Hypoglycemia remains a major clinical issue in the management of people with type 1 and type 2 diabetes. Research in basic science is only beginning to unravel the mechanisms that: 1) underpin the detection of hypoglycemia and initiation of a counterregulatory defense response; and 2) contribute to the development of defective counterregulation in both type 1 and type 2 diabetes, particularly after prior exposure to repeated hypoglycemia. In animal studies, the central nervous system has emerged as key to these processes. However, bench-based research needs to be translated through studies in human subjects as a first step to the future development of clinical intervention. This Update reviews studies published in the last 2 yr that examined the central nervous system effects of hypoglycemia in human subjects, largely through neuroimaging techniques, and compares these data with those obtained from animal studies and the implications for future therapies. Based on these studies, it is increasingly clear that our understanding of how the brain responds and adapts to recurrent hypoglycemia remains very limited. Current therapies have provided little evidence that they can prevent severe hypoglycemia or improve hypoglycemia awareness in type 1 diabetes. There remains an urgent need to increase our understanding of how and why defective counterregulation develops in type 1 diabetes in order for novel therapeutic interventions to be developed and tested. (J Clin Endocrinol Metab 97: 1–8, 2012)
hypothalamus). A direct connection to downstream integrators allows the glucose signal to be incorporated with and influenced by inputs from other brain regions (e.g. circadian rhythms) before a motor output is generated via one of a number of effector mechanisms (e.g. epinephrine or glucagon release) leading to the restoration of glucose homeostasis.

The cells or neurons that sense glucose are unique in that they are able to translate a change in extracellular glucose into a change in neurotransmitter or hormone release. Key steps in the translation of the glucose signal appear to be glucokinase, AMP-activated protein kinase, and the SUR-1 subtype of the ATP-sensitive potassium channel, and there is also evidence of a direct effect of glucose on neuronal firing (1). There are clear parallels between glucose sensing by these neurons and the classical glucose sensor, the pancreatic β-cell, suggesting that they share similar mechanisms for detecting changes in extracellular glucose. However, glucose-sensing neurons release neurotransmitters or neuropeptides rather than insulin. Intriguingly, a number of studies have now shown an important role for the inhibitory neurotransmitter, γ-aminobutyric acid (GABA), which is also cosecreted with insulin from the pancreatic β-cell, in select hypothalamic glucose-sensing regions during hypoglycemia (e.g. Ref. 4). Other neurotransmitters such as norepinephrine and serotonin have also been studied and shown to influence hypoglycemia counterregulation (1).

A major “effector” of the body’s counterregulatory response to hypoglycemia is glucagon secreted by the pancreatic α-cell. The portal insulin:glucagon ratio is the major determinant of hepatic glucose output, and during hypoglycemia insulin suppression and glucagon release act to stimulate hepatic glucose production. A hallmark of type 1 (5) and advanced type 2 diabetes (6) is the inability to secrete glucagon specifically during hypoglycemia (8). This phenomenon likely explains why intensive therapy aimed at normalizing glucose control leads to individuals with both type 1 (10) and type 2 diabetes (11) developing impaired symptom awareness and counterregulatory defenses against hypoglycemia and contributes to the higher incidence of severe hypoglycemia seen in the intensive arms of the type 1 Diabetes Control and Complications Trial (12) as well as in, for example, the recent type 2 Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial (13). The fact that strict avoidance of hypoglycemia can lead to a reversal of this effect (e.g. Ref. 14) is also evidence of the key role played by antecedent hypoglycemia.

There is some debate as to whether this phenomenon represents a maladaptive or adaptive response, and much of this is beyond the scope of this review. The association between defective hormonal counterregulation, altered thresholds for counterregulatory hormone release, and impaired hypoglycemia awareness is usually termed “hypoglycemia-associated autonomic failure” and is seen as maladaptive because it increases an individual’s risk of severe hypoglycemia (e.g. Ref. 15). However, at a cellular
level, hypoglycemia triggers a series of metabolic and stress responses that may in fact be adaptive, enabling the organisms to better withstand subsequent hypoglycemia stress (8). In particular, important regulatory roles for glucocorticoid and the CRH family of neuropeptides have emerged, as well as for AMP-activated protein kinase and ATP-sensitive potassium channel, and these systems play critical roles in preconditioning the organism or cell, enabling it to better withstand future exposure to the same stressor. This is often called “stress habitation” or “tolerance” and is ultimately a protective response at a cellular level. Figure 1 illustrates how this system may lead to the development of an impaired autonomic response to hypoglycemia. This does not mean the individual is fully protected from the consequences of hypoglycemia. The problem is that the appearance of hypoglycemia in diabetes occurs when there is a marked hyperinsulinemia rather than hypoinsulinemia. Hyperinsulinemia blocks peripheral generation of alternate fuels, suppresses hepatic glucose production, and, in the presence of impaired counterregulation, is more likely to induce severe and prolonged hypoglycemia. Under these conditions, brain extracellular fluid glucose levels are extremely low, and thus there is the potential for cellular damage or even death. This is why the inability to exert feedback inhibition of insulin release and action during hypoglycemia is one of the key counterregulatory defects of type 1 diabetes.

