

Glucosensing neurons in the ventromedial hypothalamic nucleus (VMN) and hypoglycemia-associated autonomic failure (HAAF)

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Summary

Hypoglycemia is a profound threat to the brain since glucose is its preferred fuel. Thus, decreases in plasma glucose must be sensed and appropriate hormonal and neuroendocrine responses generated to restore glucose to safe levels (i.e. counterregulatory responses (CRR) to hypoglycemia). Recurrent hypoglycemia impairs these protective mechanisms, resulting in a potentially life-threatening condition known as hypoglycemia-associated autonomic failure (HAAF). During HAAF, the glyceic threshold is reset so that glucose levels must fall further before the CRR is initiated. The brain plays a critical role in sensing hypoglycemia and initiating the CRR. Additionally, many neurons may sense changes in plasma and extracellular glucose. However, the way in which central glucose sensing is integrated to lead to effective initiation of the CRR is unknown. Furthermore, the mechanisms by which this system becomes impaired during HAAF are also unknown. Glucosensing neurons in the ventromedial hypothalamic nucleus (VMN) are poised to serve an integrative function in glucose homeostasis. First, they sense glucose. Second, the VMN receives input from other glucose-sensing areas. Finally, the VMN projects to areas linked to the regulation of the sympathoadrenal system that mediates the CRR. This review discusses VMN glucosensing neurons relative to their capacity to play a role in the regulation of the CRR and the generation of HAAF. Glucosensing neurons in the hindbrain as well as peripheral glucosensors are also considered. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords hypothalamus; hypoglycemia; counterregulatory response; obesity; diabetes; KATP channels

Introduction

Intensive insulin therapy is used clinically to obtain optimal glyceic control in type 1 diabetic patients and to avoid the long-term complications of hyperglycemia [1]. These complications include central and peripheral nervous system damage, kidney disease, and adult-onset blindness [1–3]. However, intensive insulin therapy also causes a clinically adverse effect – hypoglycemia. The Diabetes Control and Complications Trial (DCCT) reported a nearly threefold increase in severe hypoglycemia with intensive insulin therapy compared to conventional therapy [1]. The brain is particularly vulnerable to hypoglycemia since glucose is its preferred fuel [4]. Powerful hormonal and neuroendocrine (sympathoadrenal) counterregulatory mechanisms that prevent and correct hypoglycemic conditions protect the brain from hypoglycemia [5,6]. These corrective mechanisms,

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known as the counterregulatory response (CRR) to hypoglycemia, involve the release of glucagon and corticosterone, as well as increasing epinephrine release from the adrenal medulla. Together, these hormones work to restore blood glucose to acceptable levels. However, these mechanisms can become impaired as a result of repeated hypoglycemic episodes, a potentially life-threatening condition known as hypoglycemia-associated autonomic failure. During HAAF, the glycemic threshold for the CRR shifts to lower glucose levels, and the mechanisms that restore blood glucose and provide warning signals of impending hypoglycemia become severely impaired. As a result, glucose levels may proceed undetected to dangerously low or lethal levels [5,6]. Thus, recurrent hypoglycemia is a major limiting factor in the management of type 1 diabetes mellitus.

Hypoglycemia is associated with altered brain function, brain damage, and death [7]. In fact, even one prior bout of moderate hypoglycemia in adults is sufficient to produce apparent apoptotic cell death in the hypothalamus [8]. Hypoglycemia-induced neuronal damage occurs with prolonged coma-producing hypoglycemia and is most severe in layers III and V of the cerebral cortex, the hippocampus, and the striatum [2,7]. Thus, episodes of severe hypoglycemia have potentially devastating effects on the brain, including impairment of memory and cognitive function. This is especially problematic during nervous system development [7].

