



ing from a clinical standpoint. It is reminiscent of the oft-noted clinical findings in hypertrophic cardiomyopathy wherein the degree of diastolic impairment is often described as “out of proportion” to the observed ventricular hypertrophy, thus suggesting a significant myocellular contribution to disease pathogenesis. It is interesting to speculate that the well-known salutary effects of postinfarction treatment with metoprolol may reflect the molecular findings in the current paper. Thus the results presented by Perrino et al. lay the groundwork for a robust 2-pronged therapeutic approach based on relief of the inciting pathogenic stimulus and direct treatment of the underlying molecular derangements, perhaps via novel compounds to displace active PI3K isoforms from the receptor complex.

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Mechanisms of sympathoadrenal failure and hypoglycemia in diabetes

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A reduced sympathoadrenal response, induced by recent antecedent hypoglycemia, is the key feature of hypoglycemia-associated autonomic failure (HAAF) and, thus, the pathogenesis of iatrogenic hypoglycemia in diabetes. Understanding of the mechanism(s) of that reduced response awaits new insight into its basic molecular, cellular, organ, and whole-body physiology and pathophysiology in experimental models. In this issue of the JCI, McCrimmon and colleagues report that application of urocortin I (a corticotrophin-releasing factor receptor–2 agonist) to the ventromedial hypothalamus reduces the glucose counterregulatory response to hypoglycemia in rats (see the related article beginning on page 1723). Thus, hypothalamic urocortin I release during antecedent hypoglycemia is, among other possibilities, a potential mechanism of HAAF.

Nonstandard abbreviations used: HAAF, hypoglycemia-associated autonomic failure; T1DM, type 1 diabetes mellitus; VMH, ventromedial hypothalamus.

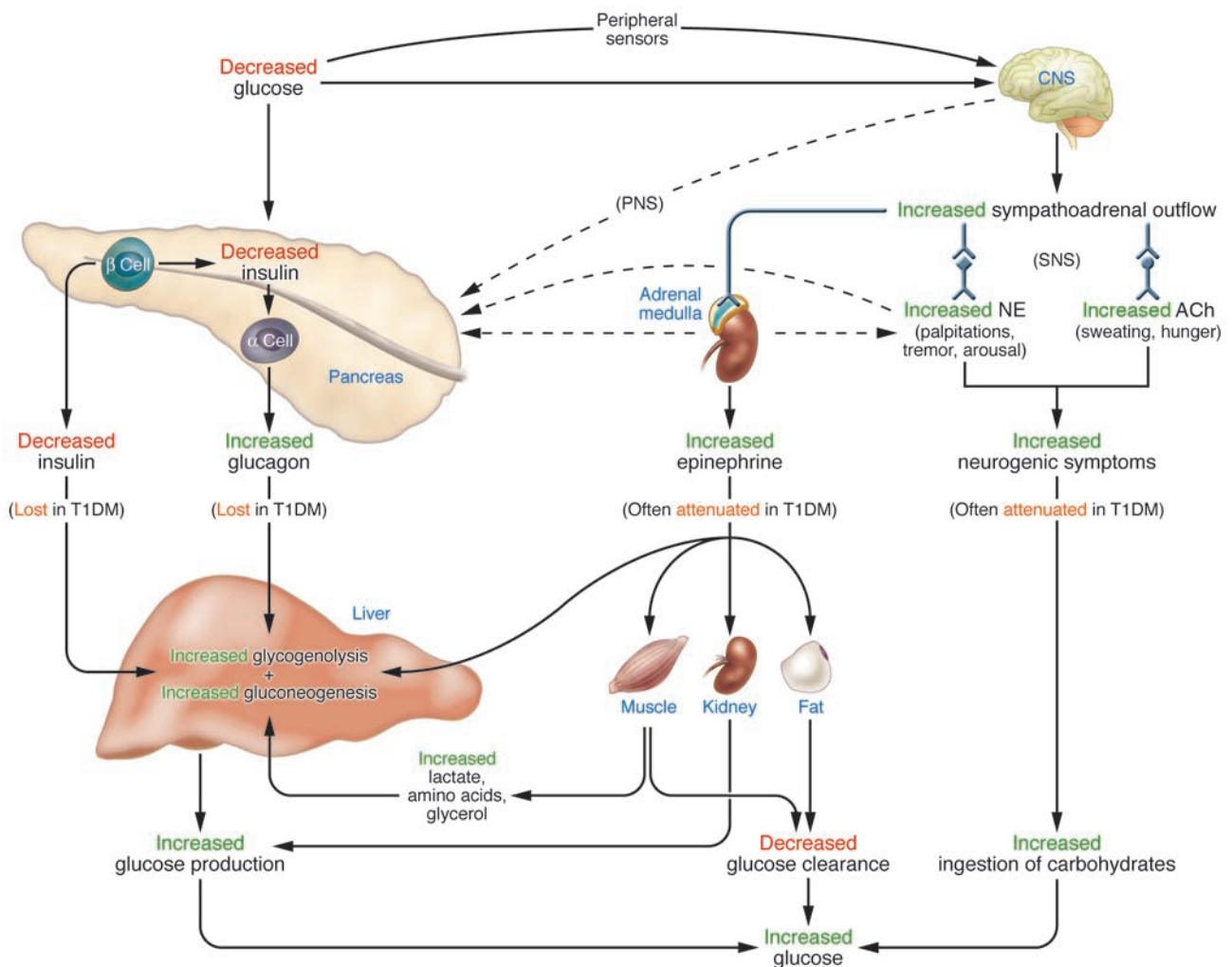
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The clinical problem of iatrogenic hypoglycemia in people with diabetes mellitus (1) has stimulated bidirectional translational research. That included, initially, patient-oriented research based on the then-existing body of knowledge and, more recently, basic research into the molecular, cellular,

