

Mechanisms of Hypoglycemia-Associated Autonomic Failure and Its Component Syndromes in Diabetes

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Iatrogenic hypoglycemia is a problem for people with diabetes. It causes recurrent morbidity, and sometimes death, as well as a vicious cycle of recurrent hypoglycemia, precluding maintenance of euglycemia over a lifetime of diabetes. Improved therapeutic approaches that will minimize both hypo- and hyperglycemia will be based on insight into the pathophysiology of glucoregulation, specifically glucose counterregulation, in insulin-deficient (type 1 and advanced type 2) diabetes. In such patients, hypoglycemia is the result of the interplay of relative or absolute therapeutic insulin excess and compromised physiological (the syndrome of defective glucose counterregulation) and behavioral (the syndrome of hypoglycemia unawareness) defenses against falling plasma glucose concentrations. The concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes posits that recent antecedent iatrogenic hypoglycemia causes both defective glucose counterregulation (by reducing epinephrine responses to a given level of subsequent hypoglycemia in the setting of absent decrements in insulin and absent increments in glucagon) and hypoglycemia unawareness (by reducing sympathoadrenal and the resulting neurogenic symptom responses to a given level of subsequent hypoglycemia) and thus a vicious cycle of recurrent hypoglycemia. The clinical impact of HAAF is well established in type 1 diabetes; it also affects those with advanced type 2 diabetes. It is now known to be largely reversible, by as little as 2–3 weeks of scrupulous avoidance of hypoglycemia, in most affected patients. However, the mechanisms of HAAF and its component syndromes are largely unknown. Loss of the glucagon secretory response, a key feature of defective glucose counterregulation, is plausibly explained by insulin deficiency, specifically loss of the decrement in intraslet insulin that normally signals glucagon secretion as glucose levels fall. Reduced neurogenic symptoms, a key feature of hypoglycemia unawareness, are largely the result of reduced sympathetic neural responses to falling glucose levels. The mechanism by which hypoglycemia shifts the glycemic thresholds for sympathoadrenal activation to lower plasma glucose concentrations, the key feature of both components of HAAF, is not known. It does not appear to be the result of the release of a systemic mediator (e.g., cortisol, epinephrine) during antecedent hypoglycemia or of increased blood-to-brain glucose transport (although increased transport of alternative fuels is con-

ceivable). It is likely the result of alterations of brain metabolism. Although there is an array of clues, the specific alteration remains to be identified. While the research focus has been largely on the hypothalamus, hypoglycemia is now known to activate widespread brain regions, including the medial prefrontal cortex. The possibility that HAAF could be the result of posthypoglycemic brain glycogen supercompensation has also been raised. Finally, there appear to be diverse causes of HAAF. In addition to recent antecedent hypoglycemia, these include exercise- and sleep-related HAAF. Clearly, a unifying mechanism of HAAF would need to incorporate these causes as well. Pending the prevention and cure of diabetes, critical fundamental, translational, and outcomes research is needed if we are to eliminate hypoglycemia from the lives of people affected by diabetes. *Diabetes* 54:3592–3601, 2005

THE CLINICAL PROBLEM

Iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes (1). It causes recurrent morbidity in most people with type 1 diabetes and many with type 2 diabetes and is sometimes fatal. Furthermore, episodes of hypoglycemia, even asymptomatic episodes, impair defenses against subsequent hypoglycemia by causing hypoglycemia-associated autonomic failure (HAAF), the clinical syndromes of defective glucose counterregulation and hypoglycemia unawareness, and therefore a vicious cycle of recurrent hypoglycemia. In addition, the barrier of hypoglycemia precludes maintenance of euglycemia over a lifetime of diabetes and thus full realization of the benefits of glycemic control. The glycemic goals advocated by professional diabetes organizations tacitly acknowledge that barrier. None recommend a goal of a normal HbA_{1c}. While glycemic control short of euglycemia reduces (but does not eliminate) the microvascular complications of diabetes, long-term euglycemia may be necessary to reduce macrovascular complications in a substantial proportion of people with diabetes (1).

Despite steady improvements in the glycemic management of diabetes (2) and perhaps because of the impetus for glycemic control that resulted from the positive findings of the Diabetes Control and Complications Trial (3) and the U.K. Prospective Diabetes Study (4,5), population-based data indicate that hypoglycemia continues to be a problem for people with both type 1 (6) and type 2 (7,8) diabetes. The problem is not limited to type 1 diabetes (1). In insulin-treated type 2 diabetes, the event rates for severe hypoglycemia, at least that requiring emergency treatment, approach those in type 1 diabetes (7,8).

Elimination of hypoglycemia from the lives of people with diabetes and long-term maintenance of euglycemia

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CNS, central nervous system; HAAF, hypoglycemia-associated autonomic failure; K_{ATP} channel, ATP-sensitive K⁺ channel; PET, positron emission tomography; VMH, ventromedial hypothalamus.

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will undoubtedly require glucose-regulated insulin replacement or secretion. Pending that ultimate goal, there is a critical need to develop therapeutic approaches that minimize both hyper- and hypoglycemia. It is a premise that those improved approaches will be based on insight into the pathophysiology of glucoregulation, specifically glucose counterregulation, in type 1 and type 2 diabetes. That integrated pathophysiology has been reviewed (1). Here, the focus is on what is known, and what is not known, about the mechanisms of the clinical syndromes of defective glucose counterregulation and hypoglycemia unawareness, and the concept of HAAF, in insulin-deficient (type 1 and advanced type 2) diabetes.

