The physiology and pathophysiology of the neural control of the counterregulatory response

Craig Beall, Michael L. Ashford, and Rory J. McCrimmon
Medical Research Institute, Division of Cardiovascular and Diabetes Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom
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Beall C, Ashford ML, McCrimmon RJ. The physiology and pathophysiology of the neural control of the counterregulatory response. Am J Physiol Regul Integr Comp Physiol 302: R215–R223, 2012. First published November 9, 2011; doi:10.1152/ajpregu.00531.2011.—Despite significant technological and pharmacological advancements, insulin replacement therapy fails to adequately replicate β-cell function, and so glucose control in type 1 diabetes mellitus (T1D) is frequently erratic, leading to periods of hypoglycemia. Moreover, the counterregulatory response (CRR) to falling blood glucose is impaired in diabetes, leading to an increased risk of severe hypoglycemia. It is now clear that the brain plays a significant role in the development of defective glucose counterregulation and impaired hypoglycemia awareness in diabetes. In this review, the basic intracellular glucose-sensing mechanisms are discussed, as well as the neural networks that respond to and coordinate the body’s response to a hypoglycemic challenge. Subsequently, we discuss how the body responds to repeated hypoglycemia and how these adaptations may explain, at least in part, the development of impaired glucose counterregulation in diabetes.

hypoglycemia; hypothalamus; hypoglycemia unawareness; epinephrine; glucagon

Type 1 diabetes mellitus (T1D) is characterized by an absolute requirement for insulin replacement therapy that follows the loss of endogenous insulin secretion due to an autoimmune process. Despite significant technological advancements, insulin replacement therapy fails to adequately replicate β-cell function, and so glucose control in T1D is frequently erratic. A major consequence of this is the experience of intermittent hypoglycemia, which is associated with significant long-term physical and psychological morbidity (79). Indeed, people with T1D fear hypoglycemia to the same extent as sight-threatening complications.

On average, patients with T1D will experience two bouts of moderate hypoglycemia per week and one bout of severe hypoglycemia annually (35). During moderate hypoglycemia, individuals experience both autonomic (e.g., sweating, palpitations) and neuroglycopenic symptoms (e.g., confusion, disorientation), which, with progressively more severe hypoglycemia can culminate in seizures, coma and, in very extreme cases, brain damage and death. Moreover, the risk of severe hypoglycemia is increased in those individuals who experience repeated hypoglycemia. This occurs because repeated bouts of hypoglycemia provoke a change in counterregulatory hormone and symptomatic responses, such that they are both reduced in magnitude and initiated at lower glucose levels; a condition referred to as impaired hypoglycemia awareness (IHA), which affects around 25% of people with type 1 diabetes (48). Understanding the mechanisms that result in IHA in T1D is critical to the development of better therapies for hypoglycemia avoidance and restoration of the glucose counterregulatory response (CRR).

Physiology of Hypoglycemia

In healthy individuals, insulin secretion from β-cells in the pancreas is inhibited as glucose levels fall below ~4.4 mM. This results in loss of a repressive effect of insulin, GABA, and zinc on α-cell function (120, 121), rapidly increasing glucagon secretion (59). Glucagon acts on the liver to increase hepatic glycogenolysis and gluconeogenesis. If glucose levels fall further (<3.8 mM), epinephrine and norepinephrine are released both from the adrenals and directly into interstitial fluid from nerve terminals exerting a tonic influence on glucose levels by further suppressing insulin secretion, increasing glucagon secretion and decreasing peripheral glucose utilization in muscle and increasing lipolysis in fat (5). Additional responses include growth hormone and cortisol secretion, which occurs below ~3.7 mM (43, 84, 105) and are initiators of the adaptive response to hypoglycemia (such as during prolonged starvation), as the glucose-raising actions are much slower in onset (several hours). These hormone responses stimulate lipolysis, ketogenesis, and gluconeogenesis. For nondiabetic healthy individuals, this system has several fail-safes, meaning that hypoglycemia is rarely experienced and would only occur during starvation or ultra-endurance sports.

