33 nmol/L to 24.84 ± 3.46 nmol/L by minute 105. TSV animals demonstrated a similar epinephrine response, with a 20-fold increase from basal, reaching a peak value of 26.73 ± 4.79 nmol/L, not significantly different from control (P > 0.05). In contrast, animals with CSMG, thereby lesioning spinal innervation to the
PMV, experienced a 68% suppression in the peak epinephrine response to hypoglycemia compared with control or TSV animals (P < 0.0001). Groups demonstrated similar norepinephrine responses to hypoglycemia, with control and TSV demonstrating a 4.5-fold increase above basal at minute 105, while peak norepinephrine responses for CSMG were suppressed by 61% compared with control values (P < 0.01). In response to hypoglycemia, glucagon values increased to 344.02 ± 5.0 pmol/L to a hyperinsulinemic plateau of 5,194 ± 510 pmol/L. No significant differences were observed in basal (P > 0.10) or terminal (P > 0.10) insulin concentrations between control and CSMG groups. In both groups, arterial glucose was rapidly lowered from a mean value of 5.66 ± 0.2 mmol/L to 2.44 ± 0.08 mmol/L within 20 min (Fig. 5). By design, these arterial glucose values were matched; thus there were no significant differences between control and CSMG animals at any sampling point.

In response to rapid-onset hypoglycemia, epinephrine values in control animals increased ~40-fold from 0.55 ± 0.14 to 21.85 ± 2.45 nmol/L by minute 110. In contrast to the results for slow-onset hypoglycemia (Fig. 3), CSMG animals demonstrated epinephrine responses to rapid-onset hypoglycemia that were not significantly different from control (P > 0.05). As with epinephrine, norepinephrine and glucagon both demonstrated robust responses to hypoglycemia in control animals that were not significantly impacted by CSMG. Consistent with their CRRs to rapid-onset hypoglycemia, the glucose infusion rates were not significantly different between groups at any point during the clamp. Substantial Fos expression was observed in the hindbrain following rapid-onset hypoglycemia but was not significantly different between control and CSMG (Fig. 6).

**DISCUSSION**

The existence of a hepatoportal–brain–sympathoadrenal neural network was postulated over 20 years ago (6,7,26), but its relevance to hypoglycemic detection remained speculative, with little compelling supportive evidence (8,27). Toward this end, we now provide the first evidence that activation of hindbrain neurons by slow-onset hypoglycemia (~0.05 mmol/L/min) requires glucosensory input from peripheral glucose sensors located in the PMV. When hypoglycemia developed slowly over 60 min, portal-mesenteric deafferentation via capsaicin dramatically suppressed hindbrain Fos-ir neuronal activation in regions that contain glucose-sensing neurons, i.e., the NTS (192%), DMX (165%), and AP (186%). Concomitant with reduced hindbrain Fos expression, there was substantial blunting of the sympathoadrenal response, i.e., epinephrine (174%) and norepinephrine (132%), together with a 47% reduction in the glucagon response to slow-onset hypoglycemia. Furthermore, severing all vagal input below the diaphragm had no significant effect on hindbrain Fos expression, while CSMG produced a reduction comparable with PMV deafferentation via capsaicin. Thus, as has been shown for the sympathoadrenal response, the ascending trajectory used for portal-mesenteric hypoglycemic sensory information to the hindbrain appears to be spinal, not vagal. Finally, we demonstrated that both hindbrain
neuronal activation and the sympathoadrenal response to rapid-onset hypoglycemia (−0.16 mmol/L/min) appear independent of peripheral glucosensory input. These data provide compelling evidence that a functional PMV–hindbrain–sympathoadrenal network is required for CRRs to slow-onset hypoglycemia. 

