Bringing Light to the Dark Side of Insulin
A Journey Across the Blood-Brain Barrier

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I think it’s remarkable that only a few months after Banting introduced insulin to the world of diabetes, on the heels of one of medicine’s greatest discoveries, Elliot Joslin wrote, “insulin is not a cure for diabetes, but a potent preparation alike... for evil and for good.” Today, 85 years later, with all the advances in insulin therapy, there is still a dark side to this potent preparation, and it is hypoglycemia. Hypoglycemia is the major barrier preventing insulin from achieving its full, therapeutic promise.

My story begins 30 years ago. Bill Tamborlane came to me with a portable, battery-powered pump being used in pediatrics to infuse the iron-chelating drug desferrioxamine continuously via the subcutaneous route into children with thalassemia major. He hoped to use it to treat children with glycogen storage disease, a study we never performed. This relatively small pump, the Autosyringe Model AS2C, was at that time relatively unique. It had an “instant dose” button that allowed for bolus dose administration as well as the ability to deliver solutions continuously. In short, it was a “perfect” vehicle to infuse insulin in diabetes. Improved glucose control had been demonstrated earlier in the inpatient setting in type 1 diabetic patients given continuous intravenous insulin delivered at preprogrammed basal rates with increments before meals. Such systems were not practical outside of a controlled environment. This device, however, was small enough to be used for continuous subcutaneous insulin delivery.

My enthusiasm for this idea was based on experiments Luigi Sacca and I performed 2 years earlier in the mid-1970s. We demonstrated that overnight basal insulin infusion in doses sufficient to normalize fasting glucose the next morning restored the ability of hyperglycemia to suppress hepatic glucose production in type 1 diabetic patients, despite their failure to release insulin (1). Those studies suggested that if insulin were delivered at more physiological basal rates (something that was not easy to achieve with the intermediate-acting insulin available at the time), it would not only provide better regulation of fasting glucose but also minimize postprandial hyperglycemia after a breakfast meal. The second study made me think that open-loop continuous insulin delivery might be relatively safe. In that study, we infused insulin to “conventionally” (which at that time was poorly) treated type 1 diabetic subjects in doses that approached 2 units/h (2). Only mild hypoglycemia (59 ± 5 mg/dl) was produced; glucose stabilized at levels comparable with those seen in nondiabetic control subjects. Since these doses of insulin exceed usual basal requirements overnight, I assumed that if insulin were delivered continuously by the subcutaneous route in relatively small basal doses, even if dosage errors occurred and plasma glucose fell during sleep, the patient would still be able to muster adequate counterregulatory defenses to protect against severe hypoglycemia.

Our initial inpatient insulin pump studies conducted by Bill Tamborlane and our team of dedicated nurses were highly successful and were trumpeted in the New York Times. The “insulin pump” produced near normalization of blood glucose throughout the day and night in youngsters with type 1 diabetes that had been difficult to control outside of the hospital (3). The improvement in glucose regulation with the pump was achieved without an increase in daily insulin doses and was associated with improved lipid and protein metabolism as well as growth potential (4,5). We saw these studies as confirming the idea that such an approach would not promote excessive hypoglycemia and said so in our report in 1979 (3): “Noteworthy, was the fact that mean concentrations, as well as fluctuations in plasma glucose, were reduced without the development of chemical, or clinical hypoglycemia. The failure to observe hypoglycemia during treatment with the infusion pump, may be related to the physiologic nature of between-meal basal insulin administration.”

This conclusion, unfortunately, proved incorrect. After our patients left the hospital, while this “more physiological” insulin delivery system markedly improved glycemic control (6) and prompted longer-term collaborative clinical trials (7), it did not take long for the dark side of insulin, hypoglycemia, to appear. Contrary to our optimistic predictions, patients had frequent hypoglycemia, and to our surprise, frequently they were totally unaware of it. As with many “new” clinical observations, they are often not as new as they seemed. Fredrick Banting in his 1925 Nobel Laureate lecture stated, “The level at which hypoglycemic symptoms occur, is slightly higher in the diabetic with marked hyperglycemia... As a patient becomes accustomed to a normal blood glucose, the threshold of these reactions, becomes lower.”

The importance of hypoglycemia, as a complication of intensive insulin therapy, became fully apparent when the benefits of such treatment in the Diabetes Control and Complications Trial (DCCT) were announced, more than a decade later (8). The DCCT follow-up analysis (9) showed that one-half of severe hypoglycemic events occurred while patients were asleep. In those patients who were awake at the time of the hypoglycemic event, one-half were not aware of impending hypoglycemia and therefore
could not take protective action. It is for this reason that, for many patients, the immediate fear of severe hypoglycemia exceeds the fear of long-term diabetes complications. This reduces the commitment of patients, their families, and their physicians to intensive insulin treatment.