organ, and whole-body physiology of glucose counterregulation — the mechanisms that normally prevent or rapidly correct hypoglycemia — and its pathophysiology in experimental models (2). The latter is exemplified by the report from McCrimmon and colleagues (3) in this issue of the *JCI*. In the aggregate, such basic research will undoubtedly lead to more informative studies in humans and, hopefully, the ultimate elimination of hypoglycemia from the lives of people with diabetes.

The clinical problem is clear to millions of people with diabetes and, increasingly, to their caregivers: iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes (1, 2). It causes recurrent morbidity in most people with type 1 diabetes mellitus (T1DM) and many with T2DM and is sometimes fatal. The barrier

**Figure 1**

Physiological and behavioral defenses against hypoglycemia. Decrements in insulin and increments in glucagon are lost and increments in epinephrine and neurogenic symptoms are often attenuated in insulin-deficient — T1DM and advanced T2DM — diabetes. SNS, sympathetic nervous system; PNS, parasympathetic nervous system; NE, norepinephrine; ACh, acetylcholine; α cell, pancreatic islet α cells; β cell, pancreatic islet β cells.

of hypoglycemia precludes maintenance of euglycemia over a lifetime of diabetes and, thus, full realization of the now well-established vascular benefits of glycemetic control (4, 5). Even asymptomatic episodes of hypoglycemia impair defenses against subsequent hypoglycemia by causing hypoglycemia-associated autonomic, specifically sympathoadrenal, failure and thus a vicious cycle of recurrent hypoglycemia.

Compromised defenses against hypoglycemia

The physiological defenses against falling plasma glucose concentrations (Figure 1) include: (a) decreased pancreatic islet β cell insulin secretion; (b) increased pancreatic islet α cell glucagon secretion; and, absent the latter, (c) increased adrenomedullary epi-

nephrine secretion (6). The behavioral form of defense (Figure 1) is the ingestion of food prompted by symptoms of hypoglycemia (6). All of these defenses are compromised (1, 2) in T1DM (7) and advanced T2DM (8). This involves both peripheral (pancreatic islet) and CNS alterations. Both decrements in insulin and increments in glucagon are lost. The former is the result of β cell failure. The latter is plausibly attributed to loss of the decrement in intra-islet insulin that normally signals increased glucagon secretion (9–11). In the setting of absent insulin and glucagon responses, attenuated epinephrine responses cause the clinical syndrome of defective glucose counterregulation that is associated with a 25-fold or greater increased risk of severe hypoglycemia (1, 2). Attenuated sympathoadrenal, largely sym-

pathetic neural (12), responses also cause the clinical syndrome of hypoglycemia unawareness that, by compromising the behavioral defense, is also associated with an increased risk of severe hypoglycemia (1, 2). Thus, hypoglycemia is the result of the interplay of absolute or relative (to exogenous glucose delivery, endogenous glucose production, or insulin sensitivity) therapeutic insulin excess and compromised glucose counterregulation (1, 2, 13).