THE PATHOPHYSIOLOGY OF GLUCOSE COUNTERREGULATION IN DIABETES

The key components of the physiology of glucose counterregulation, the mechanisms that normally prevent or rapidly correct hypoglycemia, are 1) a decrease in pancreatic islet β -cell insulin secretion, 2) an increase in pancreatic islet α -cell glucagon secretion, and, absent the latter, 3) an increase in adrenomedullary epinephrine secretion (9). All three of these defenses against developing hypoglycemia are compromised in insulin-deficient (type 1 and advanced type 2) diabetes (1). In such patients, iatrogenic hypoglycemia is the result of the interplay of relative or absolute insulin excess, which must occur from time to time because of the pharmacokinetic imperfections of all therapeutic insulin regimens, as well as compromised glucose counterregulation. As plasma glucose concentrations fall 1) plasma insulin concentrations do not decrease (in the absence of endogenous insulin secretion, these are simply the result of the absorption and clearance of administered insulin), 2) plasma glucagon concentrations do not increase (likely the result of the absence of the intraislet insulin signal, a decrease in intraislet insulin and thus in tonic α -cell inhibition by insulin [10–13], as discussed later), and 3) plasma epinephrine concentrations increase, but the increment is typically attenuated (the glycemic thresholds for sympathoadrenal activation are typically shifted to lower plasma glucose concentrations). The latter, a critical feature of the pathophysiology of glucose counterregulation, is generally the result of recent antecedent iatrogenic hypoglycemia, although sleep, and to some extent prior exercise, have a similar effect (1).

An attenuated adrenomedullary epinephrine response to falling plasma glucose concentrations, in the setting of absent β -cell insulin and α -cell glucagon responses, causes the clinical syndrome of defective glucose counterregulation (1). Affected patients are at 25-fold or greater increased risk for severe iatrogenic hypoglycemia during aggressive glycemic therapy (14,15). An attenuated sympathoadrenal response (largely an attenuated sympathetic neural [16] response, as discussed later) causes the clinical syndrome of hypoglycemia unawareness (1). Affected patients are at about sixfold increased risk for severe iatrogenic hypoglycemia during aggressive glycemic therapy (17).

HAAF IN DIABETES

The concept of HAAF in type 1 (18) and advanced type 2 (19) diabetes posits that recent antecedent iatrogenic hypoglycemia causes both defective glucose counterregulation (by reducing epinephrine responses to a given level of subsequent hypoglycemia in the setting of absent dec-

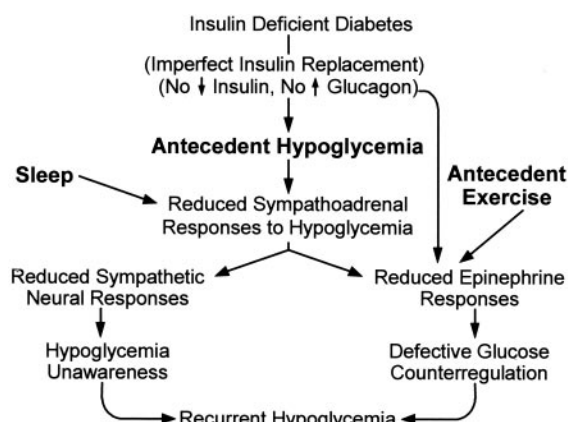


FIG. 1. Hypoglycemia-associated autonomic failure in type 1 diabetes. Modified from ref. 1. © 2004 by the MA Medical Society.

rements in insulin and absent increments in glucagon) and hypoglycemia unawareness (by reducing sympathoadrenal and the resulting neurogenic symptom responses to a given level of subsequent hypoglycemia) and thus a vicious cycle of recurrent hypoglycemia (1) (Fig. 1). The concept of HAAF has been extended to include sleep- and exercise-related HAAF (1) (Fig. 1). Since both defective glucose counterregulation (14,15) and hypoglycemia unawareness (17) result in a substantially increased frequency of severe hypoglycemia, autonomic, specifically sympathoadrenal, failure caused by recent antecedent hypoglycemia, or by exercise or sleep, is associated with a high frequency of clinical hypoglycemia in people with insulin-deficient (type 1 and advanced type 2) diabetes (1).

Reduced sympathoadrenal actions play a key role in the pathogenesis of both defective glucose counterregulation (reduced adrenomedullary epinephrine actions) and hypoglycemia unawareness (largely reduced sympathetic neural [adrenergic and cholinergic] actions) and thus HAAF in diabetes (1). These reduced actions are largely, perhaps exclusively, the result of reduced sympathoadrenal activation and the resulting reduced release of its biologically active products (e.g., epinephrine, norepinephrine, acetylcholine) in response to a given level of hypoglycemia (i.e., a shift of the glycemic thresholds to lower plasma glucose concentrations) rather than reduced tissue sensitivity to the actions of these hormones/neurotransmitters. There is substantial evidence that whole-body metabolic (and hemodynamic) sensitivity to epinephrine is not reduced in patients with type 1 diabetes (20,21). Similarly, local adipose and skeletal muscle metabolic sensitivity to a β_2 -adrenergic agonist (terbutaline) does not appear to be reduced (22). On the other hand, there is some evidence that reduced β -adrenergic sensitivity might contribute to the pathogenesis of hypoglycemia unawareness (23–26). That evidence includes reduced cardiac chronotropic sensitivity to the nonselective β -adrenergic agonist isoproterenol in patients with type 1 diabetes and hypoglycemia unawareness (23,24) and reversal of both reduced β -adrenergic sensitivity and unawareness following a period of scrupulous avoidance of iatrogenic hypoglycemia (25). Fritsche et al. (26) assessed the impact of recent antecedent hypoglycemia on cardiac chronotropic sensitivity to isoproterenol. Their data indicated that hypoglycemia was followed by increased cardiac β -adrenergic sensitivity in nondiabetic subjects but decreased cardiac β -adrenergic sensitivity in patients with type 1 diabetes. de Galan et al.