The role of the ventromedial hypothalamic nucleus (VMN) in the CRR

That the brain is involved in the initiation of the CRR is clear; however, the mechanisms by which neurons sense and respond to a fall in blood glucose are less certain. The hypothalamus, particularly the ventromedial hypothalamus (VMH), monitors glucose status and initiates a sympathoadrenal response [9,10]. VMH glucosensing neurons increase their action potential frequency in response to iontophoretic application of glucose [11] or peripheral glucose infusion [12,13]. Electrical stimulation of the VMH activates the sympathoadrenal system in a manner similar to that seen during the CRR [14]. Local VMH glucopenia causes the release of the counterregulatory hormones epinephrine and glucagon, while glucose infusion into the VMH suppresses their release during systemic hypoglycemia [10,15]. Glucose infused into the forebrain activates the sympathetic nervous system [16]. On the other hand, VMH lesions decrease sympathetic and increase parasympathetic tone [17]. This increases basal and glucose-stimulated insulin release from the pancreas, as well as increasing pancreatic blood flow [18]. Thus, the VMH is an integral regulator of autonomic activity that directly effects glucose metabolism [17,19]. The VMH also responds to changes in glucose availability. For example, insulin-induced hypoglycemia alters VMH

monoamine metabolism [20] and increases the release of γ -aminobutyric acid (GABA) within the VMH [21]. Clearly, neurons within the VMH are capable of both monitoring changes in glucose balance and initiating an appropriate sympathoadrenal response to alter glucose metabolism. This suggests a critical role for the VMH as a primary center for regulation of glucose homeostasis, especially as it pertains to the CRR.

The VMH consists of two distinct nuclei – the VMN and the arcuate (ARC). Most studies of the VMH do not distinguish between these nuclei. However, in recent years the role of the hypothalamic ARC-paraventricular hypothalamic nucleus (PVN) axis in the regulation of food intake and energy balance has been the focus of considerable work. The PVN and ARC project directly to the sympathetic preganglionic cell bodies in the spinal cord and parasympathetic (vagal) nuclei in the brainstem [22,23]. Thus, the ARC and PVN are two important final common pathways for the sympathoadrenal regulation of energy balance. The precise role of the VMN remains unclear. However, there is compelling evidence that the VMN is an important integrator of glucose homeostasis and perhaps modulates these output nuclei. The VMN possesses receptors for virtually all neurotransmitters and peptides known to influence energy balance and glucose homeostasis. These include receptors for orexins [24], melanocortins [25], neuropeptide Y [26], leptin [27], insulin [28], corticotropin releasing factor [29], cholecystokinin [30], the monoamine transmitters (norepinephrine, epinephrine, dopamine, serotonin [31–33]), GABA [34], somatostatin [35], and the interleukins [36], as well as the newly discovered cannabinoid receptors [37]. Many of these VMN receptors are altered when glucose homeostasis is disturbed [4,31,38]. The VMN also possesses receptors for the sex hormones (e.g. progesterone, estrogen) that may regulate variations in glucose homeostasis and energy balance during the estrus cycle [39,40]. Interestingly, there are significant gender differences in the magnitude of the CRR [41,42]. The VMN projects to autonomic outflow areas of the spinal cord via a brainstem projection to the periaqueductal gray and the reticular formation [19,43]. VMN neurons also project to a variety of hypothalamic regions including the ARC, dorsomedial hypothalamic nucleus (DMN), subparaventricular zone, and preoptic area [19,43–45]. The VMN receives input from limbic regions (e.g. central and dorsomedial amygdalar nuclei [44,46,47]), limbic cortex [48], other hypothalamic nuclei [19,49,50], and brainstem monoaminergic centers [31–33]. Thus, in addition to directly sensing glucose, the VMN is well situated to coordinate sympathoadrenal output in response to limbic, brainstem, and hypothalamic inputs.

VMN glucosensing neurons

For many years, we have known that glucose regulates neuronal activity. Glucose sensors exist both peripherally

and centrally. Peripherally, they are found in the portal vein and carotid body [51–54]. Neurons whose activity is altered by changes in extracellular glucose are widespread throughout the central nervous system [4,55–63]. In 1964, two separate laboratories described neurons within the hypothalamus that change their action potential frequency in response to changes in plasma glucose [55,64]. Later studies showed that direct application of glucose increased the action potential frequency of ‘glucose-responsive’ or GR neurons, while decreasing the action potential frequency of ‘glucose-sensitive’ or GS neurons [61]. GR neurons utilize the ATP-sensitive K^+ (KATP) channel to sense glucose [56]. That is, similar to the pancreatic β -cell, rising glucose levels increase the intracellular ATP to ADP ratio and close the KATP channel. This depolarizes the β -cell and activates voltage-sensitive calcium channels that mediate insulin secretion [65]. However, glucose-initiated depolarization of GR neurons increases action potential frequency. Less is known about the mechanism by which GS neurons sense glucose, although decreased activity of the Na^+/K^+ ATPase with decreased ATP has been suggested [61].