The maintenance of euglycemia is achieved by the complex integration of various glucose-sensing systems in both the
periphery and central nervous system (CNS). Fluctuations in peripheral glucose are detected by glucose-sensing neurons in the oral cavity, gut, portal/mesenteric vein (PMV), and carotid body. PMV neurons detect changes in blood glucose prior to entry into the liver from the gut. This information is then relayed through the vagus nerve and spinal cord to the hindbrain and from there to higher brain centers, such as the hypothalamus (119). In addition, the hypothalamus, due to its location adjacent to the third ventricle (3V) and median eminence, may sample factors from peripheral circulation, including glucose, as well as hormones, such as insulin and leptin. Integration of these glucose-sensing systems (Fig. 1) is discussed below; however, it is important to note that this homeostatic mechanism appears to fail at several stages in diabetes.

Abnormal Hypoglycemia Detection in Type 1 (and Type 2) Diabetes

Unregulated hyperinsulinemia. The first major abnormality in diabetes is unregulated hyperinsulinemia caused by either injected insulin or by ingestion of oral hypoglycemic agents that stimulate insulin secretion by a nonglucose-dependent mechanism (i.e., sulfonylureas). This provides a continuous glucose-lowering stimulus by increasing glucose uptake into muscle, fat, and liver and limits gluconeogenesis by repressing the gluconeogenic genes PEPCK and G6Pase. Insulin also inhibits lipolysis, preventing the release of alternate fuel stores and suppresses glucagon secretion through direct action on pancreatic α-cells (30) or by central mechanisms (94). Thus, the failure of insulin to dissipate is the first counterregulatory defect in T1D.

Altered glucagon secretion. For reasons still not completely understood, glucagon secretion in response to hypoglycemia is lost in T1D and advanced T2D (107). Alpha-cell glucagon secretion is directly regulated by a number of products of the pancreatic islet, as well as via extensive intra-islet and CNS neural inputs (113). Studies in animal models, such as the mouse with genetic ablation of insulin receptors from the pancreatic α-cell, confirm a role for insulin in the direct regulation of glucagon release during hypoglycemia (65), while there is also strong support for independent regulatory roles for zinc (121), and GABA (120) coreleased with insulin from the β-cell, as well as for the δ-cell releasable product, somatostatin (50). Others have noted evidence for an intra-islet sympathetic neuropathy (86), or a central defect, with selective loss of glutamate release in mouse hypothalamic SF1 neurons leading to a profound suppression of glucagon release during hypoglycemia or fasting (116). In addition, in non diabetic models, recurrent hypoglycemia (RH) provokes suppression of glucagon secretion, thought to be via a central action, while hypothalamic modulation can restore this response (41). However, in human studies in individuals with T1D, hypoglycemia avoidance restores autonomic but not glucagon secretion to subsequent hypoglycemia (44). Also in T1D, loss of glucagon secretion to hypoglycemia parallels loss of c-peptide secretion in humans (46) and animals (60), while induction of hypoinsulinemic hypoglycemia restores glucagon secretion in an animal model of T1D (77). Thus, in T1D, evidence strongly favors an intra-islet factor as the likely mediator of defective glucagon secretion during hypoglycemia. Further evidence is required to clarify whether this relates to a single or multiple products of the pancreatic β-cell. Interestingly, glucagon responses to other stimuli, such as amino acids and exercise, persist in T1D, while epinephrine-induced glucagon secretion is enhanced in T1D (51), indicating that α-cells are still functional, albeit insensitive to insulin-induced hypoglycemia.