(27) found no difference in the vasodilatory (increase in forearm blood flow) response to brachial artery infusion of a β_2 -adrenergic agonist (salbutamol) in patients with hypoglycemia unawareness compared with those with awareness and nondiabetic control subjects. If reduced sensitivity plays a role in the pathogenesis of hypoglycemia unawareness, one would need to postulate that in addition to reduced β -adrenergic sensitivity, reduced cholinergic sensitivity explains reduced cholinergic symptoms such as sweating.

Integrated (whole-body) glycemic sensitivity to epinephrine (increments in glucose appearance rates [endogenous glucose production] and plasma glucose concentrations produced by the hormone) is increased in type 1 diabetes (20). In a study of patients with type 1 diabetes and nondiabetic individuals, Aftab Guy et al. (28) measured responses to epinephrine infusion in a dose that raised plasma epinephrine concentrations 25-fold during 2-h hyperinsulinemic-euglycemic clamps. During the final 30 min of the clamps, the patients with type 1 diabetes exhibited smaller increments in glucose appearance rates from the insulin-suppressed rates in the absence of epinephrine infusion and smaller decrements in glucose disappearance rates from the (partially) insulin-stimulated rates in the absence of epinephrine infusion. However, the lower glucose appearance rates were associated with lower plasma glucagon concentrations in the patients, and the glucose disappearance finding was the result of lower insulin-stimulated glucose disappearance rates (i.e., insulin resistance) in the absence of epinephrine infusion in the patients. The conclusion that patients with type 1 diabetes exhibit decreased glycemic responsiveness to epinephrine under markedly hyperinsulinemic conditions conflicts with evidence that glycemic sensitivity to epinephrine, from a plasma epinephrine concentration dose–glucose appearance and plasma glucose concentration response study in patients with type 1 diabetes and control subjects with and without fixed basal insulin and glucagon concentrations (20), is increased, not decreased, in type 1 diabetes. Berk et al. (20) confirmed that glycemic sensitivity to epinephrine is increased in patients with type 1 diabetes but found it to be comparable in patients with type 1 diabetes and nondiabetic individuals when the latter, like the patients, could not increase insulin secretion and plasma glucagon concentrations were comparable. Thus, the increased glycemic sensitivity to epinephrine in type 1 diabetes is largely, perhaps exclusively, the result of the inability to increase insulin secretion.

The clinical impact of HAAF is well established in type 1 diabetes (1,18,29–31). Recent antecedent hypoglycemia, even asymptomatic nocturnal hypoglycemia (29,30), reduces sympathoadrenal epinephrine and neurogenic symptom responses (18,30,31) and cognitive dysfunction responses (30,31) to subsequent hypoglycemia. It also impairs glycemic defense against hyperinsulinemia (18) and reduces detection of hypoglycemia in the clinical setting (31). Perhaps the most compelling support for the concept of HAAF is the finding, in three independent laboratories, that as little as 2–3 weeks of scrupulous avoidance of iatrogenic hypoglycemia reverses hypoglycemia unawareness and improves the reduced epinephrine component of defective glucose counterregulation in most affected patients (32–35).

The clinical impact of HAAF is less well established in type 2 diabetes (1,19). However, in people with advanced (i.e., insulin-deficient) type 2 diabetes, the glucagon re-

TABLE 1

Mechanisms of the fundamental components of hypoglycemia-associated autonomic failure in diabetes

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| Loss of the glucagon response: the inraislet insulin hypothesis |
| Reduced awareness: the sympathetic neural hypothesis |
| Reduced sympathoadrenal responses |
| The central nervous system premise |
| The systemic mediator hypothesis |
| The brain fuel transport hypothesis |
| The brain metabolism hypothesis |
| The brain glycogen supercompensation hypothesis |

sponse to hypoglycemia is lost, as it is in type 1 diabetes, and the glycemic thresholds for epinephrine and neurogenic symptom responses are shifted to lower plasma glucose concentrations by recent antecedent hypoglycemia in type 2 diabetes, as they are in type 1 diabetes (19). Thus, people with advanced type 2 diabetes are also at risk for both components of HAAF.

In contrast to the clinical impact of HAAF, the mechanisms of HAAF and its fundamental components (Table 1) are largely unknown (1). Recent insights into the mechanism of the loss of the glucagon secretory response to falling plasma glucose concentrations in insulin-deficient diabetes, a key feature of the syndrome of defective glucose counterregulation, and that of the loss of the neurogenic symptoms of hypoglycemia, a key feature of the syndrome of hypoglycemia unawareness, are first summarized in the below text. Then, studies of potential mechanisms of the shift of glycemic thresholds for sympathoadrenal responses to lower plasma glucose concentrations caused by recent antecedent hypoglycemia, the key feature of both components of HAAF, are discussed.