With the possible exception of spinal afferents, the critical components of a PMV–hindbrain–sympathoadrenal neural network are well described. The NTS is the major recipient of the sensory input from both vagal and spinal afferents innervating a variety of systemic organs (28). It also receives afferent input from adjacent glucosensory structures, e.g., the AP, a circumventricular organ ostensibly capable of detecting changes in circulating plasma glucose levels (29). Adachi et al. (10,30) have identified glucose-sensing neurons in various regions of the hindbrain, including the NTS, AP, and DMX. The DMX provides parasympathetic preganglionic control to the gut and other organs. Direct microinjection of 5-thioglucose into the brain reveals numerous hindbrain glucopenic sensing loci, primarily in the ventrolateral and dorsomedial medulla proximal to the AP or within the NTS (31). Most of these same cell groups demonstrate Fos activation in response to 2-deoxyglucose injection (32). Adrenomedullary chromaffin cells are innervated by sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord (33). In turn, these are innervated by a small number of hindbrain structures: the caudal raphe nuclei, the ventromedial and rostral ventrolateral medulla, and the A5 cell group (34). Antidopamine-β-hydroxylase saporin injected into the spinal cord selectively eliminates those hindbrain catecholaminergic neurons critical to the sympathoadrenal response to 2-deoxyglucose (35). Further, experiments with decerebrate animals show that the hindbrain alone is sufficient to retain the sympathoadrenal response to a 2-deoxyglucose challenge (36). Thus critical elements already exist for a simple neural network that may be sufficient to monitor and maintain euglycemia in the face of moderate or slowly developing hypoglycemic challenges.

Prior models describing a portahepatic–CNS–sympathoadrenal neural axis assumed that all visceral glucose sensory input to the brain was effected via vagal afferents (6–8). While there is little doubt that glucose-sensing vagal afferents exist, their role in hypoglycemic detection remains questionable (15–17). Outside of two extreme hypoglycemic/glucopenic conditions, i.e., 0 mmol/L glucose and large portal 2-deoxyglucose injections (9,37), evidence for vagal afferent glucose sensing has been restricted to euglycemia and hyperglycemia (9,38,39). Vagotomies, both acute and chronic, have repeatedly failed...
to affect CRRs to hypoglycemia (15–17). That the vagal glucose-sensing afferents of the PMV may subserve a different function, e.g., hyperglycemic sensing, is supported by observations indicating a linear response for these neurons across a wide range of glucose concentrations, 0–27 mmol/L (9,38,39). Alternatively, CSMG suppresses CRRs to hypoglycemia (17). While vagal afferents of the celiac branch are likely severed in this procedure, this occurs caudal to the total subdiaphragmatic lesion (which has no impact on CRR), suggesting that the impact of CSMG is achieved through the sectioning of spinal glucose sensory afferents. Consistent with a spinal origin, peak epinephrine, norepinephrine, and glucagon values for CSMG and capsaicin animals were not significantly different in the current study (the apparent discrepancy in Figs. 1C and 3C derive from the slightly lower control norepinephrine values, though again control peak values were not significantly different). Additional support for a spinal origin of those glucose sensors critical for detecting hypoglycemia derives from the effectiveness of topical capsaicin in ablating PMV glucose sensing. Capsaicin acts through the TRPV1 receptor, which is largely restricted to spinal afferents at the level of the abdomen (40). Calcitonin gene-related peptide, a marker for spinal afferents at this level (41), is largely eliminated from the PMV by topical capsaicin or CSMG, but not TSV, along with the sympathoadrenal response to hypoglycemia (5,19,42). Spinal afferents are known to innervate a number of visceral organs and associated vasculature, and while most often associated with mechanoreception or nociception, there is evidence for their association with a variety of chemoreceptors, e.g., osmoreceptors (43) and glucose sensors (44).