While it is attractive to hypothesize that GR and GS neurons play a role in glucose homeostasis, it is important to note that the majority of studies of these neurons used glucose levels outside the physiologic range (0 to 10 or 20 mM) [27,28,56,61,66,67]. An extracellular glucose level of 0 mM would be incompatible with life. Moreover, virtually all studies of the central nervous system use 10 mM glucose or higher in the solutions that bathe neurons. However, recent evidence demonstrates that

brain glucose concentrations are significantly lower than 10 mM [12]. For example, at 7.6 mM plasma glucose in a fed rat, extracellular brain glucose was only 2.5 mM. Decreasing plasma glucose to 2 to 3 mM (~40 mg/dL) or increasing it to 15.2 mM (~270 mg/dL) caused brain glucose levels of 0.16 mM and 4.5 mM respectively [12]. The former levels of plasma glucose are sufficient to initiate the CRR, while the latter occur during untreated type 1 diabetes mellitus [1,5]. One caveat must be mentioned. If the neurons under investigation are in or near a region lacking a blood–brain barrier, brain glucose levels may approximate plasma levels. Neurons in these regions may be normally exposed to higher levels of extracellular glucose. ARC neurons, which have processes that extend into one such area, the median eminence, may be included in this category [68]. However, under physiologic conditions the majority of neurons are unlikely to be exposed to extracellular glucose levels above 5 mM.

Recently, we described five novel subtypes of VMN glucose-sensing neurons that interact via a complex convergence of pre- and postsynaptic influences (Figure 1). These neuronal subtypes respond to physiologically relevant changes in extracellular glucose [63]. Such sensitivity is significant because, as mentioned above, previous studies of glucose-sensing neurons utilized nonphysiological levels of extracellular glucose [61,66]. Thus, our data showing that approximately 50% of VMN neurons in brain slices altered their activity as extracellular glucose levels either increased to 5 mM or decreased to 0.1 mM from a steady state level of 2.5 mM support the hypothesis

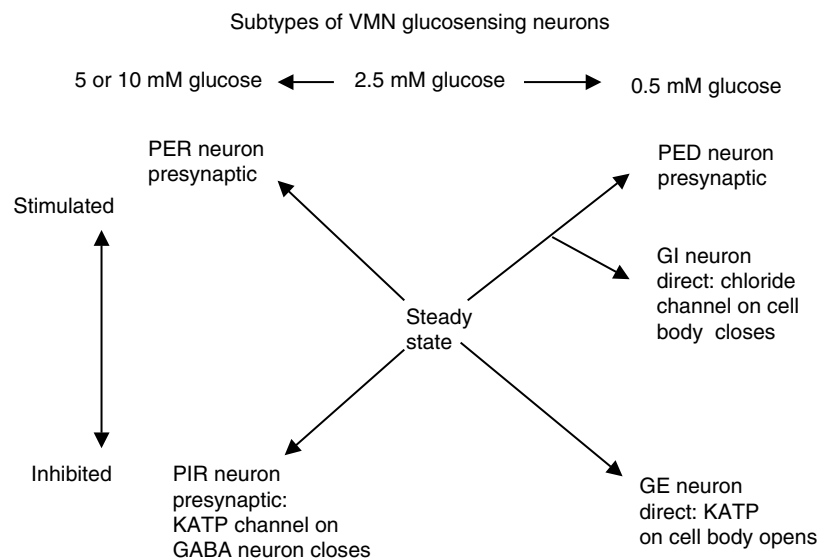


Figure 1. Schematic overview of the subtypes of VMN glucosensing neurons and mechanisms by which they sense glucose. Glucose concentration increases from right to left and neuronal activity increases from bottom to top of the diagram. The mechanism is listed below the neuronal subtype. Thus, PED and GI neurons are shown to the upper right, indicating that they are excited, presynaptically or via closure of a chloride channel respectively, as extracellular glucose levels decrease. GE neurons are shown on the lower right, indicating that they are inhibited by decreased extracellular glucose directly via opening of a KATP channel. PIR neurons are shown on the lower left, indicating that they are inhibited as extracellular glucose levels are raised (presynaptically via closure of a KATP channel on a GABA neuron), and PER neurons are shown on the upper left, indicating that they are (presynaptically) excited as extracellular glucose is raised [63]. Copyright© 2001 American Diabetes Association published in *Diabetes* 2001; 50: 2673–2681. Reprinted with permission from *The American Diabetes Association*

that the VMN is a physiologically relevant integrator of glucose homeostasis.