THE MECHANISM OF THE LOSS OF THE GLUCAGON RESPONSE TO HYPOGLYCEMIA IN INSULIN-DEFICIENT DIABETES: THE INTRAISET INSULIN HYPOTHESIS

The fact that patients with type 1 diabetes do not secrete glucagon in response to hypoglycemia was first reported by Gerich et al. (36) in 1973. Loss of the glucagon secretory response to falling plasma glucose concentrations in type 1 diabetes (36,37) and advanced type 2 diabetes (19) is a key feature of defective glucose counterregulation and, thus, HAAF (1). This is a selective defect. Glucagon responses to other stimuli are largely, if not entirely, intact (38–40). Therefore, it must be a signaling, rather than a structural, α -cell abnormality. Loss of the glucagon response is tightly correlated with the loss of endogenous insulin secretion (19,41) but not with the presence of classical diabetic autonomic neuropathy (18). Although often associated with functional autonomic failure (i.e., HAAF [1]), the glucagon response is absent in some patients with a normal epinephrine response (37). Clearly, the mechanisms of these two features of defective glucose counterregulation are different.

While acknowledging that loss of the glucagon response to hypoglycemia in insulin-deficient diabetes cannot be attributed to classical diabetic autonomic neuropathy, Mei and colleagues (42,43) have suggested that it might be the result of an early sympathetic neuropathy that is limited to the pancreatic islets and unique to autoimmune diabetes. That suggestion is based on their finding of a reduced marker of sympathetic nerve terminals (vesicular monoamine transporter 2) in the islets of BB, but not streptozotocin-induced, diabetic rats (42) and reduced glucagon

responses to sympathetic nerve stimulation in BB diabetic rats (43). Clearly, that mechanism remains to be established in humans with type 1 diabetes. It would not explain loss of the glucagon response in advanced type 2 diabetes (19). Furthermore, the denervated human (44) and dog (45) pancreas releases glucagon in response to hypoglycemia. Therefore, we have focused on a nonneural, peripheral mechanism.

First formulated by Samols et al. (46) and supported by data from studies of the perfused rat pancreas (47–49), rat islets (50), and rats in vivo (51), the inraislelet insulin hypothesis posits that a decrease in β -cell insulin secretion, and thus a decrease in inraislelet insulin and a decrease in tonic inraislelet α -cell inhibition by insulin, is normally a signal for increased glucagon secretion in response to hypoglycemia. The inraislelet insulin hypothesis has now been documented in humans (10–13). The finding that inraislelet hyperinsulinemia, produced by infusion of the β -cell secretagogue tolbutamide, prevents the glucagon response to hypoglycemia in nondiabetic humans (10) is consistent with the hypothesis, but it is conceivable that the prevention of the glucagon response was the result of inraislelet hyperinsulinemia per se rather than the absence of a decrease in inraislelet insulin. However, the finding that a reduced decrement in inraislelet insulin during induction of hypoglycemia, produced by suppression of baseline insulin secretion with the ATP-sensitive K^+ channel (K_{ATP} channel) agonist diazoxide, reduces the glucagon response to hypoglycemia (11) provided direct support for the inraislelet insulin hypothesis. Notably, basal glucagon concentrations, the brisk glucagon response to intravenous arginine, and the autonomic (adrenomedullary, sympathetic neural, and parasympathetic neural) responses to hypoglycemia were not altered by diazoxide. Additional evidence includes the findings that baseline suppression of insulin (and glucagon) secretion with somatostatin, which eliminated a decrement in inraislelet insulin during the induction of hypoglycemia, reduces the glucagon response to hypoglycemia (12) and that baseline stimulation of insulin secretion with tolbutamide, which increased the decrement in inraislelet insulin during the induction of hypoglycemia after tolbutamide was discontinued, increases the glucagon response to hypoglycemia (13). Thus, a decrease in inraislelet insulin, in concert with a decrease in α -cell glucose, is a signal for the glucagon secretory response to hypoglycemia in healthy humans. The absence of that inraislelet insulin signal plausibly explains loss of the glucagon response to falling plasma glucose concentrations in endogenous insulin-deficient (type 1 [36,37] and advanced type 2 [19]) diabetes.

THE MECHANISM OF HYPOGLYCEMIA UNAWARENESS IN DIABETES: THE SYMPATHETIC NEURAL HYPOTHESIS

The fact that some patients with insulin-treated diabetes tolerate low plasma glucose concentrations with few symptoms was recognized with the advent of insulin therapy of diabetes in 1922 (52), and the clinical syndrome of hypoglycemia unawareness in diabetes (loss of the warning symptoms of developing hypoglycemia that previously allowed the patient to recognize and abort the episode by eating) was well described by Lawrence (53) in the mid-20th century. Hypoglycemia unawareness, or impaired awareness of hypoglycemia (a more precise description since there is a spectrum ranging from complete

awareness to complete unawareness), was generally thought to be a permanent long-term complication of diabetes until it was found to be reversible, by scrupulous avoidance of iatrogenic hypoglycemia, in many affected patients 70 years after the advent of insulin therapy (32–35).

