Published in final edited form as:

Trends Endocrinol Metab. 2013 December ; 24(12): 616–624. doi:10.1016/j.tem.2013.08.005.

Influence of VMH Fuel Sensing on Hypoglycemic Responses

Owen Chan¹ and Robert Sherwin¹

¹Yale University School of Medicine, Department of Internal Medicine - Section of Endocrinology, New Haven, CT, 06520 U.S.A

Abstract

Hypoglycemia produces complex neural and hormonal responses that restore glucose levels to normal. Glucose, metabolic substrates and their transporters, neuropeptides and neurotransmitters alter the firing rate of glucose sensing neurons in the ventromedial hypothalamus (VMH) which monitor energy status and regulate the release of neurotransmitters that instigate a suitable counterregulatory response. Under normal physiological conditions, these mechanisms maintain blood glucose concentrations within narrow margins. However, antecedent hypoglycemia and diabetes can lead to adaptations within the brain that impair counterregulatory responses. Clearly, the mechanisms employed to detect and regulate the response to hypoglycemia and the pathophysiology of defective counterregulation in diabetes are complex and need to be elucidated so therapies can be developed to prevent or reduce the risk of hypoglycemia.

Keywords

brain; glucose sensing; diabetes; recurrent hypoglycemia; ventromedial hypothalamus; hypoglycemia

Physiological Response to Hypoglycemia

Hypoglycemia elicits a multifaceted hierarchical hormone and autonomic nervous system response that restores glycemic levels to normal. As plasma glucose levels begin to fall and go below 80 mg/dl, insulin secretion rapidly decreases, which serves to reduce β -cell inhibition of neighboring α -cell function. Decreased inhibition of the β -cell reduces the secretion of zinc and gamma-aminobutyric acid (GABA), which normally inhibit glucagon secretion^{1–4}. Together, this allows for the rapid release of glucagon into the portal circulation, where it acts on the liver to stimulate glycogenolysis and gluconeogenesis. If glucose levels continue to decline into the 60–70mg/dl range, epinephrine is released and sympathetic nervous activity begins to increase, which acts in concert to reverse the decline in plasma glucose levels by enhancing glucagon secretion and exerting insulin's antagonistic effects on liver, adipocytes and muscle. In cases of prolonged and/or more severe hypoglycemia, growth hormone and cortisol are mobilized to further stimulate lipolysis and gluconeogenic enzymes as well as inhibit peripheral glucose utilization⁵. Together, this

© 2013 Elsevier Ltd. All rights reserved.

Please address correspondences to: Dr. Robert Sherwin, Yale University School of Medicine, Department of Internal Medicine – Section of Endocrinology, 300 Cedar Street, TAC S141D, New Haven, CT, U.S.A. Telephone: (203) 785-4183, robert.sherwin@yale.edu.

No potential conflicts of interest relevant to this article were reported.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

coordinated hormone and neuronal response constitutes a redundant failsafe mechanism that helps prevent glucose levels from falling to dangerously low levels. In non-diabetic individuals, glucagon and epinephrine are usually very effective at restoring plasma glucose levels to normal that additional responses are rarely required.

BOX 1

Clinical Complications of Hypoglycemia

Impaired glucose counterregulation commonly occurs in patients with longstanding type 1 diabetes (T1D) or in insulin-treated individuals with antecedent exposure to hypoglycemia⁶. Administration of insulin or oral hypoglycemic agents that enhance insulin release (sulfonylureas, in particular) results in loss of the ability to reduce circulating insulin levels at the outset of hypoglycemia. Hence, the stimulus to lower glucose cannot be appropriately withdrawn. To compound the problem, within the first five years after the onset of T1D, the primary defense against hypoglycemia, the release of glucagon, either becomes markedly attenuated or is completely absent and this impairment appears to be specific for the stimulus of hypoglycemia⁷. Therefore, these patients primarily depend on the release of epinephrine as their main defense against hypoglycemia. Unfortunately, with longer duration of diabetes and/or frequent episodes of iatrogenic insulin-induced hypoglycemia, epinephrine secretion becomes compromised, making patients even more vulnerable to the threat of hypoglycemia⁵. Hence, for patients with diabetes, hypoglycemia arises from both a relative excess of insulin and a defective counterregulatory response to decrements in plasma glucose. Moreover, recent antecedent hypoglycemia reduces the autonomic and symptomatic responses (which normally prompt behavioral defenses such as eating) to subsequent bouts of hypoglycemia⁸. Thus begins the vicious cycle of recurrent hypoglycemia (RH) where hypoglycemia leads to further impairment of counterregulatory responses, which in turn begets more hypoglycemia and so forth. As a result, hypoglycemia has emerged as a major limiting factor to attaining the microvascular, cardiovascular, and neurological benefits of optimal glycemic control in both type 1 and 2 diabetes⁵. This point is underscored by outcomes of the Diabetes Control and Complications Trial (DCCT) which showed that when T1D patients were put on intensive insulin therapy, though they had improved outcomes for diabetic complications, they exhibited a 3-fold higher risk of experiencing severe hypoglycemia compared to those on conventional insulin therapy⁹. Because of the imperfections of current insulin therapies, those patients attempting to achieve glycemic control suffer an untold number of asymptomatic hypoglycemic episodes. Current estimates of symptomatic hypoglycemic episodes range from 2–3 incidences per week on average and severe, debilitating episodes where assistance from others is required occurs once or twice each year¹⁰. Therefore, understanding how the body senses falling blood glucose levels and initiates counterregulatory mechanisms will be crucial if we are to prevent or eliminate hypoglycemia.

Neural Networks that Regulate Glucose Homeostasis

The ability to respond appropriately to hypoglycemia and maintain normal glucose levels depends on the integration of metabolic signals derived from glucose sensors located in both the central nervous system (CNS) and the periphery, including the gut, the portal/mesenteric vein and the carotid body¹¹. Peripheral glucose sensors located in the portal/mesenteric vein detect changes in glucose levels and relay this information to the nucleus of the solitary tract (NTS), the lateral hypothalamus and the paraventricular nucleus (PVN) through activation of vagal afferents^{12,13}. Signals from the hindbrain in turn, are carried forth to hypothalamic centers where the predominant glucose sensors are located, and the information is

integrated. While peripheral glucose sensors may play a role in mediating the immediate counterregulatory responses to hypoglycemia, it is thought that glucosensors located within the brain may serve a redundant regulatory and/or modulatory role in ultimately regulating glucose counterregulatory responses.