Two of the five subtypes of physiologically relevant VMN glucose-sensing neurons that we found responded directly to a decrease in extracellular glucose. Glucose-excited (GE) neurons increase and glucose-inhibited (GI) neurons decrease their action potential frequency as extracellular glucose increases from 0.1 mM to 2.5 mM [63]. While the action potential frequency of GI neurons is inversely related to extracellular glucose levels as defined for GS neurons, GI neurons do not appear to be similar to GS neurons. For example, the exact mechanism whereby GI neurons respond to glucose is uncertain; however, our data indicate that they may use a chloride channel to sense glucose. Furthermore, GS neurons have a high action potential frequency in 0 mM glucose and become quiescent when glucose levels are shifted directly to 10 mM. In contrast, GI neurons are quiescent only in 2.5 mM glucose with a high action potential frequency in both 0.1 and 10 mM glucose. The excitatory response to 10 mM glucose in GI neurons is due to presynaptic modulation by glucose as discussed below. On the other hand, GE neurons do appear to be similar to the previously described GR neurons. That is, they are either quiescent or firing at a low action potential frequency in 0.1 mM glucose, and slowly increase firing as glucose levels increase to 2.5 mM where they appear to be maximally stimulated (i.e. their action potential frequency is high in both 2.5 and 10 mM glucose). In addition, GE neurons use the KATP channel to sense glucose [63]. The KATP channel is an octomeric complex consisting of four sulfonylurea receptors (SUR) and four K⁺ channel subunits (Kir 6.2 or 6.1) [69]. There are at least two types of SUR, SUR 1 and SUR 2 (A & B), with differing sensitivities to sulfonylureas. SUR 1 and SUR 2 may be differentially expressed on cell bodies versus terminals [70], allowing for differential glucose sensitivity of cell bodies versus terminals [70,71]. Support for a role of VMN GE neurons in the CRR comes from the work of Miki *et al.* [72] who demonstrate that counterregulatory responses are impaired in Kir 6.2 knockout mice lacking functional KATP channels. The remaining three subtypes of physiologically relevant glucosensing neurons rely upon glucose-controlled presynaptic inputs [63]. One subtype is presynaptically excited as extracellular glucose levels decrease below 2.5 mM (PED neurons). The other two subtypes of presynaptically modulated glucosensing neurons respond to increasing extracellular glucose from 2.5 to 5 mM. Neurons that are presynaptically excited as extracellular glucose is raised to 5 mM are referred to as PER neurons, and those that are presynaptically inhibited under the same conditions are referred to as PIR neurons. The synaptic mechanisms mediating PED and PER neuronal responses to glucose are unclear. However, PIR neurons receive glucose-controlled inhibitory GABAergic input. The antidiabetic sulfonylurea drug, tolbutamide, which closes the KATP channel, mimics the effect of increased glucose on PIR neurons. This suggests KATP channel involvement. We hypothesize that this

KATP channel is located on the terminal neurite of the GABAergic neuron. Such presynaptic channels may be present in the substantia nigra where glucose has been shown to regulate GABA release [73–75]. The interactions between pre- and postsynaptic influences are complex. For example, some GE neurons exhibit biphasic responses to changes in extracellular glucose. That is, GE neuron action potential frequency declines as a result of inhibitory glucose-controlled presynaptic inputs when glucose increases above 2.5 mM. These GE neurons (by definition) directly sense a decrease in extracellular glucose below 2.5 mM and decrease their action potential frequency. Biphasic responses have also been observed for virtually all GI and PED neurons [63]. In this case, the neurons are quiescent at 2.5 mM extracellular glucose and excited when glucose levels increase or decrease. Thus, neurons in the VMN that directly sense glucose are also modulated by glucose-sensing neurons originating elsewhere in the brain. Furthermore, VMN neurons lacking intrinsic glucose-sensing ability themselves also receive input from glucose-sensing neurons. It is noteworthy that VMN glucose-sensing neurons that respond to decreased extracellular glucose are presynaptically modulated by glucose-sensing neurons that respond to increased extracellular glucose. This observation, coupled with the fact that type 1 diabetic patients experience fluctuations in plasma glucose levels from insulin-induced hypoglycemia to hyperglycemia, makes it essential to understand the regulation of all five of these subtypes of neurons if we are to understand the role of the VMN as a regulator of glucose homeostasis.