The reason for the higher rate of hypoglycemia became evident when we adapted the euglycemic-hyperinsulinemic clamp for studies of hypoglycemia. This technique, developed a decade earlier when I worked as a postdoctoral fellow in the laboratory of Reuben Andres (10), allowed us to produce a standardized hypoglycemic stimulus, providing a means to compare responses between nondiabetic and diabetic subjects (11,12). These studies taught us that nondiabetic subjects are exquisitely sensitive to very small reductions in circulating glucose. When glucose falls below 80 mg/dl, endogenous insulin secretion is nearly totally suppressed. A further decline below 70 mg/dl provokes glucagon and epinephrine secretion, the two hormones most critical for the rapid restoration of blood glucose, whereas a decline to below 55-60 mg/dl produces symptoms. This multihormonal hypoglycemia defense system is severely compromised in type 1 diabetes, even in the absence of intensive insulin treatment. These patients, by definition, are unable to shut off the delivery of endogenous insulin into the portal circulation, and more importantly, they fail to secrete glucagon specifically when they need it most, during hypoglycemia (at other times glucagon secretion may be inappropriately excessive). This poorly understood defect, originally reported by Jack Gerich (13), makes type 1 diabetic patients totally dependent on epinephrine for their defense against hypoglycemia. Unexpectedly, we found that this alternative defense is compromised in many patients within a short time after initiating intensive insulin therapy. Epinephrine release, and the appearance of symptoms, only occurred when plasma glucose reached dangerously low levels, commonly below 50 mg/dl (Fig. 1). Thus, loss of symptom awareness was linked to a lowering of the glucose level required to trigger epinephrine secretion.

Complementary experiments by Stephanie Amiel, then a postdoctoral fellow in the lab, showed that the impairment of glucose counterregulation induced by intensive insulin treatment interfered with the ability of patients to effectively counteract even relatively small doses of infused insulin (14). As we observed earlier (2), when insulin was infused continuously in relatively low doses, conventionally treated type 1 diabetic patients were indeed able to defend against severe hypoglycemia. However, after insulin pump therapy, their response changed. They developed severe hypoglycemia due to the combined effects of inadequate release of epinephrine and improved insulin sensitivity.

It is noteworthy that the levels of glucose bathing the brain are very different from those we measure in the systemic circulation. Remarkably, glucose levels in human brain interstitial fluid during hypoglycemia are only ~25% of those measured in plasma (15). As a result, brain glucose may fall to concentrations that are rate limiting for glucose metabolism in areas of brain in which activity is locally increased. For glucose, there really is a blood-brain barrier.

The reason that severe hypoglycemia most commonly occurred during sleep in the DCCT became apparent when Tim Jones performed hypoglycemic clamp experiments during the day and in the middle of the night (16). At night, nondiabetic and diabetic subjects were studied both while awake and while asleep. As expected, hypoglycemia provoked epinephrine release when subjects were awake, independent of the time of day. In striking contrast, the same hypoglycemic stimulus had little effect on epinephrine release when either group of subjects was asleep. It took a much greater glucose fall to generate symptoms of sufficient intensity to awaken them.

Why intensively treated patients released epinephrine and experienced symptoms at lower glucose levels became apparent when Simon Heller and Philip Cryer reported that the body adapts to acute hypoglycemia by lowering the glucose level at which counterregulatory hormones are released and symptoms appear the next day (17). This scenario predicts that recurrent insulin-induced hypoglycemia would create a vicious cycle, progressively magnifying the defect in counterregulation and symptom awareness. They called the phenomenon “hypoglycemia-associated autonomic failure” (HAAF). Subsequently, Geri Boll’s group showed that HAAF could, over time, be reversed by scrupulous avoidance of hypoglycemia (18). This is, of course, not easy to achieve in clinical practice.