Many of the glucose sensing components found in pancreatic β -cells, including the type 1 sulfonylurea receptor (SUR1) subunit of ATP-sensitive potassium (K_{ATP}) channels, glucose transporters (GLUT) 2, 3 and 4, glucokinase and monocarboxylate transporters (MCTs) have been identified in the hindbrain¹⁴. However, it is still unclear whether hindbrain catecholaminergic neurons are directly or indirectly activated by glucose deprivation. Local administration of the potent antimetabolite 5-thiogluconase into the hindbrain elicits a rise in plasma glucose and an increase in feeding, suggesting that this region of the brain can respond to glucose deprivation¹⁵. Moreover, in response to a glucoprivic stimulus stemming from administration of 2-deoxyglucose (2-DG), the hindbrain also exhibits greater Fos immunoreactivity in very distinct catecholaminergic cell groups, indicating these neurons may play a role in the brain's response to glucose deficit¹⁶.

BOX 2

Ventromedial Hypothalamus (VMH)

The ventromedial hypothalamus is a bilateral nucleus located adjacent to the third ventricle, near the base of the hypothalamus and its role in the regulation of energy balance and feeding are well documented. As we obtain a better appreciation of the cell types and molecules that are expressed in the VMH, so too have we recognized the diverse roles of the VMH. In addition to its well documented role in energy homeostasis, autonomic output from the VMH alters cardiovascular tone and neuroendocrine responses¹⁷; the VMH affects nociceptive behavior¹⁸ and VMH neurons expressing orexin receptors regulate gastric acid secretion¹⁹. In addition, neurons in the VMH that express estrogen and progesterone receptors also affect sexual behavior in both male and female animals^{20,21}. Given the diverse functions that are served by the VMH, a better understanding of VMH fuel sensing mechanisms can only be achieved by appreciating the different types of neural and blood-borne information that it receives, and then attempting to determine how this information is integrated to obtain an appropriate output signal.

Central glucose sensors are also located in the lateral hypothalamus, the paraventricular nucleus, the dorsal hypothalamus, the amygdala and the ventromedial hypothalamus (VMH)²². Hypothalamic glucose sensors and more specifically, those located in the VMH, are crucial for detecting falling blood glucose levels and regulating the counterregulatory responses²³ and are probably the best characterized (reviewed in²⁴). The VMH is ideally situated to receive information regarding the metabolic status of the organism. Being located next to the third ventricle and median eminence, the VMH has projections that allow it to sample glucose and hormones which regulate metabolism. Two types of glucose sensing neurons have been identified in the VMH – “glucose-excited” or GE neurons that increase their firing rate in response to rising glucose levels, and “glucose-inhibited” or GI neurons which decrease their firing rate in response to increasing glucose levels. While glucose sensing neurons respond to changes in extracellular glucose levels by altering their firing rate, the mechanisms used by each subclass of neurons are believed to be quite different²⁴.

Glucose Excited Neurons

The glucose excited neurons in the VMH are the best characterized and they contain much of the same glucose sensing machinery as pancreatic β -cells^{25,26}. They predominantly express GLUT3 and to a lesser extent GLUT2, as well as glucokinase (GK) which regulates the first rate-limiting step in glycolysis - the phosphorylation of glucose to glucose-6-phosphate (G6P). The importance of glucokinase as a regulator of glucose sensing is supported by data showing that pharmacological inhibition or RNA interference reduces the sensitivity of VMH glucose sensing neurons²⁷ and that its expression is increased in rodent models of defective glucose sensing^{28,29}.

Once glucose is phosphorylated to G6P, this intermediate is metabolized to generate ATP. An increase in the [ATP]/[ADP] ratio causes ATP-sensitive potassium (K_{ATP}) channels to close which depolarizes the neuron membrane and opens voltage-gated calcium channels. The influx of extracellular Ca^{2+} leads to exocytosis of vesicular contents (Figure 1). Thus, as glucose levels rise, these neurons continue to fire. In contrast, during hypoglycemia, these neurons are largely silenced due to lack of glucose, production of G6P and thus ATP production. Glucose excited neurons express the SUR1-containing ATP-sensitive K^+ channel (K_{ATP}), that couples changes in energy status with electrical activity of GE neurons. In this regard, K_{ATP} channels in VMH neurons may serve a particularly important role in transducing the glucoprivic signal and initiating counterregulatory responses. Evans and colleagues demonstrated that inhibition of K_{ATP} channel activity with glibenclamide or tolbutamide attenuates the counterregulatory responses to hypoglycemia, whereas McCrimmon *et al.* showed that the local delivery of the K_{ATP} channel opener, diazoxide, into the VMH can amplify the counterregulatory responses^{30,31}. The importance of the K_{ATP} channel was further supported by studies where the K_{ATP} channel openers, NN414 and diazoxide, were used to improve the counterregulatory hormone responses seen in recurrently hypoglycemic rats with impaired glucose counterregulation³².

Glucose Inhibited Neurons

The mechanism by which GI neurons respond to changes in ambient glucose levels is not as well understood, but is thought to be regulated by a tandem-pore K^+ channel and/or closure of the cystic fibrosis transmembrane regulator (CFTR), a chloride channel^{33–37}. The latter mechanism³⁵, suggests that a reduction in glucose metabolism resulting from decreases in extracellular glucose raises intracellular AMP levels. An increase in AMP then activates the metabolic fuel sensor, AMP-activated protein kinase (AMPK) which in turn, triggers the production of nitric oxide (NO) by neuronal nitric oxide synthase (nNOS). Activation of guanylate cyclase by NO further activates AMPK which in turn, reduces chloride conductance through CFTR, leading to greater neuronal firing during hypoglycemia (Figure 1).

Astrocytes/Tanycytes

In recent years, glial cells are being recognized as important regulators of glucose sensing mechanisms. Glial cells normally express GLUT2, however, *ripplut1;glut2*^{−/−} mice have an inactivated *glut2* gene but normal glucose-regulated insulin secretion stemming from transgenic expression of GLUT1 in their β -cells and therefore have normal insulin secretion and fail to secrete glucagon in response to either peripheral or central glucose deprivation from 2-DG administration³⁸. Interestingly, when GLUT2 was transgenically re-expressed specifically in glial cells and not in neurons of *ripplut1;glut2*^{−/−} mice, glucagon secretion was restored, suggesting glial GLUT2 is an important contributor to hypoglycemic glucose sensing.

In the hypothalamus, there exists a type of glial cell called tanycytes. Tanycytes are classified into four different types, $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$, according to their histological properties^{39;40;41}. $\alpha 2$ and $\beta 1$ tanycytes, the main glial cells are localized in the lower lateral wall of the third ventricle in the basal hypothalamus^{39,42,43}, and have processes which extend into the periventricular and lateral parts of the VMH. Thus, they are able to sample both the cerebrospinal fluid and the general circulation and relay this information to neighboring neurons in the VMH^{44,45}. Tanycytes express GLUT2 in the apical membrane which contacts the cerebrospinal fluid (CSF)⁴³ and also glucokinase³⁹, hence, it has been postulated that these cells may play a role in glucose sensing or in regulating glucose sensing mechanisms. Tanycytes also have a functional distribution of MCT1 and 4 which allows them to shuttle other metabolic substrates such as lactate to neighboring neurons⁴⁶. Therefore, tanycytes may be involved in detecting changing glucose concentrations in the CSF of the ventricular system and generating lactate as an intercellular messenger, informing the neurons in the periventricular VMH of glucose levels and regulating glucose sensing activities.