An important question regarding the physiologic relevance of glucose-sensing neurons involves the mechanism by which alterations in extracellular glucose levels are transduced into an intracellular signal (e.g. ATP, ADP) that regulates action potential frequency. The most likely candidate for this mechanism appears to be the rate-limiting enzyme in glycolysis (hexokinase). Most neurons contain hexokinase I that is saturated at physiologic glucose levels [76–79]. In these neurons, altered extracellular glucose within the physiologic range would not result in changes in intracellular metabolism. However, like pancreatic β -cells, glucose-sensing neurons may possess a special hexokinase known as glucokinase (GK; hexokinase IV) whose K_m for glucose is in the physiologic range for extracellular glucose [77,80]. GK is located in brain regions that contain glucose-sensing neurons [81–83]. We have shown that GK inhibitors prevent glucose-induced intracellular calcium ([Ca²⁺]_i) changes in GE and GI neurons [81]. Furthermore, the GK inhibitor alloxan increases the activity of the KATP channel in GE neurons (unpublished observation). Moreover, our preliminary data using single-cell reverse-transcription polymerase chain reaction (RT-PCR) techniques show that both VMN GE and GI neurons possess mRNA coding for GK [84]. These data indicate that VMN glucose-sensing neurons possess the necessary machinery to detect physiologically relevant alterations in extracellular glucose levels.

Extrahypothalamic glucosensors and the CRR

It is clear that the hypothalamus is involved in the CRR. However, it is not clear whether the initial detection of hypoglycemia occurs in the hypothalamus or in the brain itself. As mentioned earlier, glucosensors are widespread, both centrally and peripherally. The argument has been made that it is the peripheral glucosensors that are responsible for detecting hypoglycemia. A series of studies have been performed by Donovan *et al.*, using 'brain' and 'liver' glucose clamps [85]. These investigators found that insulin-induced hypoglycemia produced in the hepatic-portal region during central euglycemia in dogs was sufficient to elicit the sympathoadrenal response to hypoglycemia, suggesting the existence of a liver glucosensor [86]. This was later determined to be due to a glucosensor not in the liver itself but in the portal vein that communicates with the brain via sympathetic afferents [52,86–88]. In contrast, Biggers *et al.* showed that glucagon release, as well as hepatic glucose production, was significantly attenuated during insulin-induced hypoglycemia with cerebral euglycemia [89]. Interestingly, selective hypoglycemia in either the carotid or the vertebrobasilar arteries during systemic euglycemia produced the full CRR in dogs. However, selective euglycemia in either location during peripheral hypoglycemia only slightly inhibited the CRR. The authors conclude that multiple brain regions and redundant central pathways are important for the CRR [90]. Finally, while HAAF is known to produce central alterations, there are no studies that suggest that peripheral glucosensors are altered during HAAF. Thus, the relative importance of portal versus central glucose sensors is still controversial.

There is strong evidence that the brainstem plays an important role in the CRR and the development of HAAF. Several elegant studies by Ritter and coworkers illustrate this point very clearly. Glucoprivation induced by 2-deoxyglucose (2DG) increased Fos-immunoreactivity (Fos-ir; an index of neuronal activation) in a number of brain areas, notably the PVN and catecholaminergic cell groups in the brainstem [91]. This effect was abolished by repeated glucoprivation, suggesting that these areas might be important participants in the HAAF phenomenon [92]. Fos-ir was not increased in the VMH. However, this may be because hypoglycemia causes GABA release in this region [21,93]. Neuronal inhibition would not have been detectable using Fos-ir. Ritter's group then showed that selective lesions of the rostrally projecting brainstem catecholamine cell bodies abolish the glucoprivic feeding caused by 2DG, while leaving the sympathoadrenal response to glucoprivation intact [94]. In contrast, lesions of the spinally projecting catecholamine cell bodies did just the reverse [94]. Interestingly, there are a number of other hindbrain regions (e.g. raphe pallidus/obscurus) in which glucoprivation causes feeding [62]. Finally, we have found GI-like neurons in the raphe obscurus (unpublished results). Together, these findings suggest an

important role for brainstem catecholamine cell groups in both the CRR and HAAF.