Symptoms of hypoglycemia are classified as neuroglycopenic, brain dysfunction attributable to central nervous system (CNS) glucose deprivation per se, or neurogenic (54,55). Neurogenic (or autonomic) symptoms are the result of the perception of physiological changes caused by the CNS-mediated sympathoadrenal discharge triggered by hypoglycemia (55). They include both adrenergic symptoms (e.g., palpitations, tremor, arousal/anxiety) mediated by catecholamines (norepinephrine, epinephrine) released from sympathetic postganglionic neurons, the adrenal medullae, or both and cholinergic symptoms (e.g., sweating, hunger, paresthesias) mediated by acetylcholine released from sympathetic postganglionic neurons (55). Awareness of hypoglycemia is largely the result of the perception of neurogenic, rather than neuroglycopenic, symptoms in nondiabetic individuals; it is reduced by at least 70% by administration of antagonists of the classical endogenous autonomic agonists (norepinephrine, epinephrine, and acetylcholine) (55). (The failure to eliminate awareness completely may have been the result of incomplete blockade by the competitive antagonists, the additional perception of neuroglycopenic symptoms, or both.) From this, it follows that hypoglycemia unawareness in diabetes must be largely the result of reduced autonomic, specifically sympathoadrenal, activation by a given level of hypoglycemia.

Bilaterally adrenalectomized individuals, who have no adrenal medullae and exhibit virtually no plasma epinephrine response to hypoglycemia, have typical adrenergic and cholinergic symptoms during hypoglycemia (16). Therefore, the neurogenic symptoms of hypoglycemia are largely the result of sympathetic neural, rather than adrenomedullary, activation, and the loss of neurogenic symptoms in patients with diabetes and hypoglycemia unawareness must be largely the result of reduced sympathetic neural activation. Interestingly, the data from bilaterally adrenalectomized individuals also suggest that, while circulating norepinephrine is largely derived from sympathetic neurons in the basal euglycemic state and under some stimulated conditions, the plasma norepinephrine response to hypoglycemia is largely derived from the adrenal medullae (16). In addition, several hemodynamic responses (e.g., increments in heart rate and forearm blood flow and decrements in diastolic blood pressure) appear to be largely mediated by increased adrenomedullary epinephrine secretion (16).

THE MECHANISM OF THE SHIFT OF THE GLYCEMIC THRESHOLDS FOR SYMPATHOADRENAL ACTIVATION TO LOWER PLASMA GLUCOSE CONCENTRATIONS BY RECENT ANTECEDENT HYPOGLYCEMIA

Hypoglycemia reduces neuroendocrine, symptomatic, and cognitive dysfunction responses to a given level of subsequent hypoglycemia in nondiabetic individuals (29,56–58), people with type 1 diabetes (18,30,31), and those with type 2 diabetes (19). It shifts the glycemic thresholds for these responses, including the sympathoadrenal responses, to lower plasma glucose concentrations. As discussed earlier, the reduced sympathoadrenal response to a given level of hypoglycemia caused by recent antecedent iatrogenic hypoglycemia plays a key role in the pathogenesis of

both defective glucose counterregulation (the result of reduced epinephrine responses in the setting of absent insulin and glucagon responses) and hypoglycemia unawareness (the result of reduced neurogenic symptom responses) and, thus, HAAF in diabetes (1). The mechanism, or mechanisms, of this hypoglycemia-induced shift of the glycemic thresholds is not known. Several current mechanistic hypotheses are discussed below.

The CNS premise. The sympathoadrenal response to hypoglycemia, sensed in the periphery (e.g., the portal vein [59,60] and the carotid bodies [61]), as well as in both the forebrain and hindbrain (62), is mediated through, and controlled by, the CNS (1). It is generally thought that the shift of the glycemic thresholds for sympathoadrenal responses to lower plasma glucose concentrations caused by recent antecedent hypoglycemia is the result of an as-yet-to-be-identified alteration within the CNS. Indeed, the data of Sivitz et al. (63) can be interpreted to show a reduced increment in adrenal sympathetic nerve activity during hypoglycemia following recent hypoglycemia in conscious rats. Nonetheless, the reduced sympathoadrenal response might be, at least in part, the result of an alteration at the level of the peripheral afferents or the efferent components of the sympathoadrenal system (64,65). Evidence for the latter possibility includes that from measurements of plasma concentrations of metanephrine in humans with type 1 diabetes (64) and of the adrenal mRNAs for catecholamine biosynthetic enzymes in rats with streptozotocin-induced diabetes (65).

More than 90% of circulating metanephrine, the 0-methylated metabolite of epinephrine, is derived from the adrenal medullae (66). Thus, plasma metanephrine concentrations can be conceptualized as a reflection of adrenomedullary stores of epinephrine and to provide a measure of the adrenomedullary capacity to secrete epinephrine (66). de Galan et al. (64) found ~25% lower basal plasma metanephrine concentrations (as well as reduced plasma metanephrine responses to hypoglycemia) in patients with type 1 diabetes who were known to have reduced epinephrine responses to hypoglycemia (and a mean HbA_{1c} level of 7.3%). The authors interpreted these findings as, admittedly indirect, evidence of a reduced adrenomedullary capacity to secrete epinephrine in such patients, an additive component to the resetting of the glycemic thresholds. On the other hand, the data do not provide insight into the capacity to release norepinephrine from sympathetic postganglionic neurons. Resting plasma concentrations of normetanephrine, the 0-methylated metabolite of norepinephrine, were not reduced in the patients with type 1 diabetes (64), but ~30% of circulating normetanephrine is derived from the adrenal medullae and much of the remainder is the result of extraneuronal metabolism of norepinephrine (66).