Hypoglycemia-associated autonomic failure

The concept of hypoglycemia-associated autonomic failure (HAAF) in T1DM (7) and advanced T2DM (8) (Figure 2) posits that recent antecedent iatrogenic hypoglycemia causes both defective glucose counterregu-

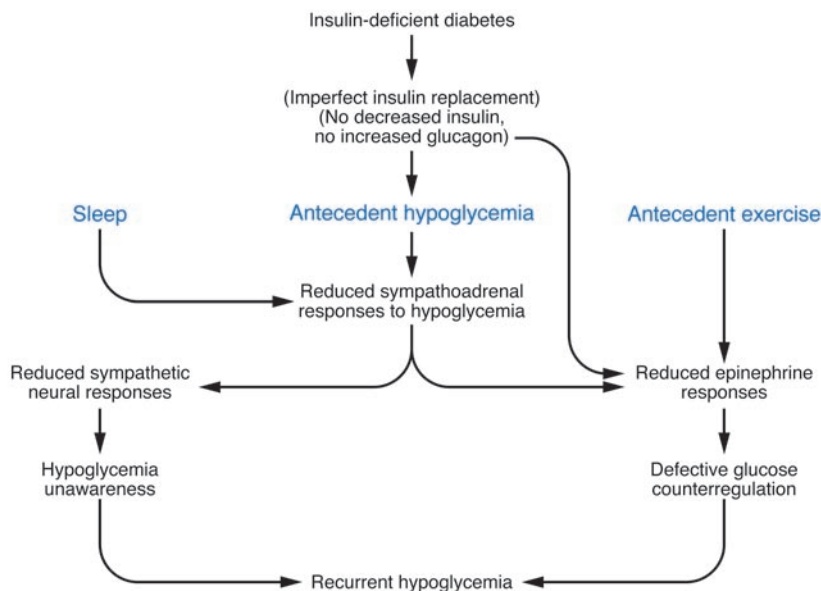


Figure 2

HAAF in T1DM and advanced T2DM. Figure modified from the *New England Journal of Medicine*, with permission from the Massachusetts Medical Society (1).

lation and hypoglycemia unawareness and, thus, a vicious cycle of recurrent hypoglycemia (1, 2). It causes defective glucose counterregulation by reducing epinephrine responses to a given level of subsequent hypoglycemia in the setting of absent decrements in insulin and increments in glucagon. It causes hypoglycemia unawareness by reducing sympathoadrenal and the resulting neurogenic symptom responses to a given level of subsequent hypoglycemia. Sleep and antecedent exercise cause a similar phenomenon (1) (Figure 2). The clinical impact of HAAF, including the finding that hypoglycemia unawareness and the reduced epinephrine component of defective glucose counterregulation are reversed by as little as 2–3 weeks of scrupulous avoidance of hypoglycemia in most affected patients, is well established (1, 2). However, the mechanism(s) of the key feature of HAAF, the attenuated sympathoadrenal response to falling plasma glucose concentrations, are unknown (2).

The shift of the glycemic thresholds for sympathoadrenal responses to lower plasma glucose concentrations caused by recent antecedent hypoglycemia (or by sleep or prior exercise) could be the result of alterations in the peripheral afferent or efferent components of the autonomic nervous system or within the CNS (2). Much of the recent research has focused on the latter, especially the hypothalamus (2, 3). However, hypoglycemia activates wide-

spread brain regions including the medial prefrontal cortex (14).

The potential CNS mechanisms of the reduced sympathoadrenal response, and thus of HAAF, have been reviewed (2). In my view (2), the balance of evidence, including more recent evidence (15), weighs heavily against the systemic mediator (e.g., cortisol) hypothesis and the brain fuel (e.g., glucose) transport hypothesis, although increased transport of other fuels remains to be explored fully (16). Thus, assuming a primary CNS alteration, the global brain metabolism hypothesis is the most attractive. The array of potential mechanisms within the brain (reviewed in ref. 2) range from post-hypoglycemic increased glucokinase activity in critical hypothalamic neurons (17) to posthypoglycemic brain glycogen supercompensation (18). In addition, alterations of ATP-sensitive K^+ channel function, AMP-activated protein kinase activity, γ -amino butyric acid release, insulin signaling, and expression of *angiotensinogen* and related genes as well as of paraventricular nucleus activity, cerebral glucose metabolism, and cerebral blood flow have been proposed (2). The findings of McCrimmon and colleagues (3) raise another possibility. They report that ventromedial hypothalamus (VMH) application of urocortin I, a corticotrophin-releasing factor receptor-2 agonist, suppresses the counterregulatory response to hypoglycemia (an effect that lasted for at least 24 hours), whereas that of cortico-

trophin-releasing factor, a predominant corticotrophin-releasing factor receptor-1 agonist, amplifies that response in rats. Urocortin I was also shown to alter the glucose sensitivity of VMH glucose-sensing neurons in whole-cell current clamp experiments in brain slices. Thus, increased hypothalamic urocortin I release during antecedent hypoglycemia could explain a decreased sympathoadrenal response to subsequent hypoglycemia. However, that causal connection was not demonstrated and, therefore, remains a provocative theoretical possibility.