Abnormal catecholamine secretion. The third major abnormality in T1D and advanced T2D is an attenuated epinephrine response to hypoglycemia, which is reduced in magnitude and activated at a lower glucose concentration (107). As for glucagon, epinephrine responses to other stimuli, such as exercise, persist (7, 57). Importantly, during total insulin deficiency, control of hepatic glucose output during hypoglycemia and exercise is mediated primarily by catecholamines rather than glucagon (7, 97); thus, maintenance of this response is critical. Adrenal output is under the control of the autonomic nervous system (17, 111), which is, in turn, regulated by various physiological and pathophysiological stimuli (17). A single episode of hypoglycemia is sufficient to engender defective epinephrine (and glucagon) secretion to a subsequent bout of hypoglycemia in healthy humans (55), with similar outcomes observed in individuals with hyperinsulinemia and poor glucose control.

Unlike glucagon, the defect in epinephrine secretion is primarily mediated by altered sympathetic drive. Indeed, there is strong evidence to suggest the central nervous system plays an important role in the regulation of the CRR to hypoglycemia.

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**Figure 1. Glucose-sensing neural network.** The ventromedial hypothalamus (VMH) projects to the arcuate nucleus (ARC), dorsomedial nucleus (DMN), lateral hypothalamic area (LHA), and paraventricular nucleus (PVH) to integrate glucose sensing. Regions containing intrinsically glucose-sensing cells are highlighted in red. Hypothalamic glucose sensing is influenced by, but not limited to, inputs from the medial amygdalar nucleus (MAN), PVH, and hindbrain (shown in green), with both norepinephrine (NE) and epinephrine (EPI) neurons projecting to the PVH. Some hindbrain EPI neurons also express neuropeptides Y (NPY). Integration of information from these centers occurs in the hindbrain, which, in turn, provides input to motor neurons engaged with peripheral outputs to the pancreas and adrenal gland. Hindbrain neurons also directly interact with the portal/mesenteric vein (PMV) glucose-sensing neurons and pancreas (not shown).

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and is the main driver of defective CRR following RH. In general, the greater the depth, duration, or frequency of hypoglycemic episode, the greater the loss of the CRR, an outcome deemed to be mediated by defective central sensing of hypoglycemia. In summary, although there are defects at the islet level in T1D, which contribute to defective CRR, it is thought that central mechanisms play the dominant role in the changes provoked by RH, as defective CRR is observed in nondiabetic models. Interestingly, this phenomenon has been reported in various vertebrate and nonvertebrate models (e.g., human, rhesus monkey, dog, rat, mouse, and Caenorhabditis elegans) (54, 55, 61, 80, 98), indicating that its occurrence is highly conserved among different species.