Alterations in central nervous system function during HAAF

While there is a large body of evidence concerning the effects of hypoglycemia on the central nervous system, little is known about the mechanism by which even one bout of antecedent hypoglycemia can disrupt the ability of the brain to sense and respond to subsequent bouts. It is known that HAAF is associated with a number of changes in central nervous system function, predominantly in the hypothalamus and the brainstem. For example, the first bout of insulin-induced hypoglycemia increases tyrosine hydroxylase (TH) activity in brainstem and hypothalamic catecholaminergic neurons. The increased TH activity was abolished with subsequent episodes of hypoglycemia only in the hypothalamus [95]. Furthermore, just one bout of mild hypoglycemia causes DNA fragmentation associated with decreased neuropeptide Y and proopiomelanocortin mRNA expression in the ARC [8]. This suggests that ARC neurons may be more vulnerable to damage caused by hypoglycemia and that damage to these neurons might play a pathophysiological role in HAAF. We have shown that GK mRNA expression is significantly increased in the VMN and medial amygdaloid nucleus 48 h after a single bout of insulin-induced hypoglycemia sufficient to induce HAAF. This suggests that an alteration in the function of VMN glucosensing neurons may occur during HAAF [81]. Finally, Simpson *et al.* [96] showed that chronic hypoglycemia increases brain microvascular glucose transporter 1 (GLUT1) mRNA and protein and increases brain glucose uptake. However, blood to brain glucose transport, cerebral glucose metabolism, and cerebral blood flow did not increase after hypoglycemia, suggesting that the HAAF-induced alterations are past the blood–brain barrier [97]. Together, these data indicate that central nervous system function is altered during HAAF. Moreover, the increased expression of GK in the VMN following a hypoglycemic episode sufficient to generate HAAF suggests a role for glucosensing neurons in this region in the pathogenesis of HAAF.

Finally, there is evidence that the glucocorticoids are mediators in the induction of HAAF. Hypoglycemia causes a rise in cortisol levels [6]. Peripheral and central infusion of cortisol, as well as peripheral infusion of adrenocorticotrophic hormone (ACTH), causes an attenuation of the CRR to subsequent hypoglycemia in humans and rats [98–100]. Conversely, humans with adrenomedullary failure did not secrete cortisol in response to hypoglycemia nor did they develop HAAF [101]. The laboratories of Cryer and Davis hypothesized that a physiological secretion of cortisol by exercise should similarly induce HAAF [102,103]. While the studies from these two groups are not in complete agreement, two conclusions are apparent. First, their data are consistent

overall with the hypothesis that the increased cortisol secretion in response to hypoglycemia may be a causal factor in the development of HAAF. However, since there was significant variability in the ability of exercise-induced cortisol release to impair the CRR, elevated cortisol is probably not the only factor involved in the development of HAAF [102,103]. Currently, there are no data concerning the effects of glucocorticoids on glucosensing neurons. If the above hypothesis is correct, then one might further postulate that cortisol would lower the glucose sensitivity of glucose-sensing neurons.

In conclusion, it is certain that alterations in plasma glucose are sensed at a number of different levels. Glucosensors in the portal vein, brainstem, hypothalamus,

and probably other regions play a role in the CRR. It is tempting to hypothesize that different aspects of the CRR are initiated or controlled by distinct sensors. That is, the CRR consists of a range of responses that are initiated sequentially as plasma glucose levels fall [104]. It is possible that the different glucosensors have different thresholds and thus would be responsible for initiation of unique aspects of the CRR. Thus, initial hypoglycemia may be sensed peripherally, while HAAF and hypoglycemia unawareness may occur centrally. This suggests a high degree of integration in glucosensing. The complex integration of pre- and postsynaptic modulation of VMN glucosensing neurons is consistent with this view. These hypotheses are summarized in Figure 2. However, the only clear conclusion is that much work is needed