This counterintuitive observation raised the question, why would the body adapt to hypoglycemia by suppressing its defense against it? My search for answers led me to a little-explored path, away from the conventional view of glucose metabolism—one that led to territory first explored by Claude Bernard long before the discovery of insulin. Bernard proposed, one and a half centuries ago, that the control center for glucose homeostasis resides in the brain (19). A more current stimulus for my change in direction was a study published by Alan Cherrington’s laboratory (20). Insulin-induced hypoglycemia was performed in two groups of dogs; in one group glucose was delivered directly into the vessels perfusing the brain to prevent glucose from falling in the brain. In these dogs the rise in epinephrine and glucagon secretion was nearly abolished, despite hypoglycemia in the rest of the body, suggesting that the brain is the primary site for hypoglycemia detection. This prompted me to ask three basic questions: 1) Where in the brain is the glucose sensor? 2) What mechanisms does it use to activate hypoglycemic counterregulation? 3) Why does hypoglycemia begin hypoglycemia?

WHERE IN THE BRAIN IS THE GLUCOSE SENSOR?
To address this question, I turned to studies performed by Oomura and coworkers, nearly 40 years ago (21). Oomura demonstrated the existence of specialized neurons in the
brain with the unique ability to alter their firing rate when glucose levels changed. These neurons were predomi-
nantly located in the hypothalamus, a critical brain region for many basic survival functions such as reproduction, energy metabolism, and feeding. In particular, they were concentrated in the ventromedial hypothalamus (VMH), a brain area that encompasses the ventromedial and arcuate nuclei. To determine if the VMH was the location of the brain glucose sensor, Jerry Shulman and I joined forces to adapt the hypoglycemic clamp, originally developed in humans for studies in awake, chronically catheterized rodents. This allowed us to test the effect of selective bilateral VMH lesions on counterregulatory responses under identical hypoglycemic conditions in awake, freely moving rats (22). Hypoglycemia increased glucagon and epinephrine secretion in sham-operated control rats as well as in rats with lesions elsewhere in brain. In contrast, VMH lesions caused a striking reduction in hormonal responses to hypoglycemia, indicating that the VMH was a place to explore in more detail. The clinical relevance of these observations in rodents was subsequently supported in a 1999 case report of a patient with the same dramatic suppression of hormonal responses to hypoglycemia in which sarcoid lesions had destroyed the VMH (23).

The complexity and small size of the VMH meant we needed a new way to navigate—one that allowed us to selectively alter the local environment of the VMH. For this purpose, Walter and Monica Borg conducted experiments in which guide cannulae were inserted in close proximity to the VMH. After the animals recovered, microdialysis probes were inserted to deliver test substances directly to the VMH. Hypoglycemic clamp studies could then be performed while simultaneously manipulating the local microenvironment of the VMH. In one study, VMH glucopenia was selectively produced by local delivery of 2-deoxy-glucose (24). This caused glucose to rise in conjunction with a marked increase in counterregulatory hormone secretion. Next, D-glucose (metabolically active) and L-glucose (a metabolically inactive control) were delivered directly to the VMH to selectively prevent hypoglycemia in the VMH, while the rest of the brain and extra-cerebral tissues were exposed to hypoglycemia (25). VMH perfusion with D-glucose markedly suppressed both glucagon and epinephrine release, while inactive L-glucose control had no effect. Interestingly, VMH perfusion with L-lactate (but not metabolically inactive D-lactate) also abolished the hormone response to hypoglycemia (26). Taken together, the data suggest VMH neurons are critical for the activation of glucose counterregulation and that they may act as fuel and not simply as glucose sensors.

It should be emphasized that our data do not mean that glucose sensing is restricted to neurons in the VMH. The system is undoubtedly much more complex. Glucose sensors have been identified in peripheral tissues, such as the portal vein and carotid body (27,28), as well as elsewhere in the brain (29–31). The VMH may act as a central integrator of glucose-sensing signals that are both locally generated and come from a network of sensors widely distributed throughout the body during hypoglycemia. Its function is to integrate this information and to activate downstream signals to rapidly restore and maintain glucose homeostasis.

We next tested whether the capacity of the VMH to sense hypoglycemia becomes impaired in rats after exposure to antecedent hypoglycemia; rats, like humans, develop defective glucose counterregulation under these conditions (32). Delivery of 2-deoxy-glucose to block VMH glucose metabolism in rats exposed to recurrent insulin-induced hypoglycemia failed to activate glucagon and epinephrine secretion, whereas in control animals, 2-deoxy-glucose, as expected, provoked counterregulatory hormone secretion (33). These findings prompted us to examine the mechanisms used by the VMH to sense glucose and to determine if they are altered by antecedent hypoglycemia.

WHAT MECHANISMS DOES THE VMH USE TO ACTIVATE COUNTERREGULATION?