**Neuroimaging during Hypoglycemia**

Having briefly reviewed current concepts of the mechanisms that underpin glucose sensing during hypoglycemia, we can now consider recent human studies seeking to translate this more basic research in the CNS aspects of hypoglycemia. The study of brain metabolism or function during hypoglycemia in human subjects relies primarily on a number of different neuroimaging techniques. Page et al. (16) demonstrated that mild hypoglycemia increased regional cerebral blood flow in the hypothalamus. Using pulsed arterial spin labeling with magnetic resonance imaging, which provides a measure of absolute blood flow responses, they examined the effect of lowering blood glucose to mean levels of 77 ± 2 mg · dl⁻¹ in a small group of nine nondiabetic subjects. They reported that during this mild hypoglycemic stimulus, there was a 2-fold increase in hypothalamic blood flow. They also reported significantly increased blood flow in a number of forebrain regions and significantly decreased blood flow in cerebellum and right pars opercularis. Interestingly, there was no significant increase in counterregulatory hormone release (epinephrine, norepinephrine, glucagon, and cortisol) at this glucose level, although there was a significant reduction in plasma c-peptide (0.47 ± 0.02 to 0.34 ± 0.02 pmol · liter⁻¹; \( P < 0.001 \)) (16). Whether the hypothalamus contributes directly to the suppression of c-peptide under these conditions is, however, speculative, and a direct effect of glucose in the islet is likely to predominate. This report was also consistent with an earlier study by Musen et al. (17) who used BOLD functional magnetic resonance imaging in type 1 diabetic and nondiabetic subjects. They reported activation of the hypothalamic region at 68 ± 9 mg/dl in control subjects and 76 ± 8 mg/dl in diabetic patients and also saw activation in the brainstem, anterior
cingulate cortex, uncus, and putamen. Both studies confirm the presence of a number of brain regions in humans sensitive to small changes in glucose.

A more recent innovation has been the application of water positron emission tomography (PET) to the study of hypoglycemia. Water-PET can be measured over short time intervals and so can allow investigators to examine the temporal pattern of changes in brain activation. Teh et al. (18) compared CNS responses during either hyperinsulinemic hypoglycemia (~50 mg · dl⁻¹; n = 10) with those seen during hyperinsulinemic euglycemia (~90 mg · dl⁻¹; n = 7) in two matched groups of nondiabetic subjects. During hypoglycemia, there was an early cerebral response bilaterally in the anterior cingulate gyrus and pulvinar region of the thalamus, with deactivation in the posterior parahippocampal gyrus. Later activation responses were also seen in the anterior insula, ventral striatum, and pituitary. These findings were generally comparable with previous reports that used single photon emission computed tomography (19) or water-PET (20) to examine human subjects during hypoglycemia, particularly in the changes seen in the pulvinar and anterior cingulate. The pulvinar region of the thalamus is thought to relay arousal-enhanced integrated sensory information to other cortical areas and might be important in facilitating behavioral responses to hypoglycemia, whereas activation of the anterior cingulate is associated with autonomic activation (18). A further interesting observation in the water-PET study by Teh et al. (18) was that pulvinar and posterior thalamic activation, high during all of hypoglycemia, fell to below baseline levels in recovery. The authors speculated that this implied a reversal of stress-induced activation to below baseline levels, i.e. represented an adaptation in the range and set-point of responses to the stressor, a finding consistent with animal studies pointing to stress habituation or tolerance as a key pathophysiological adaptation in recurrent hypoglycemia (18).