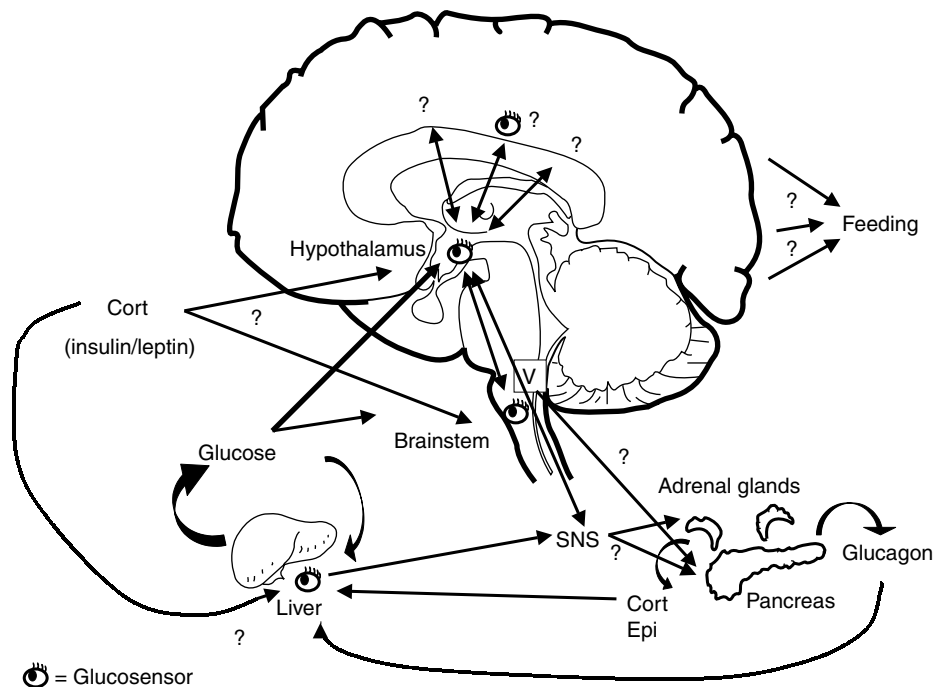


Figure 2. Putative roles of central and peripheral glucosensors in the response to hypoglycemia. Glucosensors exist in a variety of locations, including the hypothalamus, the brainstem and the portal vein of the liver. The portal vein glucosensor directly senses a change in plasma glucose in the vicinity of the liver and communicates this information to the brain via sympathetic afferents [52]. In contrast, brainstem and hypothalamic glucosensors sense changes in brain extracellular glucose concentration. The levels of glucose sensed by the central glucosensors are much lower than those of the periphery. Changes in brain glucose reflect changes in peripheral glucose; however, these changes may lag behind those detected by the portal vein glucosensors [12,13]. In addition, changes in brain extracellular glucose levels may reflect regional changes due to differences in neuronal activity [105,106]. Finally, glucosensors in regions of the brain adjacent to a leaky blood–brain barrier might sense peripheral glucose levels [68]. During hypoglycemia, these glucosensors detect a drop in extracellular glucose. Hypothetically, this initiates the counterregulatory response to hypoglycemia (CRR), resulting in a well-characterized sympathoadrenal (e.g. epinephrine, cortisol) and behavioral (feeding) response that restores blood glucose to normal levels [5,6]. Glucagon release is stimulated directly by hypoglycemia and possibly also by sympathetic and parasympathetic activation [107]. The net result is increased glucose production by the liver. Cortisol may alter the function of the glucosensors or glucosensor circuitry such that detection of subsequent bouts of hypoglycemia is altered, resulting in hypoglycemia-associated autonomic failure [98,101]. Finally, glucosensors respond to metabolic status (e.g. insulin, leptin) [27,28]. The physiological need for multiple glucosensors is unknown, as is their relative importance in the CRR. One hypothesis is that they possess different glucose thresholds and are thus responsible for the sequential activation of the CRR [104]. It is also possible that the central glucosensors compare the magnitude and time course of changes in glucose levels in the brain (or even changes within different regions of the brain) with those occurring in the periphery. They then integrate a whole-body response (including feeding) and prioritize glucose needs. Many other brain regions (e.g. substantia nigra, amygdala) may possess glucosensors in order to monitor local need and forward this information to the hypothalamus where it is integrated. Thus, multiple glucosensors may represent a hierarchical system that senses and integrates alterations in extracellular glucose throughout the brain and the periphery. This information is compared with the overall energy status and perhaps previous alterations in glucose balance in order to affect long-term glucose homeostasis. CORT cortisol; Epi, epinephrine; SNS, sympathetic nervous system; V, dorsal motor nucleus of the vagus.

before a specific role for VMN glucosensing neurons in HAAF can be assigned.

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