Inouye et al. (65) found ~25% decreased adrenal tyrosine hydroxylase (the rate-limiting enzyme in catecholamine biosynthesis) mRNA in streptozotocin-induced diabetic rats, but the level of that message was unaffected by recent antecedent hypoglycemia. In contrast, adrenal phenylethanolamine *N*-methyltransferase (the enzyme that converts norepinephrine to epinephrine) mRNA was unaffected by diabetes but reduced by ~40% by recent antecedent hypoglycemia. Assuming that the catecholamine biosynthetic enzyme protein and enzymatic activities paralleled the mRNA levels and that the reduced biosynthetic enzyme activities were sufficient to reduce adrenomedullary catecholamine contents, these data are consistent

with the hypotheses that 1) reduced tyrosine hydroxylase activity contributes to reduced catecholamine responses to hypoglycemia in diabetes and 2) reduced phenylethanolamine *N*-methyltransferase activity contributes to further reduced epinephrine responses to hypoglycemia following recent hypoglycemia. Alternatively, as the authors acknowledge, the observed reduced mRNA levels could have been the result of, rather than the cause of, the blunted catecholamine responses.

Finally, with respect to the possibility of an extra-CNS alteration, Mevorach et al. (67) have suggested that an additional nonglucagon, nonepinephrine mechanism, perhaps impairment of glucose autoregulation, may contribute to compromised glucose counterregulation in type 1 diabetes. That was based on their finding that a low-dose insulin-induced reduction of the plasma glucose concentration to 70 mg/dl (3.9 mmol/l), with glucagon fixed at baseline levels and with minimal elevation of epinephrine levels, was associated with an ~20% lower, partially insulin-suppressed, rate of endogenous glucose production in patients with type 1 diabetes compared with nondiabetic individuals. However, as the authors pointed out, plasma epinephrine concentrations tended to be lower in the patients.

Based on the CNS premise, the status of four generic hypotheses (Table 1) concerning CNS alterations that could result in reduced sympathoadrenal responses to a given level of hypoglycemia and, thus, explain the pathogenesis of both components of HAAF, in the setting of absent decrements in insulin and absent increments in glucagon, in insulin-deficient diabetes is discussed below.

The systemic mediator hypothesis. The systemic mediator hypothesis posits that increased cortisol levels (or those of another systemic factor), stimulated by recent antecedent hypoglycemia, act on the brain to reduce sympathoadrenal responses to a given level of subsequent hypoglycemia and thus mediate HAAF in insulin-deficient diabetes. It was formulated by Davis et al. (68) and supported by their finding that cortisol infusions (during euglycemia), like bouts of hypoglycemia, reduced adrenomedullary epinephrine and muscle sympathetic nerve activity responses to hypoglycemia the following day in healthy subjects. (Notably, effects on symptomatic responses to hypoglycemia were not reported.) It was further supported by their finding that the effect of antecedent hypoglycemia was not apparent in patients with primary adrenocortical failure, who could not release cortisol during antecedent hypoglycemia (69). The generic phenomenon was confirmed when we found that supra-physiological cortisol levels (to ~45 µg/dl), produced by infusions of a pharmacological dose of α_{1-24} adrenocorticotrophic hormone, led to reduced adrenomedullary and neurogenic symptom responses to hypoglycemia the following day (70). However, the relevance of the phenomenon to the pathogenesis of HAAF has been questioned. Using an experimental design generally similar to that initially used by Davis et al. (68) but with lower cortisol infusion doses, we found that cortisol elevations comparable to, indeed somewhat above, those that occur during hypoglycemia (~26 µg/dl) did not reduce the adrenomedullary or neurogenic symptom responses to hypoglycemia the following day in healthy individuals (71). Furthermore, an adrenocortical glucocorticoid response does not appear to be critical to the effect of antecedent hypoglycemia. Despite the absence of a corticosterone response to hypoglycemia, recent antecedent hypoglycemia reduced

epinephrine and glucagon responses to subsequent hypoglycemia in corticotropin-releasing hormone knockout mice (72). Thus, the cortisol response to antecedent hypoglycemia does not appear to be the mediator of HAAF.

Despite the apparent confirmation, albeit pharmacological, of the phenomenon in humans (70), effects of central glucocorticoid actions to reduce sympathoadrenal responses to subsequent hypoglycemia in rodents remain controversial. Corticosterone administered subcutaneously (73), intravenously (74), or into the third cerebral ventricle (75) has been reported not to reduce the sympathoadrenal response to hypoglycemia, whereas cortisol administered into a lateral cerebral ventricle (76) and dexamethasone administered peripherally or into the fourth cerebral ventricle (but not into the third cerebral ventricle) (77) has been reported to reduce that response.

There is also evidence that recent antecedent epinephrine elevations do not cause HAAF (78). Epinephrine infusions did not reduce the plasma epinephrine (or glucagon) and neurogenic symptom responses to subsequent hypoglycemia in nondiabetic subjects.

The brain fuel transport hypothesis. The brain fuel transport hypothesis posits that recent antecedent hypoglycemia increases blood-to-brain glucose transport (or that of alternative fuels) by upregulating blood-brain barrier transporters and that increased fuel transport into the brain reduces responses, including sympathoadrenal responses, to subsequent hypoglycemia and is, therefore, the mechanism of HAAF. It is based on a substantial body of evidence in rodents indicating that ≥ 3 days (not hours) of hypoglycemia increases brain microvascular GLUT-1 mRNA and protein and brain glucose uptake (79–83). However, the relevance of that evidence to HAAF is questionable since the HAAF phenomenon can be produced by hours of antecedent hypoglycemia in humans (56–58).