**Brain Metabolism after Repeated Hypoglycemia**

Glucose-sensing neurons are responsive to a number of energy substrates in addition to glucose (e.g. lactate). After recurrent hypoglycemia, counterregulatory responses are significantly blunted, and the glucose level at which glucose-sensing neurons in the hypothalamus are activated is lowered (8). Theoretically, this adaptation might reflect an increase in glucose and/or alternate fuel transport or metabolism. Examining this question, Henry et al. (21) used ¹³C nuclear magnetic imaging to measure cerebral oxidative metabolic rate in a small group of individuals (n = 5) with type 1 diabetes and hypoglycemia unawareness [glycosylated hemoglobin (HbA1c) <7.5% and self-reported hypoglycemia unawareness/biochemical hypoglycemia] and compared this with nondiabetic control subjects (n = 5). All subjects were infused iv with insulin, glucose, and somatostatin to achieve stable glucose plateaus of 200 mg · dl⁻¹ as well as an infusion of ¹³C glucose, and measures of cerebral metabolic rate of glucose oxidation were made under steady-state conditions. In this study, metabolic fluxes between control and diabetic patients did not differ, indicating no overall difference in the rate of glucose oxidation in the brain. A previous study by the same group (22) had reported higher steady-state glucose levels in a similar population of type 1 subjects, and as such the authors interpreted their current findings as indicating an overall increased rate of glucose transport in the unaware type 1 diabetic population. Although an attractive hypothesis, this is not consistent with the findings of others (23), and because subjects were not studied under hypoglycemic conditions or directly compared, it remains speculative.

An alternate explanation for defective sensing in the brain is that glucose-sensing neurons might obtain additional metabolic substrates from more local sources, such as brain glycogen. Although glycogen is present in much smaller quantities in the brain compared with muscle or liver, it still represents a potential additional source of fuel. Öz et al. (24), again using ¹³C nuclear magnetic imaging in conjunction with ¹³C-glucose, examined the impact of acute hyperinsulinemic hypoglycemia (~45 mg · dl⁻¹) on brain glycogen mobilization and of antecedent hypoglycemia on glycogen synthesis rates in two groups of five nondiabetic volunteers. They found that brain glycogen content was reduced by approximately 15% during hypoglycemia when compared with the control euglycemic state. They also reported that brain glycogen content was increased when compared with euglycemic control studies after exposure of the nondiabetic subjects to 120 min of hypoglycemia. On the basis of these findings and prior work from the same group, the authors concluded that brain glycogen does represent an additional source of energy substrates during hypoglycemia and that “supercompensation” of brain glycogen content after a period of hypoglycemia might contribute to the suppression of counterregulatory responses during subsequent hypoglycemia (by now providing an additional fuel source). However, brain glycogen levels are much lower in the brain than in muscle or liver, and it is questionable how much fuel is actually available from glycogen under hypoglycemic conditions and whether the small increases in glycogen content seen after hypoglycemia could make a meaningful contribution to energy supply in the CNS. However, the studies remain of great interest and at the very least
have raised interest in the therapeutic potential of alternate fuels.

This very question was examined by Page et al. (16). Prolonged fasting and recurrent hypoglycemia cause adaptive changes in the brain that increase its ability to use alternate fuels to support metabolism (25, 26). Exploiting this metabolic adaptation, Page et al. (16) examined whether supplementation with oral medium-chain triglycerides (MCT; constituents of coconut and palm oils) would act to enhance cognitive function during acute hyperinsulinemic hypoglycemia in 11 intensively treated (mean HbA1c, 6.9 ± 0.6%, and history of frequent hypoglycemia) subjects with type 1 diabetes. In this randomized crossover study, ingestion of MCT prevented the development of hypoglycemia-induced cognitive dysfunction (on working memory tasks). Interestingly, whereas MCT ingestion resulted in a 4-fold increase in free fatty acid and a 14-fold increase in β-hydroxybutyrate levels, there were no differences in the counterregulatory response to hypoglycemia between groups. These findings appear to indicate that there are regional differences in the ability of the brain to use alternate fuels and that whereas MCT could support brain regions involved in cognition, they did not affect subcortical regions such as the hypothalamus (i.e. they appear to have had no obvious effect on glucose-sensing neurons).