In vitro electrophysiology studies (34–36) have identified two distinct types of glucose-sensing VMH neurons: 1) glucose-excited (GE) neurons that increase their activity with a rise in glucose and are suppressed by a fall in glucose and 2) glucose-inhibited (GI) neurons that decrease their activity when glucose rises and increase their activity when glucose falls. Interestingly, these glucose-sensing neurons appear to employ mechanisms to detect glucose excess and deficiency that are very similar to those used in peripheral tissues, such as β-cells and muscle. Current data suggest that GE neurons may sense glucose via its effects on ATP-sensitive K(+) channel (KATP channel) activity, much like pancreatic β-cells (34–36). Interestingly, VMH neurons express the same SUR1 KIR-6.2 KATP channel isoform as the pancreatic β-cell (37). VMH GI neurons, on the other hand, may use the AMP kinase to sense fuel deficit (38,39). This enzyme is regulated by the relative cellular concentrations of AMP and ATP and functions as a fuel gauge in and outside the brain (e.g., muscle) to protect against energy depletion (40). These in vitro findings prompted us to explore if these glucose-sensing mechanisms were used by the VMH in vivo.

K(+)ATP channel activity. To evaluate whether KATP channel activity of VMH neurons alters glucose counterregulation, KATP channel openers (KCOs) (activators), KATP channel closers (blockers), or vehicle (control) were bi-laterally microinjected directly into the VMH of awake, chronically catheterized nondiabetic rats before a hypoglycemic clamp study (41,42). VMH microinjection of diazoxide, a KCO, reduced by 50% the glucose infusion rate needed to maintain hypoglycemia and increased the response of both epinephrine and glucagon to hypoglycemia three-fold (P < 0.05). Similar enhancement of glucose counterregulation was observed when a SUR1-specific KCO was delivered to the VMH instead. Conversely, closure of VMH KATP channels with the sulfonyleurea glibenclamide suppressed counterregulatory hormone secretion during hypoglycemia. Thus, the activity of KATP channels in VMH neurons, much like in β-cells, modulates hormone responses during hypoglycemia (Fig. 2).

HOW DO CHANGES IN VMH K(+)ATP CHANNEL ACTIVITY MODULATE GLUCOSE COUNTERREGULATION?

To address this issue, we focused on the inhibitory neurotransmitter γ-aminobutyric acid (GABA), based upon the link between intra-islet GABA inhibitory tone and the secretion of glucagon (43). Initial studies showed that acute hypoglycemia rapidly reduced GABA levels in VMH interstitial fluid, implying that a fall of this inhibitory neurotransmitter might activate a VMH signal. To test this hypothesis further, Owen Chan (Yale University, New Haven, CT) measured GABA levels in VMH interstitial fluid...
while we altered VMH K<sub>ATP</sub> channel activity during acute hypoglycemia (44). Opening K<sub>ATP</sub> channels with diazoxide produced a much more pronounced reduction in GABA levels in VMH interstitial fluid and at the same time augmented hormone release. Conversely, closure of K<sub>ATP</sub> channels by local delivery of a sulfonylurea to the VMH caused a paradoxical increase in VMH GABA levels in conjunction with suppression of the counterregulatory hormone response to hypoglycemia (Fig. 2). Furthermore, modulation of VMH GABA tone using GABAA receptor agonists suppressed, whereas GABAA<sub>δ</sub> receptor antagonists augmented, glucagon and epinephrine responses to hypoglycemia (45). Taken together, it would appear that K<sub>ATP</sub> channels in glucose-sensing VMH neurons modulate local GABAergic inhibitory tone, which in turn modulates the magnitude of the counterregulatory response.

**AMP kinase activity.** Rory McCrimmon explored the role of AMP kinase in VMH glucose sensing. For this purpose, AICAR (5-aminoimidazole-4-carboxamide) was delivered into the VMH to locally stimulate the enzyme. This increased glucose counterregulation (46). Conversely, he selectively reduced AMPK<sub>α2</sub> gene expression by ~40% in the VMH using a selective short hairpin RNA (shRNA) processed in an adeno-associated virus (AAV) (47). This caused a marked reduction in epinephrine and glucagon release during hypoglycemia (Fig. 3), suggesting that VMH AMP kinase activity has an important influence on the magnitude of the counterregulatory response, as well. This conclusion is also supported by in vitro data from Vanessa Routh’s laboratory demonstrating that the activation of VMH glucose-inhibited neurons is linked to the activation of AMP kinase within these neurons (48).