Boyle et al. (84) found that brain glucose uptake (calculated from the product of arteriovenous glucose differences across the brain and cerebral blood flows; the Kety-Schmidt technique) is preserved during hypoglycemia after ~ 56 h of interprandial hypoglycemia in healthy individuals and in patients with well-controlled (i.e., frequently hypoglycemic) type 1 diabetes (85). Again, however, the relevance of the former finding to HAAF is questionable given that it was demonstrable only after > 2 days of hypoglycemia. On the other hand, the brain glucose transport hypothesis has been directly tested in a model of HAAF (86) and in patients with type 1 diabetes with and without hypoglycemia unawareness (87). With respect to the model, using [^{11}C]glucose and positron emission tomography (PET), we found that global blood-to-brain glucose transport was not increased after ~ 24 h of interprandial hypoglycemia (which reduced the sympathoadrenal and neurogenic symptom responses to hypoglycemia substantially) in healthy subjects (86). In patients with type 1 diabetes, Bingham et al. (87) found no difference in global blood-to-brain glucose transport, measured with [^{11}C]3-O-methylglucose and PET, between patients with and without hypoglycemia unawareness. These data do not exclude increased regional blood-to-brain glucose transport but, as discussed above, the hypothesis was based on evidence of increased global glucose transport in rodents (79–83) and humans (84,85). Furthermore, in rodents, short-term recent antecedent hypoglycemia, which reduces sympathoadrenal responses to subsequent

hypoglycemia just as it does in humans, does not increase regional brain extracellular glucose concentrations, as measured by microdialysis in the ventromedial hypothalamus (VMH) (88), the brain stem (89), or the hippocampus (90), during subsequent hypoglycemia. Indeed, in humans, recent antecedent hypoglycemia did not increase brain glucose concentrations, as measured with nuclear magnetic resonance spectroscopy, significantly during subsequent hypoglycemia (91).

Thus, the accumulating evidence does not support the notion that increased blood-to-brain glucose transport is the mechanism of HAAF. Nonetheless, the finding of slightly higher brain glucose concentrations (a mean of 5.5 $\mu\text{mol/g}$ compared with 4.7 $\mu\text{mol/g}$ in nondiabetic individuals), measured with nuclear magnetic resonance during substantial hyperglycemia, in patients with tightly controlled type 1 diabetes and probable hypoglycemia unawareness (92) could be interpreted to be consistent with the hypothesis. However, using the same method and the same hyperglycemic conditions, in a separate study, these investigators reported a mean brain glucose concentration of 5.3 $\mu\text{mol/g}$ in nondiabetic individuals (not significantly different from that of 4.7 $\mu\text{mol/g}$ in patients with poorly controlled type 1 diabetes) (93). Furthermore, using [^{11}C]3-O-methylglucose and PET, Bingham et al. (87) found no difference in whole-brain glucose content in patients with and without hypoglycemia unawareness.

Increased transport of a fuel other than glucose into the brain could theoretically explain HAAF. In that regard, Mason et al. (94) have presented evidence of increased blood-to-brain acetate transport in patients with type 1 diabetes, raising the possibility that increased blood-to-brain monocarboxylate (e.g., lactate) transport might be a mechanism of HAAF. Clearly, the latter remains to be directly demonstrated and to be shown to be specific for patients with hypoglycemia unawareness.

The brain metabolism hypothesis. The brain metabolism hypothesis posits that recent antecedent hypoglycemia in some way alters brain metabolism, which ultimately results in reduced CNS-mediated responses, including sympathoadrenal responses, to subsequent hypoglycemia. Much of the research into the pathogenesis of HAAF in diabetes is focused on the VMH (95). VMH glucopenia (2-deoxyglucose administration) activates the sympathoadrenal system and increases glucagon secretion (96) and VMH glucose perfusion suppresses these responses during systemic hypoglycemia (97). Nonetheless, activation of glucose counterregulatory systems involves widespread regions of the brain in experimental animals (98,99) and humans (100,101).

We used [^{15}O]water and PET to measure regional cerebral blood flow, a marker of synaptic activation, during hypoglycemia in healthy humans (100). One novel finding was a small (6–8%) generalized (i.e., cerebrum, brain stem, and cerebellum) decrease in cerebral blood flow, with a more marked decrease (25%) in the hippocampus, which may reflect reduced brain metabolism due to glucose deprivation and the vulnerability of the hippocampus. On the other hand, typical sympathoadrenal and symptomatic responses to hypoglycemia were associated with synaptic activation in widespread but discrete brain regions, including the medial prefrontal cortex, another novel finding, as well as the thalamus and the periaqueductal gray. (These findings have seemingly been confirmed [101]). The medial prefrontal cortex and its connections provide the major cortical output to auto-

onomic structures in the hypothalamus and periaqueductal gray (100,102). These findings raise the possibility of cortical modulation of the sympathoadrenal response to a simple low-level physiological process: defense against hypoglycemia. At the very least, they indicate that widespread brain regions must be considered in studies of the CNS mechanisms of HAAF in diabetes. These findings suggest that metabolic alterations, caused by recent antecedent hypoglycemia and resulting in altered synaptic activities during subsequent hypoglycemia, occur in widespread brain regions, including the cortex, and may be involved in the pathogenesis of HAAF in diabetes. Interestingly, Tkacs et al. (103) found evidence of cellular (presumably neuronal) death in rostral brain regions, including the medial prefrontal cortex (but not the hippocampus), which was associated with a reduced epinephrine response to hypoglycemia, in rats subjected to plasma glucose levels of 30–35 mg/dl for 75 min 48 h earlier. Notably, evidence of brain cellular death was not found following glucose levels of ~45 mg/dl.