Ultimately, the problem of hypoglycemia (and that of hyperglycemia) in diabetes will likely be solved by the development of safe and effective methods that provide plasma glucose-regulated insulin replacement or secretion. That will probably involve closed-loop insulin replacement (online glucose sensor–computer interface–insulin infusion pump) until implantation of glucose-responsive, insulin-secreting cells becomes feasible for widespread use. Pending that, there is clear a need for new insight into the fundamental mechanisms of the physiology of glucose counterregulation and its pathophysiology in experimental models that leads to clinical strategies that are then shown to both reduce the risk of hypoglycemia and facilitate glycemic control in people with diabetes (i.e., bidirectional translational research).

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IPEX and the role of FOXP3 in the development and function of human Tregs

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Genetic defects in the transcription factor forkhead box protein P3 (FOXP3) cause immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX). IPEX is thought to be due to a defect in naturally arising CD4⁺ Tregs. In this issue of the *JCI*, Bacchetta and colleagues demonstrate that patients with IPEX and missense mutations in FOXP3 provide insight into the role of various domains of FOXP3 in the development and function of Tregs (see the related article beginning on page 1713).

In the past decade, naturally arising CD4⁺CD25⁺ Tregs have emerged as being critical for the maintenance of peripheral immunological tolerance (1). Neonatal thymectomy in mice and thymic hypoplasia in humans (DiGeorge syndrome) result in impaired Treg generation and in the development of organ-specific autoimmune diseases (2, 3). Treg generation in the thymus requires interaction with MHC class II molecules expressed by cortical epithelial cells (4). Other signals have also been implicated, including those delivered by CD28, CD40 ligand, and the cytokines TNF- α and TGF- β (5). However, IL-2, which is necessary for Treg survival

in the periphery, is dispensable for their development (6, 7).

Tregs represent a small subset of CD4⁺ T cells (5–10%). They express several surface markers including CD25 (the IL-2 receptor α -chain), CTL-associated antigen 4 (CTLA4), and glucocorticoid-induced TNF receptor (GITR). However, these molecules are upregulated in naive CD4⁺CD25⁻ T cells following TCR stimulation and cannot therefore serve as absolute Treg markers. Recently, several groups identified the forkhead family transcription factor forkhead box protein P3 (FOXP3) as a specific molecular marker of Tregs (8, 9). FOXP3 expression seems to be largely restricted to a small subset of TCR $\alpha\beta$ T cells and defines 2 pools of Tregs with suppressive activity: CD4⁺CD25^{high} cells and a minor population of CD4⁺CD25^{low/-} T cells. Thus, FOXP3 expression correlates better with suppressor activity than CD25 expression (10). Ectopic expression of Foxp3 in vitro and in vivo is sufficient to convert naive murine CD4⁺ T cells to Tregs (11).

In contrast, overexpression of FOXP3 in human CD4⁺CD25⁻ T cells in vitro is not sufficient to generate potent suppressor activity, suggesting that additional factors are required for bona fide Treg activity (12). Foxp3 expression cannot be readily induced in murine T cells by stimulation in vitro. In humans, cross-linking of the TCR and CD28 stimulation, or antigen-specific stimulation, can induce CD4⁺CD25⁻ FOXP3⁻ cells to become Tregs and express FOXP3 and exert suppressor function (11, 13). These results suggest that de novo generation of Tregs may be a natural consequence of the immune response in humans.

Tregs are anergic in vitro. They fail to proliferate and secrete IL-2 in response to TCR ligation, and they depend on IL-2 generated following activation of CD4⁺CD25⁻ T cells to survive and exert their function. An in vitro assay that measures the ability of CD4⁺CD25⁺ T cells to suppress CD4⁺CD25⁻ T cell proliferation is commonly used to assess Treg function (14). The mechanisms mediating the suppression of immune responses in vivo by Tregs are still unclear. Several studies support the involvement of contact-dependent inhibition, while others suggest that Tregs can exert their function by secreting immunosuppressive cytokines such as IL-10 or TGF- β or by directly killing their target in a perforin-dependent manner (15).

Nonstandard abbreviations used: FKH, forkhead; FOXP3, forkhead box protein P3; GITR, glucocorticoid-induced TNF receptor; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked.

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