Hypoglycemia and Cognition

A major concern of patients with type 1 diabetes is whether recurrent hypoglycemia and chronic hyperglycemia lead to premature cognitive decline. This area has been controversial, but reassuringly, the DCCT/EDIC investigators reported no relationship between decline in cognitive functioning over an 18-yr period and the occurrence of one or more episodes of hypoglycemia-associated seizure or coma (27). In a follow-up report of this large (n = 1144) and very carefully monitored cohort (detailed biological measures recorded and an extensive battery of cognitive function tests that took 4–5 h to complete) of type 1 diabetics, Jacobson et al. (27) were able to provide a detailed analysis of the biomedical factors that could increase the risk of cognitive decline. The authors reported that over an 18-yr period, modest declines in cognitive function were associated with the development of microvascular complications. Additional multivariable modeling revealed that glycemic control, serious diabetic retinopathy, and renal complications were each independently associated with declining performance on measures of psychomotor efficiency. Recurrent severe hypoglycemia, the apolipoprotein E ε4 allele or measures of macrovascular disease showed no significant relationship with cognitive performance. These findings support the use of intensive insulin therapy to achieve near-normal glucose levels and imply that the beneficial effect of this on microvascular disease may extend to the brain. However, as pointed out in an accompanying commentary by Frier (28), the DCCT/EDIC cohort were highly selected and all young and healthy. They would not have been expected to show much evidence of cognitive decline, and it is not possible as yet to exclude cumulative effects of recurrent hypoglycemia in elderly subjects with type 1 diabetes or in groups thought particularly vulnerable such as children under the age of 5 yr or subjects with impaired awareness of hypoglycemia.

Improving CNS Responses to Hypoglycemia

The question for physicians looking after individuals with type 1 diabetes who are experiencing recurrent disabling hypoglycemia is how best to manage such an individual without simply relaxing glucose control. For a few subjects, pancreas transplantation, either whole organ or islet, remains the only way in which severe and distressing hypoglycemia can be prevented, but for the majority we need to examine how we might safely achieve optimal glucose control. Clearly, the first approach is to ensure that there are no significant comorbidities contributing to that risk, such as associated endocrinopathies or disorders affecting insulin clearance or glucose production (2). In the vast majority of cases, however, recurrent hypoglycemia is occurring in the context of a mismatch between insulin requirements and delivery, and understanding this will require a detailed exploration of meal patterns, exercise, alcohol intake, and insulin injection routine.

Recently, we have also seen a resurgence of interest in the development of structured education programs aimed at providing individuals with the information and skills to successfully manage intensive insulin therapy. A key stimulus to this was the Dusseldorf education and training for dietary flexibility and insulin adjustment program (29). This 5-d in-patient program was the first to really demonstrate the effectiveness of a structured approach to diabetes care. In an 1-yr evaluation of 9583 subjects with type 1 diabetes from 96 participating diabetes centers who had enrolled in the course, it was shown that mean baseline HbA1c had fallen from 8.1 to 7.3%, and yet despite this, the incidence of severe hypoglycemia (defined as a requirement for iv glucose or im glucagon) actually decreased from 0.37 to 0.14 events per patient per year, and the beneficial effects were most obvious in those patients in the lowest quartile of HbA1c. A related program in the United Kingdom, DAFNE (Dose Adjustment for Normal Eating), resulted in improved HbA1c and quality of life,
but no significant reduction in incidence of severe hypoglycemia (30). Other behavioral approaches based on symptom recognition such as blood glucose awareness training and hypoglycemia awareness and avoidance have also been tried, although as yet none of these education programs have been shown to improve hypoglycemia awareness in diabetes (2).

The next step is to consider the most effective insulin replacement regimen. The experience of most investigators in this field is that intensive insulin therapy is best achieved using flexible insulin regimens that can more closely mimic normal physiology as well as adapt to the patient’s general lifestyle. This is most commonly achieved in modern practice through the use of multiple daily injection therapy with insulin analogs or through continuous sc insulin infusions (CSII; insulin pump therapy). However, the data supporting their use to reduce frequency of severe hypoglycemia is not robust and is mostly limited to a reduction in nocturnal hypoglycemia with basal analog use (31). Support for the use of CSII over multiple daily injection analog regimens to reduce severe hypoglycemia risk and improve HbA1c is also limited, with a recent Health Technology Assessment in the United Kingdom suggesting no significant benefits in adults with type 1 diabetes (32). However, despite these limitations, it is this reviewer’s opinion that technical innovations, particularly through the use of CSII combined with real-time continuous monitoring or “closed-loop” systems, are likely to represent the immediate future of diabetes care. A recent 26-wk study of real-time continuous glucose monitors (rt-CGM) used in 129 adults and children with intensively treated (HbA1c <7.0%) type 1 diabetes found that regular use of rt-CGM was associated with a small improvement in glucose control and a reduction in glucose variability (less time spent outside the target glucose range of ≤70 or >180 mg/dl) (33). Although, no overall effect was seen in frequency of either biochemical or severe hypoglycemia, the short-term nature of the trial may have been insufficient to demonstrate these outcomes.