**Neural Networks of Hypoglycemia Detection**

Evidence that fluctuations in glucose was used by the brain as a signal was first suggested in 1953 by Jean Mayer (75). Subsequent studies of Oomura et al. in 1969 (89) indicated that neuronal activity was directly altered by physiological changes in extracellular glucose. The importance of discrete brain regions, such as the ventromedial hypothalamus (VMH), to the detection of hypoglycemia was demonstrated by Borg and colleagues in a series of in vivo studies in the Sprague-Dawley rat in the 1990s (9–11), since confirmed by many others (8, 32, 62, 78). Other regions of the forebrain and hindbrain also contain specialized glucose-sensing neurons, such as the arcuate nucleus (ARC), lateral hypothalamic area (LHA), dorsomedial nucleus (DMN), and amygdala (6, 108). Tracer studies suggest these centers may integrate to form a glucose-sensing network (Fig. 1). For example, VMH neurons project to the ARC, DMN, LHA, and paraventricular hypothalamus (PVH) (21). The PVH, LHA, and to a lesser extent the DMH are labeled by pseudorabies virus following its intraventricular hypothalamus following its intraventricular hypothalamus (PVH) and amygdala (6, 108). Tracer studies suggest these centers may integrate to form a glucose-sensing network (Fig. 1). For example, VMH neurons project to the ARC, DMN, LHA, and paraventricular hypothalamus (PVH) (21). The PVH, LHA, and to a lesser extent the DMH are labeled by pseudorabies virus following its introduction into the pancreas, indicating that these neurons can interact with premotor neurons controlling peripheral outputs (49, 70). In contrast, VMH neurons are not labeled by retrograde virus injections into the pancreas or adrenal glands (16, 66), indicating that they do not project directly onto peripheral outputs but are more likely third-order neurons (16). A further level of control is mediated by hindbrain integration into both hypothalamic and peripheral outputs. For example, hindbrain neuropeptide Y (NPY) neurons are important mediators of PVH corticotropin-releasing hormone (CRH) secretion by glucoprivation (47), and a proportion of the hypothalamic catecholamine release by hypoglycemia is mediated by hindbrain neurons (93), although local VMH norepinephrine release may also occur (45). In addition, hindbrain neurons are directly linked with PMV glucose sensors (1). Rat studies have suggested that PMV neurons must be engaged for the full activation of the CRR (74), suggesting that hypothalamic glucose-sensing regions can be fed information from peripheral glucose sensors via the hindbrain. However, the role of PMV neurons in CRRs in humans remains controversial [see commentary (36)], with some studies showing little or no effect (100), while others have shown amplification (39) or suppression (110) of the CRR/symptomatic response to hypoglycemia. For a more comprehensive review of neural networks involved in the control of glucose counterregulation, see Ref. 119. Taken together, these animal model studies support the existence of a neural network of central and peripheral glucose sensors that act in concert to maintain glucose homeostasis and coordinate the organism’s response to changes in glucose levels. The architecture of the system suggests that forebrain sensors play the major role in adaptations to recurrent insulin-induced hypoglycemia (IH).

**Intracellular Glucose-Sensing Mechanisms**

Fluctuation in brain extracellular glucose levels is directly sensed by central neurons, and this, in turn, regulates their electrical activity. Detailed molecular analysis of hypothalamic glucose-sensing (GS) neurons suggest that they resemble the α- and β-cells of the pancreas (3, 38). GS neurons increase or decrease their firing rate in response to increasing levels of glucose, termed glucose-excited (GE) or glucose-inhibited (GI) neurons, respectively, and are shown schematically in Fig. 2. These neurons predominantly express the glucose transporter GLUT3, and to a lesser extent GLUT2, and glucokinase (GK) (63), the putative “gatekeeper” of glucose-sensing mechanisms. GK catalyzes the phosphorylation of glucose to produce glucose-6-phosphate, with subsequent metabolism yielding an increased [ATP]/[ADP] ratio, which directly modulates the activity of ion channels present in the plasma membrane (3). The exact molecular identity of the ion channel(s) responsible for the regulation of GI neuron excitability has yet to be conclusively determined, with speculation that it is the cystic fibrosis transmembrane regulator (CFTR) (87) or a tandem-pore K⁺ channel (18). The sulphonylurea receptor type 1 (SUR1)-containing ATP-sensitive K⁺ channel (Kₐ₅₃) is also expressed in the majority of GE, and a significant proportion of GI, neurons (63). This channel is essential for coupling cellular energy status to electrical activity of GE neurons (3, 4). Furthermore, Kₐ₅₃ channels in VMH neurons are particularly important for the initiation of CRRs. Inhibition of channel activity with the SUR1-specific Kₐ₅₃ blocker glybenclamide blunts the CRR, whereas local VMH or systemic application of a SUR1-specific Kₐ₅₃ channel opener (NN414) or diazoxide amplifies CRR (40, 42, 76). Thus, it is likely that direct changes in electrical activity of GE and GI neurons are required for the full activation of the CRR.