There is an array of possible alterations of brain metabolism that might be the mechanism of HAAF, but none have been unequivocally established. One possibility is that recent antecedent hypoglycemia results in increased glucokinase activity in critical, perhaps VMH, glucose-sensing neurons that initiate the sympathoadrenal response (95,104–106). The resulting increased neuronal glucose metabolism would be expected to result in a reduced sympathoadrenal response to a given level of subsequent hypoglycemia. Glucokinase (and GLUT-2) expression has been demonstrated in human brain, including in the VMH (arcuate nucleus and ventromedial hypothalamic nucleus) (107). Interestingly, infusion of fructose, which might modulate glucokinase activity in relevant central neurons, has been reported to nearly normalize the plasma epinephrine response (but not the glucagon response) to hypoglycemia in patients with type 1 diabetes (108). Many other alterations in brain signaling or metabolism could contribute to the pathogenesis of HAAF. Those include increased corticotropin-releasing hormone or urocortin release (73,109), decreased hypothalamic paraventricular nucleus activity (75,110,111), K_{ATP} channel closure (112,113), decreased hypothalamic AMP-activated protein kinase activity (114–116), increased GABAergic tone (117,118), increased hypothalamic expression of the angiotensinogen and related genes (119), decreased cerebral glucose metabolism (87), increased cerebral blood flow (120), and decreased brain insulin signaling (121,122). Interestingly, Bingham et al. (87), using [^{11}C]3-O-methylglucose and PET, found a relative decrease in cerebral glucose metabolism during hypoglycemia in a small sample of patients with type 1 diabetes and hypoglycemia unawareness. Cerebral glucose metabolism tended to increase in aware patients but decrease in unaware patients, resulting in a significantly lower mean rate of glucose metabolism in the latter. However, using [^{11}C] glucose and PET, we found no difference in cerebral glucose metabolism during hypoglycemia unawareness induced by recent antecedent hypoglycemia in nondiabetic individuals (86).

The brain glycogen supercompensation hypothesis. The brain glycogen supercompensation hypothesis posits that after an episode of hypoglycemia, brain (astrocyte) glycogen levels rebound to levels that exceed the prehypoglycemic concentrations and provide an expanded source of glycolytic and ultimately neuronal oxidative fuel

(e.g., lactate), explaining the reduced sympathoadrenal response to a given level of subsequent hypoglycemia (123). It is based on findings in rats showing that brain glycogen concentrations are somewhat higher than those reported earlier (124) and that brain glycogen concentrations, measured with nuclear magnetic resonance spectroscopy, decrease during hypoglycemia but then increase to ~150% of basal levels >7 h following restoration of euglycemia (125). In addition, glycogen turnover has been reported to be relatively slow in the human brain (125,126). Clearly, the phenomenon of supercompensation is critical to this hypothesis since, even with the updated estimates, brain glycogen content is orders of magnitude lower than the glycogen content of liver and muscle, brain glycogen falls with a half-time of a few minutes during global ischemia, and brain glycogen turnover is a few percent of glucose consumption as measured in rats (127).

DIVERSE CAUSES OF HAAF IN DIABETES

Initially, we attributed HAAF in diabetes exclusively to recent antecedent iatrogenic hypoglycemia (18,19). However, it may well be that antecedent events in addition to hypoglycemia cause the HAAF phenomenon (1) (Fig. 1).

Antecedent exercise has been found to reduce the sympathoadrenal responses to subsequent hypoglycemia (128,129). Galassetti et al. (128) found that exercise reduced epinephrine and muscle sympathetic nerve activity, but not neurogenic symptom, responses to subsequent hypoglycemia. We also found that antecedent exercise reduced the epinephrine response to subsequent hypoglycemia (129). Again, however, the neurogenic symptom responses to hypoglycemia were not reduced. Thus, since antecedent exercise does not reduce symptoms, i.e., produce hypoglycemia unawareness, exercise-related HAAF appears to be a partial HAAF syndrome.

Sleep-related HAAF is a more compelling example. People with type 1 diabetes have substantially reduced sympathoadrenal responses to a given level of hypoglycemia, and their sympathoadrenal responses are reduced further during sleep (130,131). Probably because of their markedly reduced sympathoadrenal responses (132), people with type 1 diabetes are much less likely to be awakened from sleep by hypoglycemia than nondiabetic individuals (131). They slept ~75–80% of the time during hypoglycemia, whereas nondiabetic control subjects slept only ~25% of the time (131). Thus, sleeping patients with type 1 diabetes have both defective glucose counterregulation (a further reduced epinephrine response in the setting of absent insulin and glucagon responses) and a form of hypoglycemia unawareness (reduced arousal from sleep), the two components of HAAF in diabetes (1).

Thus, there are diverse causes of HAAF in diabetes: the originally recognized hypoglycemia-related HAAF, exercise-related HAAF, and sleep-related HAAF (1). Clearly, a unifying mechanism of HAAF would need to incorporate the effects of antecedent hypoglycemia, antecedent exercise, and sleep.

COMMENT

It is possible to both reduce the risk of hypoglycemia and improve glycaemic control in many patients with diabetes (1,2,133,134). Nonetheless, iatrogenic hypoglycemia continues to be a problem for people with diabetes that has not been solved. Pending the prevention and cure of diabetes, critical fundamental, translational, and outcomes

research into the problem is needed if we are to eliminate hypoglycemia from the lives of people affected by diabetes.

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