**HOW DO THESE STUDIES ALL FIT TOGETHER?**

I propose a working model of VMH glucose sensing in which a downstream VMH signal to promote counterregulatory hormone secretion is driven by the simultaneous activation of AMP kinase in GI neurons as well as by GE neuron–driven disinhibition of GABA neurons (Fig. 4). Based on recent data implicating ventromedial nucleus SF1 glutamate neurons in activating counterregulatory responses (49), it is intriguing to speculate that ventromedial nucleus SF1 glutamate neurons may be responsible for generating this downstream signal.

The model predicts that changes in VMH glucose-sensing activity in GI and GE neurons work in concert to stimulate or inhibit glucose counterregulation. In response to hyperglycemia, AMP kinase in VMH GI neurons is suppressed, reducing neuronal activity, and at the same time K<sub>ATP</sub> channels in GE neurons close and calcium enters the neuron, stimulating the release of the inhibitory neurotransmitter GABA, further suppressing the GI neuron. Conversely, hypoglycemia stimulates AMP kinase activity, stimulating VMH GI neurons, and at the same time K<sub>ATP</sub> channels open in GE neurons and calcium entry is reduced, reducing GABA tone, which in turn causes disinhibition of VMH GI neurons. Remarkably, this picture bears a striking resemblance to the crosstalk found in the islet when blood glucose levels change. The β-cell and glucose-excited neurons have much in common, as do the α-cell and glucose-inhibited neurons.

**Corticotropin-releasing factor receptors.** As with most things in biology, the story turns out to be more complex. VMH neurons are regulated during hypoglycemia...
by a second system, namely the stress response. Hypoglycemia provokes stress signals from brain regions outside of the VMH that modulate its activity via two types of corticotropin-releasing factor receptors that oppose each other. The primary stress peptide hormone corticotropin-releasing factor (CRF) predominately stimulates VMH CRF1 receptors and, in turn, counterregulatory responses (50). Local VMH delivery of a CRF1 receptor antagonist during hypoglycemia suppresses both epinephrine and glucagon responses, whereas local VMH microinjection of CRF stimulates the release of these hormones during acute hypoglycemia. The stress response also provokes a counterbalancing stress inhibitory signal urocortin III, a member of the CRF family of neuropeptides identified by Wylie Vale’s laboratory a decade ago (51). Urocortin III is a selective CRFR2 receptor agonist that is generated in the medial amygdula and released locally into the VMH, where it acts to suppress counterregulatory hormone responses to hypoglycemia. This effect is reversed by the selective CRFR2 antagonist antisauvagine (52). These observations are consistent with whole cell–current clamp recordings in brain slices showing that CRF2 receptor activation directly lowers the glucose concentration required to trigger activation of VMH GI neurons. In short, hypoglycemic stress provokes dual modulation of the neuroendocrine response via stimulation of endogenous CRF2 (inhibitory) and CRF1 (stimulatory) receptors. Based on these data, I have expanded my working model to incorporate the additional neuronal input to the VMH generated by the stress pathway during hypoglycemia. This postulated multistep process allows for the full activation of the VMH downstream neuronal signal stimulating glucose counterregulation (Fig. 5). How might such a model help us to understand why hypoglycemia begets hypoglycemia?

**WHY DOES HYPOGLYCEMIA BEGET MORE HYPOGLYCEMIA?**

The proposed model of VMH glucose sensing may serve as a starting point to help us understand why hypoglycemia begets hypoglycemia. Several putative mechanisms emerge. Recurrent antecedent hypoglycemia might reduce VMH K_ATP channel activity or increase VMH GABA or urocortin III inhibitory input or suppress VMH AMP kinase activity. To address these possibilities, we developed an animal using nondiabetic rats exposed to recurrent antecedent insulin-induced hypoglycemia for 3 h for 3 days.

**FIG. 4. Working model of VMH glucose sensing. Reciprocal changes in glucose-excited and glucose-inhibited neurons act in concert to stimulate or inhibit glucose counterregulation when blood glucose rises or falls.**

**Reduced VMH K_ATP channel activity.** To determine whether KCO delivery to the VMH could reverse defective glucose counterregulation, rodents exposed to antecedent hypoglycemia were given bilateral microinjections of either diazoxide or vehicle into the VMH, after which they underwent a hypoglycemic clamp study (41). VMH delivery of diazoxide or a SUR1-specific KCO totally reverses the animal’s defective counterregulatory response (Fig. 6). Preliminary studies suggest that the systemic delivery of KCO has similar beneficial effects on hormonal responses to hypoglycemia in animals with impaired glucose counterregulation and thus might have therapeutic benefit in patients with type 1 diabetes.