Recent evidence has emerged, which suggests that the intracellular energy-sensing kinase, AMPK is essential for glucose-sensing in hypothalamic neurons. AMPK is a heterotrimer comprising a catalytic α-subunit with regulatory β- and δ-subunits, with multiple isoforms of each (α₁, α₂, β₁, β₂, δ₁–δ₃). Similar to Kₐ₅₃, AMPK is regulated by adenine nucleotide ratios (23). Both catalytic subunits are present in the hypothalamus with the α₂ subunit more important in relation to body weight regulation and glucose sensing (81, 83). AMPK is also important in the acute regulation of GI neuron excitability via the production of the nonclassical neurotransmitter, nitric oxide (19, 85), although this latter study failed to show direct regulation of GE neuron excitability by acute pharmacological activation of AMPK (85). In contrast, genetic ablation of the α₂ subunit of AMPK in mouse GE neurons and pancreatic β-cells completely abolished their sensitivity to acute hypoglycemia (8, 32) but had no effect on responses of these neurons (POMC and AgRP) to the hormones leptin and insulin (32). These latter data strengthen the hypothesis that hypothalamic GE neurons use the same molecular components as pancreatic β-cells to sense changes in extracellular glucose. However, important differences between the two cell types also exist, such as the glucose range to which they respond electrically (~0.1–2.5 mM in GE neurons vs. 4–8 mM in β-cells) and that in GS neurons, but not β-cells, glucose sensing is modulated by lactate.
Neural Mechanisms Mediating Defective Glucose Counterregulation

Exposing hypothalamic but not hindbrain glucose-sensing regions in rats to recurrent glucoprivation induces defective counterregulation (102) and induces a left shift in the glucose-responsiveness of VMH GE neurons (i.e., are not hyperpolarized until glucose levels fall further) (64). These findings among others suggest that altered hypothalamic glucose sensing may underpin the development of defective CRR in response to hypoglycemia in T1D. Several mechanisms have been suggested to explain the neural malfunctions that develop to mediate defective counterregulation (summarized in Fig. 3).

Enhanced glucose metabolism. One theory postulates that local or regional uptake and metabolism of glucose by GS neurons is enhanced following RH. Evidence from rat models has shown that prolonged hypoglycemia increases GLUT1 and GLUT3 expression, raises glucose levels, and enhances glucose uptake in brain (37, 109). In addition, RH increases GK mRNA (64) and enhances hexokinase activity (90) in the hypothalamus, while direct pharmacological enhancement of VMH GK activity suppresses the CRR to hypoglycemia (69). Increased glucose uptake and metabolism would provide better support for intracellular energy status, but also reduce the sensitivity of glucose-sensing neurons to hypoglycemia if this is an ATP/ADP-dependent mechanism. This is an attractive hypothesis, but not all studies have observed enhanced brain glucose uptake (80), especially in human studies (106), and it is not clear why there should be regional differences (hypothalamic vs. hindbrain) in this adaptation to RH.

Alternate fuel usage. It has also been suggested that alternative fuel usage, particularly a switch from glucose to lipid metabolism or enhanced glycogen content, may underlie defective CRRs (73, 95, 96). Glycogen is stored in limited deposits in astrocytes and is mobilized during energy stress, being released as the transportable lactate, which can support neuronal activity (15, 112). Decreased brain glycogen content has been reported in mouse, rat, and

![Fig. 2. Intracellular neuronal glucose-sensing mechanisms. Under conditions of hypoglycemia, the fall in extracellular glucose leads to a concomitant reduction in glucose intracellularly. A: for glucose-excited neurons, this results in increased AMP:ATP and ADP:ATP ratios leading to activation of AMPK and K_ATP, respectively. Increased K_ATP and subsequent hyperpolarization, in turn, inactivate voltage-dependent Ca^{2+} channels (VDCC) leading to reduced intracellular Ca^{2+} and decreased neurotransmitter release. B: conversely, in glucose-inhibited neurons, although the low glucose also causes increased AMP:ATP ratio and activation of AMPK, the AMPK activity is thought to inhibit CFTR, decreasing Cl^- flux leading to activation of VDCC and an increase in neurotransmitter release. AMPK can also activate nitric oxide synthase (NOS) leading to the generation of nitric oxide (NO). GK, glucokinase; NT, (neurotransmitter).](http://www.ajpregu.org)