A key question that arises is, what is the nature of the interaction between glial cells, GE and GI neurons and more importantly, what are the downstream targets of these neurons that regulate counterregulatory hormone release during hypoglycemia? Moreover, while it is likely that direct changes in GE and GI neuron firing rates are required for full activation of the counterregulatory response, it is the resulting exocytosis of vesicles containing neurotransmitters and/or neuropeptides from these neurons that is important for conveying that signal to peripheral tissues. Several neurotransmitter candidates have been studied over the years and the role of neurotransmitters in hypoglycemic counterregulation are slowly coming to light. Of these, GABA, glutamate, norepinephrine and serotonin are the most studied.

GABA

GABA is the major inhibitory neurotransmitter in the mammalian CNS and it is important for mediating fast inhibitory post-synaptic currents. In the VMH, local blockade of GABA_A receptors with bicuculline amplified, whereas activation of GABA_A receptors with muscimol, suppressed counterregulatory responses to hypoglycemia, suggesting that GABA plays an important modulatory role⁴⁷. Similar to these findings, Moheet and colleagues used ultra-high field magnetic resonance spectroscopy at 7 tesla to show a decrease in hypothalamic GABA levels in healthy human subjects during hypoglycemia⁴⁸. In subsequent studies, it was shown that K_{ATP} channel modulators can regulate VMH GABA levels⁴⁹. Activation of K_{ATP} channels with diazoxide decreased extracellular GABA levels and this was associated with increased glucagon and epinephrine responses to hypoglycemia. Conversely, glibenclamide increased GABA levels in the VMH and suppressed glucagon and epinephrine responses. Interestingly, co-administration of diazoxide with muscimol did not amplify glucagon and epinephrine secretion and similarly, co-administration of glibenclamide with bicuculline did not suppress the counterregulatory response. This suggests that the K_{ATP} channels lie upstream of GABA_A receptors on pre-synaptic GABAergic neurons and likely act to modulate its release. In subsequent studies, it was shown that glucose was also capable of stimulating GABA release in the VMH⁵⁰. Taken together, it has been suggested that K_{ATP}-positive VMH GABAergic neurons may be glucose excited in nature. However, the exact neural pathway from VMH GABAergic neurons to the suppression of counterregulatory responses has not been identified.

Glutamate

At the other end of the spectrum is glutamate which serves as the predominant stimulatory neurotransmitter in the CNS. Glutamate acts through two main types of glutamate receptors – the metabotropic receptors which elicit their effects through activation of a G-protein coupled second messenger cascade and the ionotropic receptors which are ligand-gated ion channels. The latter are likely responsible for regulating the rapid responses to hypoglycemia and consist of NMDA, AMPA, and kainate subclasses of receptors. This system has not been as well characterized as the GABAergic system, but one study in particular demonstrated that specific knockout of the vesicular glutamate transporter, *Vglut2*, in steroidogenic factor-1 (SF-1) neurons of the VMH leads to loss of the glucagon response to hypoglycemia, suggesting that glutamate neurotransmission plays an integral part in stimulating glucagon release ⁵¹. Recent findings have revealed that EphA receptor tyrosine kinases and their ephrin ligands play an important role in contact-dependent neuron–glia communication at synapses ⁵² and Szepletowska *et. al.* reported EphA5 receptor forward signaling in the VMH can augment counterregulatory responses to hypoglycemia by enhancing glutamatergic neurotransmission in the VMH ⁵³.

Norepinephrine

In addition to GABA and glutamate, norepinephrine is also released into the VMH and regulates the counterregulatory responses during hypoglycemia. A rise in norepinephrine concentrations in VMH interstitial fluid has been reported during acute hypoglycemia ⁵⁴, and this increase is prevented when glucose is perfused into the VMH during systemic hypoglycemia ⁵⁵. In addition, it was recently demonstrated that the counterregulatory response to hypoglycemia can be enhanced through activation of β_2 -adrenergic receptors in the VMH ⁵⁶. Although local norepinephrine release into the VMH appears to be altered during hypoglycemia, the neuronal projection circuits that co-ordinate this response are not fully understood. Norepinephrine neurons originating from the brainstem as well as other hypothalamic regions that project to the VMH may be important components of the glucose-sensing network ^{22,57}. More recently, it was reported that norepinephrine can stimulate glutamatergic neurotransmission in mechanically dissociated rat VMH neurons and that this effect is mediated via activation of β_2 -adrenergic receptors ⁵⁸.

Serotonin

It is well established that serotonin modulates neuroendocrine responses that also contribute to hypoglycemia counterregulation. Recently, it was shown that a 6-day treatment of rats with the selective serotonin reuptake inhibitor (SSRI) sertraline specifically augmented the epinephrine response to an acute bout of hypoglycemia, whereas treatment for 20 days enhanced the epinephrine, norepinephrine and glucagon responses to hypoglycemia ⁵⁹. Interestingly, in that same study, both 6 and 20 day treatment with sertraline prevented loss of the epinephrine response in recurrently hypoglycemic animals, suggesting loss of serotonergic neurotransmission may contribute to counterregulatory failure. In contrast, another study showed that treatment with either the 5-HT_{1C}/5-HT₂ serotonin receptor blocker, LY 53857, or the 5-HT₁/5-HT₂ receptor antagonist, metergoline, did not diminish plasma catecholamine responses to insulin-induced hypoglycemia ⁶⁰. Differences in these findings may be due to the length of treatment and/or the site of action of the compounds used. Although the specific central site(s) of action have not been identified, a select population of serotonin neurons in the hindbrain is a potential neuroanatomical target. These caudal hindbrain serotonin neurons which express key glucose-sensing proteins ⁶¹, project to the intermediolateral cell column of the spinal cord, where they synapse on sympathetic preganglionic neurons innervating the adrenal medulla ⁶².

Corticotrophin-Releasing Hormone

In addition to neurotransmitters, the neuropeptides corticotrophin-releasing hormone (CRH) and urocortins 1–3 (UCN1–3) also appear to regulate the endocrine responses to hypoglycemia. These neuropeptides act through the type 1 and type 2 subtypes of CRH receptors in the VMH, respectively, to elicit their opposing effects. In particular, local administration of CRH into the VMH amplifies, whereas administration of either UCN1 or 3 suppresses the counterregulatory responses to hypoglycemia^{63,64}. Interestingly, UCN appears to act by lowering the glucose threshold at which GE are inactivated and GI neurons are activated. Although it has been reported that the medial amygdala nucleus expresses glucokinase and that one-third of the UCN3 neurons in this region of the brain are active during hypoglycemia, expression of glucokinase and UCN3 are mutually exclusive, suggesting UCN3 neurons themselves may not be directly glucose sensing⁶⁵. At present, the signals that activate UCN3 neurons projecting to the VMH have not been identified.