**Increased VMH GABAergic inhibitory tone.** To assess whether increased VMH GABA tone contributes to the development of defective counterregulation, we quantified the GABA synthetic enzymes GAD65 and GAD67 within the VMH of rats exposed to recurrent hypoglycemia (53). These rodents demonstrated a specific increase in GAD65 gene expression and a five- to sixfold increase in GAD65 protein expression within the VMH. We also measured GABA levels in VMH interstitial fluid in rats exposed to antecedent hypoglycemia. In keeping with the GAD65 protein findings, basal levels of GABA in VMH interstitial fluid were threefold higher in the basal state and during acute hypoglycemia. Furthermore, delivery of a GABAA receptor antagonist into the VMH of these rats reversed their defective counterregulatory hormone response (53).

**FIG. 5. Expanded VMH model for activation of glucose counterregulation to include stimulatory and inhibitory input from the CRF family of neuropeptides. The model posits that hypoglycemia provokes the activation of AMP kinase in VMH glucose-inhibited neurons. Simultaneously, the inhibitory neuronal inputs, urocortin III and GABA, are suppressed, and the CRF metabolic stress pathway is activated. Together, these changes act in concert to fully activate downstream neural pathways to restore glucose homeostasis.**

**FIG. 6. Delivery of KCOs into the VMH reverses the suppressive effect of antecedent recurrent hypoglycemia on glucagon and epinephrine secretion. Three groups of rats were studied: control (open bars), recurrent hypoglycemic (shaded bars), and recurrent hypoglycemic plus bilateral VMH KCO microinjections (black bars).**
These data suggest that antecedent hypoglycemia may enhance VMH GABAergic inhibitory tone and in turn impair glucose counterregulation.

**Increased VMH CRF2 receptor activity.** The role of altered VMH CRF receptor activity in the pathogenesis of impaired counterregulation after antecedent hypoglycemia remains to be explored. The possibility that hypoglycemia-induced increases in plasma cortisol might be the driving force (54) led us to study nondiabetic human subjects in whom antecedent hypoglycemia was produced with and without concurrent blockade of endogenous cortisol production using metyrapone (55). We observed that antecedent hypoglycemia suppressed the epinephrine response to hypoglycemia the next day regardless of whether endogenous cortisol production was selectively suppressed with metyrapone. These observations are most consistent with rodent data (50,52,56), suggesting that hypoglycemia-induced alterations in brain CRH receptor activity rather than increases in plasma cortisol are more likely to contribute to the pathogenesis of defective counterregulation following antecedent hypoglycemia.

**Suppressed VMH AMP kinase activity.** To test the potential role of suppressed VMH AMPK activity in defective counterregulation, we injected the chemical activator of AMP kinase, AICAR, into the VMH of rats exposed to recurrent hypoglycemia (57). This totally reversed the impaired hormonal response to acute hypoglycemia seen in these animals, findings consistent with data demonstrating impaired hypothalamic AMPK activation in this rodent model (58). Similar results were also observed in diabetic BB rats, a model of type 1 diabetes that exhibits a nearly absent glucagon response to hypoglycemia (59). Chemical activation of AMP kinase within the VMH increased epinephrine secretion three- to fourfold during hypoglycemia and unexpectedly stimulated glucagon release, implying that this defect in type 1 diabetes might be reversible as well (unpublished data, R. McCrimmon).

### WHY DOES HYPOGLYCEMIA BEGET HYPOGLYCEMIA?

**Lessons learned from my journey.** One might speculate, based on our VMH model of glucose counterregulation, that recurrent hypoglycemia impairs VMH glucose sensing and signaling by multiple complementary adaptations (Fig. 7), including suppressed VMH AMP kinase activity and increased activation of inhibitory neuronal circuits, mediated by GABA or CRF receptors. It is noteworthy that these adaptive responses appear to be reversible, as has been reported clinically. Most importantly, the proposed model offers a number of potential therapeutic targets to reverse the phenomenon in patients with type 1 diabetes. Of course, we are undoubtedly dealing with a much more complex system. This should not be a surprise, given that maintenance of glucose homeostasis is so important to survival.