![Fig. 3. Summary of the neuronal adaptations that may underlie the development of defective glucose counterregulation in type 1 and type 2 diabetes.](http://www.ajpregu.org)
human studies during hypoglycemia (20, 56, 68), indicating that glycogen is utilized and may act as a temporary energy source. Indirect measurement of glycogen stores by nuclear magnetic resonance in humans has indicated that these may be enhanced following RH (115). This “glycogen supercompensation” to a single or repeated bouts of hypoglycemia has also been reported in mouse (20, 92), and in a rat model of RH, although no absolute increase in glycogen content was observed, the rate of glycogen resynthesis after hypoglycemia was enhanced (56). Interestingly, increased brain glycogen concentration in rats treated with an inhibitor of glycogen phosphorylase was demonstrated to support neuronal activity on the initiation of hypoglycemia (112). Such an outcome might be expected to delay the responses of GS neurons to hypoglycemia and impede initiation of the CRR. In support of this notion, Alquier et al. (2), using a model of repeated 3V 2-deoxyglucose injection to induce recurrent glucoprivation, observed elevated hypothalamic glycogen levels, which correlated with delayed activation of AMPK. This suggests that the mobilization of glycogen on induction of glucoprivation and suppressed local AMPK activity may be linked to delayed detection of glucoprivation. It is important to note that other studies have failed to find this glycogen supercompensation, and brain glycogen represents a very small substrate pool compared with muscle or liver (56). Moreover, elevation of brain glycogen levels posthypoglycemia is not persistent and returns to baseline when CRRs are still defective (56).

**AMP-activated protein kinase.** The idea that AMPK activation is a key component of the CRR to hypoglycemia, which may become defective in RH, is intriguing. Direct pharmacological activation of AMPK, specifically in the VMH, augments the CRR to IIH (41), whereas suppression of VMH AMPK activity attenuates this response (81). Importantly, activation of VMH AMPK enhances CRRs in rats with defective counterregulation (78) and in a rat model of T1D (41). At present, it is unclear how AMPK transduces the hypoglycemic signal to initiate increased counterregulatory hormone release, although direct regulation of neuronal excitability is a strong possibility as neuronal AMPK has been shown to phosphorylate CFTR, thus regulating GI neuron excitability (87) and to be required for GE neuron GS (29). AMPK also phosphorylates Kv2.1 (58), NMDA (114) and GABA<sub>B</sub> receptors (67), and it alters intracellular metabolism via the peroxisome proliferator-activated receptor gamma coactivator 1 (53), although the contribution of AMPK to these ion channels and signaling pathways has not yet been explored in relation to hypoglycemia detection and glucose counterregulation. However, direct suppression of AMPK by enhanced metabolism (such as increased lactate release from glycogen stores in astrocytes) may contribute to defective counterregulation. A possible scenario is that following RH, neuronal AMPK activation is delayed, which, in turn, modifies GS neuron responses to hypoglycemia, resulting in altered synaptic transmission and diminished counterregulatory hormone release. The phenotype of the neurons associated with AMPK activation and altered CRRs has yet to be determined, although several neurotransmitters and neuropeptides have been implicated in suppression or amplification of CRRs.