Metabolic Fuel Sensors

AMPK is an enzyme that plays a role in cellular energy homeostasis. It consists of three subunits that together make a functional enzyme that is conserved from yeast to humans. The idea that this metabolic fuel sensor affects hypothalamic glucose homeostatic mechanisms was first demonstrated through pharmacological activation of AMPK in the VMH using 5-amino-1- β -D-ribofuranosyl-imidazole-4-carboxamide (AICAR). Interestingly, local administration of AICAR reduced exogenous glucose requirements during the hypoglycemic clamping period, and this was due almost exclusively to an increase in hepatic glucose production and not to the release of counterregulatory hormones⁶⁶. In contrast, genetic knockdown of AMPK- α 2 in the VMH resulted in suppressed glucagon (~60%) and epinephrine (~40%) responses to hypoglycemia⁶⁷. Moreover, AMPK activation does appear to augment counterregulatory hormone responses in some animal models of defective counterregulation^{68,69}. One possible mechanism by which AMPK initiates counterregulatory responses may be through the direct regulation of GI neuron excitability as described above³⁵. However, others have demonstrated that AMPK also phosphorylates GABA_B receptors, Kv2.1 and NMDA receptors^{70–72} in the brain, but these mechanisms have not yet been examined in the context of glucose sensing and counterregulation.

Alternate Fuels

Over the last two decades, it has been recognized that the brain can utilize alternative forms of fuel substrates to meet its metabolic needs, especially when glucose supplies are limited. Studies by Magistretti and Pellerin's groups argue that the effects of glucose on neurons may be secondary to the uptake and metabolism of glucose into lactate by astrocytes^{73–75}. In this model, glucose entering astrocytes through glucose transporters is converted into lactate. The expression of MCT1 and MCT4 on astrocytes, and MCT2 on neurons, allows lactate to be transferred from astrocytes to neurons for oxidation. A number of different *in vitro* studies have shown that lactate can influence the behavior of glucose sensing neurons in the VMH suggesting that in addition to glucose, lactate may be an alternatively sensed fuel within the brain^{26,76}. In this context, it has been proposed that glycolytic metabolism of glucose to lactate by hypothalamic glial cells and its subsequent release into the extracellular space and uptake by neighboring neurons may enhance neuronal oxidative metabolism, ATP synthesis and neuronal depolarization⁷⁷. Recent data suggest that lactate and pyruvate are not only critically sensed fuels within the brain^{78–81}, but they also play pivotal roles in regulating glucose homeostatic mechanisms^{82,83}. Local delivery of lactate into the VMH has been shown to dramatically suppress the counterregulatory responses to systemically induced hypoglycemia, but the precise mechanisms by which this is achieved was never

established⁸². Using NMR spectroscopy and ¹³C-labeled lactate, Waagepetersen demonstrated that lactate can be incorporated into GABA in cortical neurons^{84,85}. Recent data suggests that the uptake and utilization of monocarboxylic acids is enhanced in antecedent hypoglycemia⁸⁶ and diabetes⁸⁷ and lactate likely suppresses counterregulatory responses by raising extracellular GABA levels in the VMH⁸⁸. Hence, lactate may serve as a crucial modulator of activity in glucose sensing neurons and thus, the interaction between neurons and glial cells in the VMH will be an important determinant of the final effector signal.

Antecedent Hypoglycemia and Diabetes

While numerous factors can influence the magnitude of the counterregulatory response to hypoglycemia, both prior antecedent hypoglycemia and insulin deficient diabetes can impair these responses. Although the mechanisms underlying this defect are still not fully understood, alterations in central glucose sensing appear to contribute to this defect.

Counterregulatory failure stemming from recurrent hypoglycemia can be effectively reversed through activation of K_{ATP} channels in the VMH with diazoxide³¹ or the blockade of GABA_A receptors in the VMH with bicuculline⁸⁹ suggesting that activation of glucose sensing GABAergic neurons in this brain region plays a role. In support of this hypothesis, Hedrington and colleagues demonstrated that prior activation of GABA_A receptors in healthy human subjects can blunt the autonomic and neuroendocrine response to a bout of hypoglycemia the following day⁹⁰. In addition, the counterregulatory defects can also be improved in recurrently hypoglycemic animals through the activation of AMPK in the VMH with AICAR⁶⁸, suggesting that mechanisms that lie downstream of NO signaling in GI neurons are also affected by antecedent hypoglycemia. But what drives these adaptations?

Using *in vivo* NMR spectroscopy, Jiang and Herzog demonstrated that recurrent hypoglycemia alters cortical neuronal oxidative metabolism and increases lactate transport across the blood brain barrier to help preserve normal brain function during subsequent bouts of hypoglycemia^{86,91}. Interestingly, this effect of lactate appears, in large part, due to its capacity to maintain the same rate of glucose oxidation and neuronal TCA cycle activity, as occurs under euglycemic conditions⁸⁶. If this is the case in glucose sensing neurons, lactate would be anticipated to stimulate GE GABAergic neurons, while simultaneously inactivating AMPK and silencing GI neurons. Song and colleagues showed that recurrent hypoglycemia reduced the glucose threshold at which GI neurons became active, an effect that was replicated with the addition of lactate⁹². Together these observations suggest that lactate may play a significant role in impairing glucose sensing mechanisms in individuals with prior exposure to hypoglycemia (Figure 2).

Remarkably, very similar adaptations in central glucose sensing mechanisms were observed in T1D, as with antecedent hypoglycemia. Canabal and colleagues showed that STZ-diabetic animals have reduced NO production in VMH GI neurons⁹³. In addition, it was recently shown that both extracellular GABA and GAD₆₅ protein levels in the VMH of spontaneously diabetic BB and STZ-diabetic rats are elevated and fail to decline during hypoglycemia⁹⁴. These changes were accompanied by an inability to activate counterregulation during hypoglycemia. Similar to the situation in recurrently hypoglycemic animals, when lactate uptake or utilization in the VMH was inhibited, it is possible to reduce extracellular GABA levels and restore both the epinephrine and glucagon response to hypoglycemia to normal, suggesting lactate may also contribute to the altered brain glucose sensing and defective glucose counterregulation accompanying diabetes. Indeed, brain monocarboxylic acid transport and utilization is increased in the brains of patients with T1D compared to non-diabetic subjects⁸⁷. In a follow up study using magnetic resonance

spectroscopy with ^{13}C -labelled lactate, De Feyter and colleagues showed that individuals with T1D who experience frequent episodes of hypoglycemia have a 5-fold increase in brain lactate concentrations during hypoglycemia, but oxidation of this alternate fuel is not changed significantly, suggesting lactate may also serve additional roles beyond being a metabolic fuel ⁹⁵. Hence, it appears that adaptive changes in brain fuel metabolism occur in patients with T1D. Key to this may be how glucose and other metabolic substrates such as lactate are taken up and utilized by glucose sensing neurons in the brain, which may limit their capacity to detect hypoglycemia and thereby to trigger appropriate counterregulatory responses.