### WHY WOULD THE BRAIN ADAPT TO RECURRENT HYPOGLYCEMIA BY BECOMING MORE VULNERABLE TO IT?

The brain is not as vulnerable as it seems. It may have adapted to this recent challenge (from an evolutionary perspective) the best way it can, namely, by becoming more efficient metabolically, by increasing the transport of glucose across the blood-brain barrier, and by using alternative fuels to glucose. This view is supported by several observations. Antecedent insulin-induced hypoglycemia increases brain glucose transport and utilization in rodents (60–62) and brain glucose uptake in nondiabetic and diabetic humans rendered hypoglycemic for several days (63,64). Under conditions of more prolonged glucose deprivation, the brain most likely adapts to use a number of alternative fuels to generate ATP, such as the monocarboxylic acids lactate, ketone bodies, and acetate. All are potential energy sources. In starvation, humans can tolerate severe hypoglycemia (1 mmol/l) without an impairment of cognitive function by increasing the use of ketone bodies as an energy source for the brain (65). In keeping with this scenario, the brain utilization of monocarboxylic acids appears to be increased in type 1 diabetic patients receiving intensive insulin therapy (66). Thus, the brain exposed to antecedent hypoglycemia may adapt, much as it did long before the discovery of insulin, i.e., when glucose declined during prolonged starvation.

The adaptation to recurrent hypoglycemia is likely to serve two basic survival functions, serving initially to protect the body from the negative consequences of recurrent stress and serving long term to protect the brain from fuel deprivation, much like the phenomenon of preconditioning. The problem is that these beneficial adaptations also act to limit the brain’s ability to detect reductions in blood glucose. As a result, when it does sense hypoglycemia, it may be too little, too late.

### DO HYPOGLYCEMIA-INDUCED ADAPTATIONS IN BRAIN FUEL METABOLISM PROTECT IT FROM INJURY?

To examine this question, Ewan McNay tested spatial memory performance and brain glucose metabolism in nondiabetic and streptozotocin-induced diabetic rats exposed to hypoglycemia for 3 consecutive days (67). Remarkably, memory performance improved in both groups of rats when they were studied at euglycemia the next day, most likely due to an increase in glucose transport across the blood-brain barrier. The capacity of antecedent hypoglycemia to enhance cognition was, however, limited. When these animals were exposed to moderately severe hypoglycemia, the improvement in cognitive performance could not be sustained. Thus, a point may be reached when there is need to preserve basic functions (e.g., hypothalamic and brainstem activity) at the expense of more complex functions (e.g., spatial memory). This view is supported by studies demonstrating that antecedent hypoglycemia facilitates brainstem neural transmission during acute hypoglycemia (68).
A similar picture was observed in long-term studies using a more clinically relevant protocol (69). Hypoglycemia was induced in nondiabetic rats for 3 h once each week, a pattern similar to that seen in insulin-treated diabetic patients. Weekly bouts of insulin-induced hypoglycemia were initiated at 1 month of age and continued until the animals were 1 year of age (Fig. 8). Spatial working memory performance was tested 1 week after the animal’s last bout of hypoglycemia when they were euglycemic. At 4 months of age the rats exposed to weekly hypoglycemia, like their counterparts in the short-term study, performed better during the memory task. After 1 year, while the control animals showed the expected steep decline in memory performance, this failed to occur in rats exposed to hypoglycemia. Indeed, they performed like a 4-month-old control animal. These data suggest that there are marked differences in the immediate consequences of acute hypoglycemia and the long-term effects of recurrent hypoglycemia on higher brain function. This conclusion is consistent with the follow-up analysis of DCCT patients, nearly two decades after the start of the study (70). Detailed neuropsychiatric testing (when these patients were not hypoglycemic) failed to detect cognitive dysfunction in the intensively treated patients with more frequent severe hypoglycemia. Although these data are not definitive, they suggest that insulin, as we give it today, really has two dark sides. From an acute prospective it is the serious immediate consequences of hypoglycemia, but over the long haul, for most patients, it is hyper- rather than hypoglycemia.

VIEW OF GLUCOSE HOMEOSTASIS FROM ACROSS THE BLOOD-BRAIN BARRIER

For the 85 years since Banting’s landmark discovery, the regulation of glucose homeostasis has been viewed by the diabetes world from an islet-centric perspective. Bernard’s vision of the brain as the master control site of glucose homeostasis has, for the most part, been abandoned. My journey across the blood-brain barrier has taught me that Bernard’s vision should not be forgotten. I believe we evolved dual control centers for the regulation of glucose homeostasis, the islet and the brain, and that they are wired together and connected with the rest of the body to more precisely regulate blood glucose as fuel demands change.