Why the CNS requires PMV glucose sensory input for slow-onset hypoglycemic detection when it possesses a host of different glucosensing neurons remains unclear. Perhaps CNS glucose sensors are insensitive to slow or small glycemic changes, thereby serving as a "fail safe" mechanism for catastrophic declines in glucose. Decreasing the responsiveness of these neurons to any single molecule might, in turn, allow them to respond or integrate a variety of incoming signals to help achieve the appropriate motor response. Indeed, many of these neurons have been characterized as metabosensors responding to a variety of substrates and signaling molecules (45,46). As such, their role in glycemic detection may be secondary to other homeostatic parameters, e.g., energy expenditure. Alternatively, central glucose sensors may serve a synergistic and equally important role with peripheral glucose sensors in responding to hypoglycemia. For example, central glycermia may serve as a reference for integrative elements of the brain against which peripheral glycemic input is measured to establish appropriate CRRs. In this capacity, central glucose sensors might also act primarily to suppress CRRs once euglycemia is reestablished following a hypoglycemic event. This would further explain why clamping either portal-mesenteric or brain glycermia during general systemic hypoglycemia is equally effective in blunting the CRR (47,48).

What is clear from experiment 3 is that the brain can defend itself against large, rapid declines in blood glucose without portal-mesenteric glucose sensory input. Hindbrain Fos activation, as well as the hormonal CRRs, were unaffected by CSMG following rapid-onset hypoglycemia (Figs. 5 and 6). Since visceral spinal afferents synapse with secondary spinal neurons projecting to the hindbrain, these lesions most likely leave hindbrain neurons intact. Ritter et al. (31) have shown that neurons in the hindbrain respond to direct application of the 5-thioglucose (a glucopenic agent). Given that Fos expression in rapid- and slow-onset hypoglycemia occurs in the same loci, i.e., AP, NTS, and DMX, it may be that when there is a catastrophic decline in blood glucose, the same neurons that receive input from the PMV are fully capable of initiating a CRR without that input. However, it is not certain that these are the same neurons or that they are necessarily responding directly to local hypoglycemia; this hypothesis remains to be tested. Elucidating the neurons involved will require phenotyping, neuroanatomical tracing, and markers of neuronal activation. While Fos provides one measure of neuronal activation, it does not always correlate with neuronal firing rate, rather c-fos transcription results from...
elevated intracellular calcium subsequent to receptor activation (49).

In summary, peripheral glucose sensory input from the PMV is essential to activate hindbrain neurons in response to slow-onset hypoglycemia. Furthermore, PMV sensory neurons that provide hypoglycemic input to the hindbrain are not vagal in origin, but rather they appear to be spinal afferents. These findings are consistent with our earlier observations showing that portal-mesenteric denervated and CSMG, but not vagotomized, animals display diminished CRRs to slow-onset hypoglycemia (5,17,19). However, when the rate of fall in glycemia is rapid, i.e., 0.2 mmol/L/min, activation of both hindbrain neurons and CRRs are independent of portal-mesenteric glucose sensory input. Given the frequency of slow-onset hypoglycemia among insulin-dependent patients with diabetes (50), the PMV–hindbrain–sympathoadrenal glucosensory neural network constitutes a critical element in the body’s defense against insulin-induced hypoglycemia.

Figure 5—Arterial glucose (A), epinephrine (B), and norepinephrine (C) during a hyperinsulinemic–euglycemic clamp (−45 to 0 min) and during a rapid-onset (−0.16 mmol/L/min) hyperinsulinemic–hypoglycemic clamp (0–110 min). Glucagon (D) at the end of the basal (0 min) and hypoglycemic (110 min) periods, and glucose infusion rate (E) during euglycemia (−45 to 0 min) and deep hypoglycemia (60–110 min). Open circles and bars represent control, and closed circles and bars represent CSMG. All values are expressed as mean ± SEM. CON, control; GINF, glucose infusion rate; HYPO, hypoglycemia.

Figure 6—Fos-immunoreactive neurons within the NTS, DMX, and AP sampled at the end of a rapid-onset (−0.16 mmol/L/min) hypoglycemic clamp (110 min) (A). Open bars represent control, and closed bars represent CSMG. Photomicrographs of representative hindbrain sections for control (B) and CSMG (C) with the AP, NTS, DMX, hypoglossal nucleus, gracile nucleus, and central canal indicated. All values are expressed as mean ± SEM. C, central canal; CON, control; GR, gracile nucleus; XII, hypoglossal nucleus.
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