Concluding Remarks and Future Perspectives

While the neural networks that detect hypoglycemia still need to be worked out fully, what is clear is that VMH glucose sensing neurons not only contain their own mechanisms for detecting hypoglycemia, but there is a multitude of inputs from both the periphery and other regions within the brain that signal the metabolic status of the organism to these neurons. The convergence of these signals on VMH glucose sensing neurons act to modulate their activity and in turn the magnitude of the counterregulatory responses. What will be important in the future is to not only identify the phenotype of the GE and GI neurons within the VMH, but also the efferent signals that lie downstream of the VMH, and to understand how these metabolic signals are transduced to peripheral organs.

Hypoglycemia has emerged as the principal barrier that precludes the maintenance of normal blood sugar levels over a lifetime of diabetes and full realization of the long-term benefits of normal blood sugar levels. As 3–4% of deaths from diabetes are the direct result of severe hypoglycemia ⁹⁶, it is essential that we achieve a better understanding of how the body detects falling blood glucose levels and activates defense mechanisms against hypoglycemia. In addition, we must elucidate the mechanisms by which counterregulatory responses are impaired with prolonged diabetes and following recurrent exposure to hypoglycemia so appropriate therapeutic strategies can be developed to more effectively prevent hypoglycemic outcomes in patients with diabetes. If hypoglycemia can be prevented, it will enable physicians to treat diabetes more aggressively and allow patients to achieve more optimal glucose targets.

Acknowledgments

Portions of the work presented in this review were generously supported by research grants from the American Diabetes Association (7-11-BS-21 to OC), the Juvenile Diabetes Research Foundation (2-2008-480 to OC, 4-2010-433 to RS) and the National Institutes of Health (P30 DK45735 and R37 DK20495 to RS). O.C is the recipient of a Career Development Award from the JDRF.

References

1. Robertson RP, et al. A role for zinc in pancreatic islet beta-cell cross-talk with the alpha-cell during hypoglycaemia. *Diabetes Obes Metab.* 2011; 13(Suppl 1):106–111. [PubMed: 21824263]
2. Slucca M, et al. ATP-sensitive K⁺ channel mediates the zinc switch-off signal for glucagon response during glucose deprivation. *Diabetes.* 2010; 59:128–134. [PubMed: 19808893]
3. Xu E, et al. Intra-islet insulin suppresses glucagon release via GABA-GABAA receptor system. *Cell Metab.* 2006; 3:47–58. [PubMed: 16399504]
4. Zhou H, et al. Zinc, not insulin, regulates the rat alpha-cell response to hypoglycemia in vivo. *Diabetes.* 2007; 56:1107–1112. [PubMed: 17317764]
5. Cryer PE. The barrier of hypoglycemia in diabetes. *Diabetes.* 2008; 57:3169–3176. [PubMed: 19033403]

6. Cryer PE, et al. Hypoglycemia in diabetes. *Diabetes Care*. 2003; 26:1902–1912. [PubMed: 12766131]
7. McCrimmon RJ, Sherwin RS. Hypoglycemia in type 1 diabetes. *Diabetes*. 2010; 59:2333–2339. [PubMed: 20876723]
8. Arbelaez AM, et al. Attenuation of counterregulatory responses to recurrent hypoglycemia by active thalamic inhibition: a mechanism for hypoglycemia-associated autonomic failure. *Diabetes*. 2008; 57:470–475. [PubMed: 18003752]
9. Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group. *Diabetes*. 1997; 46:271–286. [PubMed: 9000705]
10. Cryer PE. Hypoglycemia: still the limiting factor in the glycemic management of diabetes. *Endocr Pract*. 2008; 14:750–756. [PubMed: 18996798]
11. McCrimmon R. The mechanisms that underlie glucose sensing during hypoglycaemia in diabetes. *Diabet Med*. 2008; 25:513–522. [PubMed: 18312421]
12. Adachi A, et al. Convergence of hepatoportal glucose-sensitive afferent signals to glucose-sensitive units within the nucleus of the solitary tract. *Neurosci Lett*. 1984; 46:215–218. [PubMed: 6738915]
13. Shimizu N, et al. Functional correlations between lateral hypothalamic glucose-sensitive neurons and hepatic portal glucose-sensitive units in rat. *Brain Res*. 1983; 265:49–54. [PubMed: 6850320]
14. Vavaiya KV, Briski KP. Effects of caudal fourth ventricular lactate infusion on hypoglycemia-associated MCT2, GLUT3, GLUT4, GCK, and sulfonylurea receptor-1 gene expression in the ovariectomized female rat LHA and VMH: impact of estradiol. *J Mol Neurosci*. 2008; 34:121–129. [PubMed: 18084728]
15. Ritter S, et al. Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Res*. 2000; 856:37–47. [PubMed: 10677609]
16. Ritter S, et al. Subgroups of hindbrain catecholamine neurons are selectively activated by 2-deoxy-D-glucose induced metabolic challenge. *Brain Res*. 1998; 805:41–54. [PubMed: 9733914]
17. Wanner SP, et al. Physical exercise-induced cardiovascular adjustments are modulated by muscarinic cholinceptors within the ventromedial hypothalamic nucleus. *Physiol Res*. 2010; 59:165–175. [PubMed: 19537936]
18. Cataldo G, et al. Ventromedial and medial preoptic hypothalamic ibotenic acid lesions potentiate systemic morphine analgesia in female, but not male rats. *Behav Brain Res*. 2010; 214:301–316. [PubMed: 20678986]
19. Eliassi A, et al. Role of the ventromedial hypothalamic orexin-1 receptors in regulation of gastric Acid secretion in conscious rats. *J Neuroendocrinol*. 2009; 21:177–182. [PubMed: 19207823]
20. Flanagan-Cato LM. Sex differences in the neural circuit that mediates female sexual receptivity. *Front Neuroendocrinol*. 2011; 32:124–136. [PubMed: 21338620]
21. Yang CF, et al. Sexually dimorphic neurons in the ventromedial hypothalamus govern mating in both sexes and aggression in males. *Cell*. 2013; 153:896–909. [PubMed: 23663785]
22. Marty N, et al. Brain glucose sensing, counterregulation, and energy homeostasis. *Physiology (Bethesda)*. 2007; 22:241–251. [PubMed: 17699877]
23. Borg MA, et al. Local ventromedial hypothalamus glucose perfusion blocks counterregulation during systemic hypoglycemia in awake rats. *J Clin Invest*. 1997; 99:361–365. [PubMed: 9006005]
24. Routh VH. Glucose sensing neurons in the ventromedial hypothalamus. *Sensors (Basel)*. 2010; 10:9002–9025. [PubMed: 22022208]
25. Song Z, et al. Convergence of pre- and postsynaptic influences on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes*. 2001; 50:2673–2681. [PubMed: 11723049]
26. Yang XJ, et al. Hypothalamic glucose sensor: similarities to and differences from pancreatic beta-cell mechanisms. *Diabetes*. 1999; 48:1763–1772. [PubMed: 10480606]
27. Levin BE, et al. Ventromedial hypothalamic glucokinase is an important mediator of the counterregulatory response to insulin-induced hypoglycemia. *Diabetes*. 2008
28. Levin BE, et al. Neuronal glucosensing: what do we know after 50 years? *Diabetes*. 2004; 53:2521–2528. [PubMed: 15448079]