I believe the brain, and the VMH in particular, is the dominant control center initiating counterregulatory responses when blood glucose falls below baseline. This view is consistent with the brain’s immediate dependence on glucose as an energy source and the marked differences in the concentration of glucose within brain interstitial fluid compared with levels elsewhere in the body. However, dual control is more effective. The islet supports the VMH by reducing its secretion of insulin and by working in concert with the VMH to increase glucagon secretion (Fig. 9). On the other hand, the islet serves as the dominant sensor when blood glucose rises, by virtue of its ability to increase its secretion of insulin and to reduce its secretion of glucagon. However, once again dual control is more effective in restoring glucose homeostasis (Fig. 10). The brain receives neural and hormonal signals from the gut that promote insulin secretion as well as glucose storage in the liver (71,72). The brain may also receive a signal from the islet to facilitate the suppression of hepatic glucose production (73) as well as glucagon secretion. Again, the islet and VMH are working synergistically.

How did such a dual glucose sensor system evolve? There are lessons we can learn from the fruit fly. The cells that produce insulin in the fruit fly, like other invertebrates, are located in the brain (74). These insulin-secreting neurons communicate directly with cells that secrete an anti-insulin hormone that has effects similar to glucagon (75). Thus, the ancestral home of the islet and the regulation of glucose homeostasis most likely resided
in the brain. One can imagine that, as we evolved, it was more advantageous for the islet to move from the brain to an area closer to the liver, the site where glucose is stored. Nevertheless, one can imagine that it would have been crucial for survival to retain glucose-sensing neurons with characteristics similar to β- and α-cells in the VMH to directly monitor minute-to-minute changes in brain glucose availability more closely. This might be particularly important given that the levels of glucose in brain interstitial fluid are only a small fraction (~25%) of those bathing peripheral tissues. Thus, I believe it was essential to retain glucose sensors in the VMH; only a site within the brain can provide a truly accurate assessment of its energy supply. A peripheral glucose sensor exposed to much higher glucose levels during hypoglycemia probably couldn’t do the job as well. This is crucial since the brain is the only organ that must have glucose to meet its immediate needs. As a result, insulin-induced hypoglycemia is a brain-specific complication.

**POSTSCRIPT**

I’d like to end my journey across the blood-brain barrier with a postscript. When I began my research career, I was taught that there were specific organs that responded to insulin and that the brain was not one of them. This view is now being challenged on many fronts. Dan Porte, Steve Woods, and Michael Schwartz have established that insulin modulates satiety by acting on the hypothalamus (76). Rossetti and coworkers (73) suggest the existence of an insulin-brain-liver connection that regulates glucose metabolism. Furthermore, our preliminary studies indicate that delivery of small amounts of insulin directly into the hippocampus of nondiabetic rats, a brain region critical for learning and memory and rich in insulin receptors and GLUT4 (77), significantly improves cognitive performance and stimulates local brain glucose metabolism. This effect is impaired in insulin-resistant rats with diet-induced obesity, suggesting a potential link between defective insulin-stimulated glucose metabolism in the hippocampus and the increased risk of cognitive impairment seen in type 2 diabetes.

These observations raise another important question, namely, could insulin also act directly on the VMH, altering its capacity to sense fuel deficit? This possibility is supported by evidence that VMH neurons express insulin receptors and that insulin alters the firing rate of VMH glucose-sensing neurons in vitro (78). It is intriguing to speculate that we evolved a dual glucose-sensing system based in the VMH and islet designed to defend against hypoglycemia in the setting in which it originally occurred, namely during prolonged starvation, a time when both glucose and insulin levels fall simultaneously. Thus, we may not have optimally adapted to insulin-induced hypoglycemia, a danger rarely, if ever, encountered before Banting (Fig. 11). During insulin-induced hypoglycemia, the α-cell sees a drop in local islet insulin in spite of the rise in systemic insulin levels, given the suppression of endogenous insulin production. On the other hand, the VMH gets a very different message, namely, a feeding signal: insulin. How this might impact on the homeostatic response to hypoglycemia in nondiabetic and diabetic individuals’ disease warrants careful scrutiny. It is possible that insulin-induced hypoglycemia may limit the capacity of the body to defend against the challenge of acute hypoglycemia. A maximal response to insulin-induced hypoglycemia may require both the islet and the VMH to see a drop in insulin concentration.

Six years ago in my American Diabetes Association president’s address, I challenged the diabetes community to think beyond the barriers—beyond glucose. I suggested that our journey would require engaging scientists from many different disciplines, people not currently working in diabetes. I challenge you again to think beyond the islet and to engage the burgeoning world of neuroscience. These efforts will help us unravel not only hypoglycemia but also such problems as obesity, Alzheimer’s disease, and perhaps even type 2 diabetes.

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