**Altered hypothalamic neurotransmission.** GABA is the major inhibitory neurotransmitter in the CNS and is extremely important for mediating fast changes in synaptic transmission. Local VMH blockade of GABA<sub>A</sub> receptors with bicuculline amplified, whereas stimulation of GABA<sub>B</sub> receptors with muscimol, suppressed CRRs (27). Furthermore, K<sub>ATP</sub> channel modulators regulate VMH GABA levels, such that K<sub>ATP</sub> activation with diazoxide decreased GABA levels and increased glucagon and epinephrine responses to hypoglycemia. Conversely, glybenclamide increased VMH GABA levels and suppressed glucagon and epinephrine responses. Interestingly, diazoxide did not amplify glucagon and epinephrine secretion when coapplied with muscimol, and glybenclamide did not suppress CRR in the presence of bicuculline (25), suggesting that, functionally, K<sub>ATP</sub> channels are upstream of GABA<sub>A</sub> receptors, i.e., on the presynaptic, GABA-secreting neurons. Subsequently, it was shown that although acute hypoglycemia decreases VMH GABA levels, repeated hypoglycemia prevents the fall in GABA (24), and diabetic biobreeding and streptozotocin-induced diabetic rat models have increased basal GABA levels in the VMH (26). This was associated with increased expression of the GABA-synthesizing enzyme, GAD65, specifically in the VMH. Taken together, it is tempting to speculate that K<sub>ATP</sub>-positive VMH (GE) neurons present are GABAergic and that these are the neurons that fail to hyperpolarize in response to hypoglycemia after antecedent hypoglycemia. In addition, the abnormally high VMH GABA levels in diabetic models or following RH, suggest that VMH GABA may be directly modulating the suppression of CRRs. However, the exact neural pathway from VMH GABAergic neurons to the suppression of CRR has not been mapped. Interestingly, the VMH GABAergic neurons are spatially separated from glutamatergic neurons, the main excitatory neurotransmitter in the CNS. The majority of the GABAergic neurons are located in the ARC and encapsulating the ventromedial nucleus (VMN), where they surround the glutamatergic neurons (34, 91, 116, 118). Glutamate acts on NMDA, AMPA, and kainate receptors, which are also required for glucose counterregulation (116), as afferent of the vesicular glutamate transporter VGLUT2 causes fasting-induced hypoglycemia via defective glucagon secretion (116). This study induced genetic loss of VGLUT2 in stereoidogenic factor-1 (SF-1) neurons. SF-1 neurons are essential for the development for the mediobasal hypothalamus and are a reasonably specific marker for the VMN. The exact functional relationship between the GABAergic and glutamatergic neurons and how this regulates the CRR remain to be determined.

Recent evidence has also suggested that the monoamine neurotransmitter, serotonin, is involved in CRRs. A recent study in rats has shown that the selective serotonin reuptake inhibitor (SSRI) sertraline augments epinephrine, norepinephrine, and glucagon responses to hypoglycemia and prevents blunted epinephrine responses following RH (103). Furthermore, studies in non-diabetic and T1D humans have demonstrated a similar amplification of catecholamine responses following SSRI treatment, although no change in glucagon secretion was observed (12, 13). Importantly, these studies demonstrated that muscle sympathetic nerve activity was enhanced by SSRI treatment, indicating that enhancement of CRR is mediated, at least in part, by central mechanisms (12, 13). It should be noted that, contrary to the above reports, isolated case studies have demonstrated that some patients have experienced rapid increases in hypoglycemia frequency, resulting in impaired hypoglycemia awareness on commencement of SSRIs (33, 104). Thus, whether modulation of this neurotransmitter system is a feasible therapeutic target requires further research.

**Altered VMH neuropeptide input.** Corticotrophin-releasing hormone (CRH) and urocortins 1–3 (UCN1–3) are important
regulators of the neuroendocrine stress response and function through their actions at the CRHR1 and CRHR2 receptor subtypes, respectively. CRH via the CRHR1 amplifies, whereas UCN through the CRHR2, suppresses the CRR to hypoglycemia (82). Furthermore, the VMH and tuberal area are densely innervated by UCN3 nerve terminals with the main projections from the parvicellular part of the PVH (28) and to a lesser extent the medial amygdalar nucleus (MAN) (122). UCN3-containing neurons are also present in the hypothalamic medial preoptic nucleus and rostral perifornical area. UCN3 action in the VMH blunts glucose sensing, as priming GE and GI neurons with UCN3 lowered the glucose threshold for altering their electrical activity, such that glucose levels had to decline further before any significant change in membrane potential occurred (82). The MAN expresses GK, although the GK-expressing neurons are exclusive of UCN3 mRNA, suggesting that the UCN3 neurons may not be directly glucose sensing. Furthermore, local glucoprivation in the MAN does not elicit a CRR, although MAN glucoprivation during mild systemic hypoglycemia amplifies the CRR, suggesting that the MAN does influence the CRR (122). At present, it is not known whether the MAN is directly involved in the development of IHA or, indeed, what signals activate the UCN3 neurons that project to the VMH.