29. Kang L, et al. Prior Hypoglycemia Enhances Glucose Responsiveness in Some Ventromedial Hypothalamic Glucosensing Neurons. *Am J Physiol Regul Integr Comp Physiol*. 2007
30. Evans ML, et al. Hypothalamic ATP-sensitive K⁺ channels play a key role in sensing hypoglycemia and triggering counterregulatory epinephrine and glucagon responses. *Diabetes*. 2004; 53:2542–2551. [PubMed: 15448082]
31. McCrimmon RJ, et al. Activation of ATP-sensitive K⁺ channels in the ventromedial hypothalamus amplifies counterregulatory hormone responses to hypoglycemia in normal and recurrently hypoglycemic rats. *Diabetes*. 2005; 54:3169–3174. [PubMed: 16249441]
32. Fan X, et al. Amplified hormonal counterregulatory responses to hypoglycemia in rats after systemic delivery of a SUR-1-selective K(+) channel opener? *Diabetes*. 2008; 57:3327–3334. [PubMed: 18776135]
33. Burdakov D, et al. Tandem-pore K⁺ channels mediate inhibition of orexin neurons by glucose. *Neuron*. 2006; 50:711–722. [PubMed: 16731510]
34. Guyon A, et al. Glucose inhibition persists in hypothalamic neurons lacking tandem-pore K⁺ channels. *J Neurosci*. 2009; 29:2528–2533. [PubMed: 19244527]
35. Murphy BA, et al. AMP-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. *Am J Physiol Cell Physiol*. 2009; 297:C750–C758. [PubMed: 19570894]
36. Fioramonti X, et al. Ventromedial hypothalamic nitric oxide production is necessary for hypoglycemia detection and counterregulation. *Diabetes*. 2010; 59:519–528. [PubMed: 19934009]
37. Fioramonti X, et al. Hypothalamic nitric oxide in hypoglycemia detection and counterregulation: a two-edged sword. *Antioxid Redox Signal*. 2011; 14:505–517. [PubMed: 20518706]
38. Marty N, et al. Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. *J Clin Invest*. 2005; 115:3545–3553. [PubMed: 16322792]
39. Millan C, et al. Glial glucokinase expression in adult and post-natal development of the hypothalamic region. *ASN Neuro*. 2010; 2:e00035. [PubMed: 20531973]
40. Akmayev IG, Popov AP. Morphological aspects of the hypothalamic-hypophyseal system. VII The tanycytes: Their relation to the hypophyseal adrenocorticotrophic function An ultrastructural study. *Cell Tissue Res*. 1977; 180:263–282. [PubMed: 194701]
41. Rodriguez EM, et al. Hypothalamic tanycytes: a key component of brain-endocrine interaction. *Int Rev Cytol*. 2005; 247:89–164. [PubMed: 16344112]
42. Garcia MA, et al. Elevated expression of glucose transporter-1 in hypothalamic ependymal cells not involved in the formation of the brain-cerebrospinal fluid barrier. *J Cell Biochem*. 2001; 80:491–503. [PubMed: 11169733]
43. Garcia MA, et al. Hypothalamic ependymal-glial cells express the glucose transporter GLUT2, a protein involved in glucose sensing. *J Neurochem*. 2003; 86:709–724. [PubMed: 12859684]
44. Chauvet N, et al. Transected axons of adult hypothalamo-neurohypophysial neurons regenerate along tanycytic processes. *J Neurosci Res*. 1995; 41:129–144. [PubMed: 7674374]
45. Flament-Durand J, Brion JP. Tanycytes: morphology and functions: a review. *Int Rev Cytol*. 1985; 96:121–155. [PubMed: 2416706]
46. Cortes-Campos C, et al. MCT expression and lactate influx/efflux in tanycytes involved in glia-neuron metabolic interaction. *PLoS One*. 2011; 6:e16411. [PubMed: 21297988]
47. Chan O, et al. Blockade of GABAA Receptors in the Ventromedial Hypothalamus Further Stimulates Glucagon and Sympathoadrenal but Not the Hypothalamo-Pituitary-Adrenal Response to Hypoglycemia. *Diabetes*. 2006; 55:1080–1087. [PubMed: 16567532]
48. Moheet A, et al. Initial experience with seven tesla magnetic resonance spectroscopy of hypothalamic GABA during hyperinsulinemic euglycemia and hypoglycemia in healthy humans. *Magn Reson Med*. 2013
49. Chan O, et al. ATP-sensitive K(+) channels regulate the release of GABA in the ventromedial hypothalamus during hypoglycemia. *Diabetes*. 2007; 56:1120–1126. [PubMed: 17251273]
50. Zhu W, et al. Glucose prevents the fall in ventromedial hypothalamic GABA that is required for full activation of glucose counterregulatory responses during hypoglycemia. *Am J Physiol Endocrinol Metab*. 2010; 298:E971–E977. [PubMed: 20304763]