A further development is the use of rt-CGM with preset alarms at specific glucose levels. Ly et al. (34) performed hyperinsulinemic hypoglycemic clamp studies to assess baseline counterregulatory hormones and symptom responses in a pilot trial of adolescents with type 1 diabetes and self-reported hypoglycemia unawareness. The subjects were then randomized to either standard therapy (n = 5) or rt-CGM (n = 6) for 4 wk, after which the clamp procedure was repeated. They were able to report that both the epinephrine and adrenergic symptom responses during hypoglycemia after rt-CGM were improved, with no significant deterioration in glycemic control. Buckingham et al. (35) used a closed-loop system to study 40 subjects with type 1 diabetes overnight in hospital focusing on overnight glucoregulation and hypoglycemia avoidance, a critical target given that 50% of severe hypoglycemic episodes occur during the night. The closed-loop approach uses a sc glucose monitor to control delivery of insulin via an insulin pump throughout the day (the artificial pancreas). In this study, predictive algorithms were used to assess risk of hypoglycemia based on the pattern of glucose change recorded from the sc sensor every minute. When it was predicted that the blood glucose would fall below 80 mg·dl⁻¹, the pump was suspended for 90 min. Using these predictive algorithms, the investigators found that they were able to prevent 60–80% of nocturnal hypoglycemic episodes (blood glucose <60 mg·dl⁻¹). This important therapeutic development addresses one of the primary abnormalities in hypoglycemia counterregulation in type 1 diabetes, namely the inability to shut off insulin delivery.

Future Therapies

In addition, to these behavioral and technical strategies for hypoglycemia avoidance, it is also possible that we may be able to intervene therapeutically to restore or augment hypoglycemia awareness in type 1 diabetes using approaches that target specific molecular processes involved in the detection of hypoglycemia or regulation of the counterregulatory response. To date, these approaches have included studies of β2-adrenergic agonists, methylxanthine derivatives (e.g., caffeine), sulfonylureas, GABAergic antagonists, and fluoxetine (2). More recently, Leu et al. (36) have suggested that opioid receptor antagonists may represent a useful future therapeutic option. Eight nondiabetic subjects were examined using a 2-d hyperinsulinemic glucose clamp protocol on four different occasions to determine whether opioid receptor blockade during antecedent hypoglycemia (60 mg/dl) on d 1 would prevent development of defective counterregulation on d 2. The investigators reported that, as expected, d-1 antecedent hypoglycemia produced a significant suppression of d-2 hormonal responses to hypoglycemia. However, when naloxone was injected before hypoglycemia on d 1, this effect was reversed, implicating endogenous opioids in the development of hypoglycemia-induced defective counterregulation.

Summary

Hypoglycemia remains a major clinical issue for individuals with type 1 diabetes. Definitive strategies for reducing the frequency of severe hypoglycemia while maintaining near-normal glucose control through intensive insulin
therapy remain undefined. A number of technological and behavioral interventions may lead to reduced glucose variability and reduced severe hypoglycemia risk, but these have not been subject to the sort of large-scale trials that are required to show a meaningful reduction in severe hypoglycemia rather than simply an improvement in surrogate end-points. In the short- to medium-term, targeting hypoglycemia avoidance through improved insulin analogs and insulin-delivery systems in combination with structured education programs may all help, whereas in the long-term, strategies based on a better understanding of the cellular changes evoked by repeated hypoglycemia, especially in the CNS, will be required to significantly reduce severe hypoglycemia risk. This information is urgently needed if endocrinologists and their patients are to be enabled to safely achieve normalization of glucose control.

Acknowledgments

The author thanks the postdoctoral fellows and technical staff who contributed greatly to the research that underpins this review.

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The research work of the author is supported by research grants from the Juvenile Diabetes Research Foundation.

Disclosure Summary: The author has no competing interests.

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