Opioid receptors. Opioids are important mediators of stress and increase during hypoglycemia (22, 88), exercise (52), and psychological stress (72). Studies by Caprio et al. (22) demonstrated that naloxone treatment to block opioid receptors in humans amplified the epinephrine and cortisol responses to hypoglycemia. More recently, naloxone confusión in nondiabetic human subjects during antecedent hypoglycemia was shown to prevent the development defective CRRs The mechanisms by which opioid receptor blockade prevents defects in glucose counterregulation are not known and could occur through a hypothalamic, pituitary, or adrenal effect (23). In support of the former, naloxone was recently shown to prevent the induction of mouse hypothalamic Pdk4 and Angptl4 transcripts by RH. (95). These genes are thought to induce metabolic switching from carbohydrate to fat metabolism (71), suggesting opioid antagonism prevents this adaptation leading to preserved CRR for reasons discussed above. Further research is required to elucidate the mechanisms by which opioid antagonists prevent the development of defective CRR.

Hindbrain contribution to glucose counterregulation. The hindbrain contains neurons that are activated (as measured by c-Fos immunoreactivity) by glucoprivation (99). Recent studies have shown that these neurons contain functional K\textsubscript{ATP} channels (31) and express GLUT3, GLUT4, GK, and monocarboxylate transporters (MCTs) (117). Repeated 2-deoxy-d-glucose injection attenuates the activation of neurons in the hindbrain (as well as neurons in the forebrain) (101), indicating that reduced network activity is involved in hypoglycemia detection. Furthermore, prior insulin-induced hypoglycemia increases the expression of GK, GLUTs, and K\textsubscript{ATP} (14). This study also addressed the issue of lactate exposure in the hindbrain and demonstrated that lactate suppressed glucose counterregulation, increased hypoglycemic induction of GK, GLUT3/4, and SUR1 mRNA and decreased MCT2 expression. This may indicate a shift toward increased glycolytic flux as an adaptation to hypoglycemia. However, a study examining recurrent glucoprivation using 5-thioglucose (5TG) found that only 3V recurrent 5TG induced defective CRRs, whereas 5TG injected into the hindbrain did not suppress CRRs, indicating that forebrain glucose sensors predominate in the development of IHA (102).

Perspectives and Significance

The neural mechanisms that underlie the detection of hypoglycemia and the development of defective counterregulation clearly require further elucidation. One key area will be the phenotypic characterization of GE and GI neurons present within the hypothalamus and other brain areas, as this will aid the delineation of the neural pathways leading to altered CRRs. It will also be important to determine whether the glucose-sensing mechanism is ubiquitous, the effect of the glucose signal determined instead by other characteristics of the neuron (e.g., the neurotransmitters released or neural pathway into which it feeds), as current evidence suggests or whether different brain regions employ different glucose-sensing mechanisms.

The mechanisms underpinning the development of defective CRR also remain largely unknown. Data supporting metabolic switching, altered fuel transport, and/or stress regulation are inconsistent, and especially in the case of fuel transport and metabolism, are often limited to global measures that may miss important local changes in key brain regions. Determining the molecular adaptive mechanisms in neurons and comparing and contrasting this with those in astrocytes/glial cells within different key glucose-sensing brain regions will also be important. The complex cellular and physiological responses to recurrent hypoglycemia suggest that a number of adaptive processes may be provoked and that these may show differing temporal relationships to the initial hypoglycemic stimulus. Clarifying the nature of these molecular adaptations will be important if we are ultimately to identify potential sites for effective pharmacological intervention.

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