51. Tong Q, et al. Synaptic glutamate release by ventromedial hypothalamic neurons is part of the neurocircuitry that prevents hypoglycemia. *Cell Metab.* 2007; 5:383–393. [PubMed: 17488640]
52. Murai KK, Pasquale EB. Eph receptors and ephrins in neuron-astrocyte communication at synapses. *Glia.* 2011; 59:1567–1578. [PubMed: 21850709]
53. Szepletowska B, et al. EphA5-EphrinA5 interactions within the ventromedial hypothalamus influence counterregulatory hormone release and local glutamine/glutamate balance during hypoglycemia. *Diabetes.* 2013; 62:1282–1288. [PubMed: 23274893]
54. Beverly JL, et al. Noradrenergic and GABAergic systems in the medial hypothalamus are activated during hypoglycemia. *Am J Physiol Regul Integr Comp Physiol.* 2001; 280:R563–R569. [PubMed: 11208588]
55. de Vries MG, et al. Hypoglycemia-induced noradrenergic activation in the VMH is a result of decreased ambient glucose. *Am J Physiol Regul Integr Comp Physiol.* 2005; 289:R977–R981. [PubMed: 16183631]
56. Szepletowska B, et al. Modulation of beta-adrenergic receptors in the ventromedial hypothalamus influences counterregulatory responses to hypoglycemia. *Diabetes.* 2011; 60:3154–3158. [PubMed: 22013013]
57. Watts AG, Donovan CM. Sweet talk in the brain: glucosensing, neural networks, and hypoglycemic counterregulation. *Front Neuroendocrinol.* 2010; 31:32–43. [PubMed: 19836412]
58. Lee JG, et al. beta(2)-Adrenoceptor-mediated facilitation of glutamatergic transmission in rat ventromedial hypothalamic neurons. *Neuroscience.* 2007; 144:1255–1265. [PubMed: 17175110]
59. Sanders NM, et al. The selective serotonin reuptake inhibitor sertraline enhances counterregulatory responses to hypoglycemia. *Am J Physiol Endocrinol Metab.* 2008; 294:E853–E860. [PubMed: 18334609]
60. Chaoulouff F, et al. Influence of 5-HT1 and 5-HT2 receptor antagonists on insulin-induced adrenomedullary catecholamine release. *Neuroendocrinology.* 1991; 54:639–645. [PubMed: 1784347]
61. Maekawa F, et al. Localization of glucokinase-like immunoreactivity in the rat lower brain stem: for possible location of brain glucose-sensing mechanisms. *Endocrinology.* 2000; 141:375–384. [PubMed: 10614660]
62. Strack AM, et al. CNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labeling with pseudorabies virus. *Brain Res.* 1989; 491:274–296. [PubMed: 2548665]
63. Cheng H, et al. Type 1 corticotropin-releasing factor receptors in the ventromedial hypothalamus promote hypoglycemia-induced hormonal counterregulation. *Am J Physiol Endocrinol Metab.* 2007; 293:E705–E712. [PubMed: 17578887]
64. McCrimmon RJ, et al. Corticotrophin-releasing factor receptors within the ventromedial hypothalamus regulate hypoglycemia-induced hormonal counterregulation. *J Clin Invest.* 2006; 116:1723–1730. [PubMed: 16741581]
65. Zhou L, et al. The medial amygdalar nucleus: a novel glucose-sensing region that modulates the counterregulatory response to hypoglycemia. *Diabetes.* 2010; 59:2646–2652. [PubMed: 20627933]
66. McCrimmon RJ, et al. Potential role for AMP-activated protein kinase in hypoglycemia sensing in the ventromedial hypothalamus. *Diabetes.* 2004; 53:1953–1958. [PubMed: 15277372]
67. McCrimmon RJ, et al. Key role for AMP-activated protein kinase in the ventromedial hypothalamus in regulating counterregulatory hormone responses to acute hypoglycemia. *Diabetes.* 2008; 57:444–450. [PubMed: 17977955]
68. McCrimmon RJ, et al. Activation of AMP-Activated Protein Kinase Within the Ventromedial Hypothalamus Amplifies Counterregulatory Hormone Responses in Rats With Defective Counterregulation. *Diabetes.* 2006; 55:1755–1760. [PubMed: 16731839]
69. Fan X, et al. Hypothalamic AMP-activated protein kinase activation with AICAR amplifies counterregulatory responses to hypoglycemia in a rodent model of type 1 diabetes. *Am J Physiol Regul Integr Comp Physiol.* 2009; 296:R1702–R1708. [PubMed: 19357294]

70. Ikematsu N, et al. Phosphorylation of the voltage-gated potassium channel Kv2.1 by AMP-activated protein kinase regulates membrane excitability. *Proc Natl Acad Sci U S A*. 2011; 108:18132–18137. [PubMed: 22006306]
71. Terunuma M, et al. Prolonged activation of NMDA receptors promotes dephosphorylation and alters postendocytic sorting of GABAB receptors. *Proc Natl Acad Sci U S A*. 2010; 107:13918–13923. [PubMed: 20643948]
72. Kuramoto N, et al. Phospho-dependent functional modulation of GABA(B) receptors by the metabolic sensor AMP-dependent protein kinase. *Neuron*. 2007; 53:233–247. [PubMed: 17224405]
73. Pellerin L, et al. Evidence supporting the existence of an activity-dependent astrocyte-neuron lactate shuttle. *Dev Neurosci*. 1998; 20:291–299. [PubMed: 9778565]
74. Pellerin L. Lactate as a pivotal element in neuron-glia metabolic cooperation. *Neurochem Int*. 2003; 43:331–338. [PubMed: 12742077]
75. Pellerin L, et al. Activity-dependent regulation of energy metabolism by astrocytes: An update. *Glia*. 2007; 55:1251–1262. [PubMed: 17659524]
76. Song Z, Routh VH. Differential effects of glucose and lactate on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes*. 2005; 54:15–22. [PubMed: 15616006]
77. Ainscow EK, et al. Dynamic imaging of free cytosolic ATP concentration during fuel sensing by rat hypothalamic neurones: evidence for ATP-independent control of ATP-sensitive K(+) channels. *J Physiol*. 2002; 544:429–445. [PubMed: 12381816]
78. Briski KP, et al. In situ coexpression of glucose and monocarboxylate transporter mRNAs in metabolic-sensitive caudal dorsal vagal complex catecholaminergic neurons: transcriptional reactivity to insulin-induced hypoglycemia and caudal hindbrain glucose or lactate repletion during insulin-induced hypoglycemia. *Neuroscience*. 2009; 164:1152–1160. [PubMed: 19744543]
79. Vavaiya KV, Briski KP. Effects of caudal fourth ventricular lactate infusion on hypoglycemia-associated MCT2, GLUT3, GLUT4, GCK, and sulfonylurea receptor-1 gene expression in the ovariectomized female rat LHA and VMH: impact of estradiol. *J Mol Neurosci*. 2008; 34:121–129. [PubMed: 18084728]
80. Vavaiya KV, Briski KP. Effects of caudal hindbrain lactate infusion on insulin-induced hypoglycemia and neuronal substrate transporter glucokinase and sulfonylurea receptor-1 gene expression in the ovariectomized female rat dorsal vagal complex: Impact of estradiol. *J Neurosci Res*. 2008; 86:694–701. [PubMed: 17941059]
81. Vavaiya KV, Briski KP. Caudal hindbrain lactate infusion alters glucokinase, SUR1, and neuronal substrate fuel transporter gene expression in the dorsal vagal complex, lateral hypothalamic area, and ventromedial nucleus hypothalamus of hypoglycemic male rats. *Brain Res*. 2007; 1176:62–70. [PubMed: 17889836]
82. Borg MA, et al. Local lactate perfusion of the ventromedial hypothalamus suppresses hypoglycemic counterregulation. *Diabetes*. 2003; 52:663–666. [PubMed: 12606506]
83. Lam TK, et al. Regulation of blood glucose by hypothalamic pyruvate metabolism. *Science*. 2005; 309:943–947. [PubMed: 16081739]
84. Waagepetersen HS, et al. Comparison of lactate and glucose metabolism in cultured neocortical neurons and astrocytes using ¹³C-NMR spectroscopy. *Dev Neurosci*. 1998; 20:310–320. [PubMed: 9778567]
85. Waagepetersen HS, et al. Metabolism of lactate in cultured GABAergic neurons studied by ¹³C nuclear magnetic resonance spectroscopy. *J Cereb Blood Flow Metab*. 1998; 18:109–117. [PubMed: 9428311]
86. Herzog RI, et al. Lactate preserves neuronal metabolism and function following antecedent recurrent hypoglycemia. *J Clin Invest*. 2013
87. Mason GF, et al. Increased brain monocarboxylic Acid transport and utilization in type 1 diabetes. *Diabetes*. 2006; 55:929–934. [PubMed: 16567513]
88. Chan O, et al. Lactate-Induced Release of GABA in the Ventromedial Hypothalamus Contributes to Counterregulatory Failure in Recurrent Hypoglycemia and Diabetes. *Diabetes*. 2013 In Press.

89. Chan O, et al. Increased GABAergic tone in the ventromedial hypothalamus contributes to suppression of counterregulatory responses after antecedent hypoglycemia. *Diabetes*. 2008; 57:1363–1370. [PubMed: 18375441]
90. Hedrington MS, et al. Effects of antecedent GABAA activation with alprazolam on counterregulatory responses to hypoglycemia in healthy humans. *Diabetes*. 2010; 59:1074–1081. [PubMed: 20086227]
91. Jiang L, et al. Recurrent antecedent hypoglycemia alters neuronal oxidative metabolism in vivo. *Diabetes*. 2009; 58:1266–1274. [PubMed: 19276443]
92. Song Z, Routh VH. Recurrent hypoglycemia reduces the glucose sensitivity of glucose-inhibited neurons in the ventromedial hypothalamus nucleus. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291:R1283–R1287. [PubMed: 16793940]
93. Canabal DD, et al. Glucose, insulin and leptin signaling pathways modulate nitric oxide (NO) synthesis in glucose-inhibited (GI) neurons in the ventromedial hypothalamus (VMH). *Am J Physiol Regul Integr Comp Physiol*. 2006
94. Chan O, et al. Increased GABAergic output in the ventromedial hypothalamus contributes to impaired hypoglycemic counterregulation in diabetic rats. *Diabetes*. 2011; 60:1582–1589. [PubMed: 21411513]
95. De Feyter HM, et al. Increased brain lactate concentrations without increased lactate oxidation during hypoglycemia in type 1 diabetic individuals. *Diabetes*. 2013 In Press.
96. Laing SP, et al. The British Diabetic Association Cohort Study, II: all-cause mortality in patients with insulin-treated diabetes mellitus. *Diabet Med*. 1999; 16:466–471. [PubMed: 10391393]

Outstanding questions

- What is the nature of the interaction between glial cells, GE and GI neurons and what are the downstream targets of these neurons that regulate counterregulatory hormone release during hypoglycemia?
- What is the exact neural pathway from VMH GABAergic neurons to the suppression of counterregulatory responses during hypoglycemia?
- What are the neuronal circuits that co-ordinate local norepinephrine release into the VMH during hypoglycemia?
- What are the signals that activate UCN3 neurons projecting to the VMH?
- What are the efferent signals that lie downstream of the VMH, and how are these metabolic signals transduced to peripheral organs?
- What are the origins of the GABA and glutamate neurons that act within the VMH?
- How are glucose sensors in other areas of the brain and periphery affected by antecedent episodes of hypoglycemia and how do they contribute to defective counterregulation?

Highlights

- The counterregulatory hormone response to hypoglycemia is regulated by a multitude of inputs from CNS fuel sources, neurotransmitter systems and neuropeptide systems providing information to the VMH regarding overall energy status
- Prior exposure to hypoglycemia results in central adaptations that lead to the use of alternative fuels by glucose sensing neurons.
- Similar to recurrent hypoglycemia, adaptive changes in brain fuel metabolism in diabetes limit the capacity of glucose sensing neurons from properly detecting hypoglycemia.

HYPOGLYCEMIA

Figure 1. VMH glucose sensing mechanisms during acute hypoglycemia

Schematic showing pathways in the VMH involved in glucose sensing, in response to an *acute* bout of hypoglycemia in the non-diabetic hypoglycemia-naïve condition. In glucose excited (GE) neurons it has been postulated that a decrease in glucose entering the neuron leads to a reduction in the ATP/ADP ratio. Decreases in this ratio causes ATP-sensitive potassium channel (K_{ATP}) to open which in turn, hyperpolarizes GE neurons and prevents release of the inhibitory neurotransmitter, GABA. On the other hand, hypoglycemia, which stimulates glucose inhibited (GI) neurons, leads to activation of AMPK. AMPK then increases the production of nitric oxide (NO) which in turn, further enhances the level of AMPK activation through secondary messengers. This amplification of AMPK activity then inactivates (dotted line) the CFTR chloride channel, resulting in depolarization of glucose inhibited neurons and release of a neurotransmitter, likely glutamate. In the astrocytes/tanycytes, a reduction in glucose entry leads to the breakdown of stored glycogen and the production of lactate. Lactate is exported from the astrocyte through monocarboxylic acid transporter 1 or 4 (MCT1 or 4) and enters nearby neurons through MCT2 where it is oxidized.

RECURRENT HYPOGLYCEMIA

Figure 2. VMH glucose sensing mechanisms during recurrent hypoglycemia

Schematic showing our current understanding of how defective fuel sensing mechanisms in the brain following recurrent exposure to hypoglycemia contribute to counterregulatory failure. The oxidation of glucose and the breakdown of stored glycogen in the astrocyte/tanycyte results in greater lactate formation. Lactate is then made available to both adjacent GE and GI neurons. The oxidation of lactate in GE neurons serves two purposes: 1) to generate ATP which leads to closure of the K_{ATP} channel and 2) to provide a substrate for the synthesis of GABA. Together, this enhances GABA release. A decrease in glucose along with increased oxidation of lactate in GI neurons, however, prevents intracellular AMP levels from rising and inhibits activation of AMPK, allowing CFTR channels to open and chloride to enter. Chloride entry then hyperpolarizes GI neurons and inhibits its firing, thereby reducing glutamate release. The resulting increase in GABAergic inhibition and reduction in glutamatergic excitation following recurrent exposure to hypoglycemia act synonymously to suppress the release of counterregulatory hormones during subsequent bouts of hypoglycemia.