Potential approaches to prevent hypoglycemia-associated autonomic failure

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Accepted 12 October 2017
Published Online First 14 November 2017

ABSTRACT

Clear health benefits are associated with intensive glucose control in type 1 diabetes mellitus (T1DM). However, maintaining near-normal glycemia remains an elusive goal for many patients, in large part owing to the risk of severe hypoglycemia. In fact, recurrent episodes of hypoglycemia lead to 'hypoglycemia-associated autonomic failure' (HAAF), characterized by defective counter-regulatory responses to hypoglycemia. Extensive studies to understand the mechanisms underlying HAAF have revealed multiple potential etiologies, suggesting various approaches to prevent the development of HAAF. In this review, we present an overview of the literature focused on pharmacological approaches that may prevent the development of HAAF. The purported underlying mechanisms of HAAF include: 1) central mechanisms (opioid receptors, ATP-sensitive K+(K_{ATP}) channels, adrenergic receptors, serotonin selective receptor inhibitors, γ-aminobuyric acid receptors, N-methyl D-aspartate receptors); 2) hormones (cortisol, estrogen, dehydroepiandrosterone (DHEA) or DHEA sulfate, glucagon-like peptide-1) and 3) nutrients (fructose, free fatty acids, ketones), all of which have been studied vis-à-vis their ability to impact the development of HAAF. A careful review of the current literature reveals many promising therapeutic approaches to treat or reduce this important limitation to optimal glycemic control.

INTRODUCTION

Although intensive glucose control in diabetes is associated with clear health benefits,[1] maintaining near-normal glycemia remains an elusive goal for many patients with type 1 diabetes mellitus (T1DM), in large part owing to the risk of severe hypoglycemia.[2] Hypoglycemia contributes to significant morbidity and mortality in T1DM, as it is estimated that 6%–10% of deaths in patients with T1DM may be attributed to hypoglycemia.[3] Each year, hypoglycemia accounts for an estimated 100 000 emergency room visits and 30 000 hospital admissions, with each event taking an enormous economic toll on the healthcare system[4] as well as on patients' productivity and well-being.

Patients with T1DM as well as advanced type 2 diabetes mellitus (T2DM) are susceptible to hypoglycemia because they require exogenous insulin treatment, and due to acquired defective counter-regulatory responses, including deficient glucagon release. Additionally, their vulnerability to hypoglycemia is worsened by a phenomenon known as 'hypoglycemia-associated autonomic failure' (HAAF), which is a blunting of sympathoadrenal and other counter-regulatory responses to hypoglycemia. HAAF tends to occur following recurrent episodes of hypoglycemia (eg, in patients with insulinoma[5 6]) and in patients with T1DM who observe tight glyemic control.[7] The mortality and morbidity risks associated with hypoglycemia are exacerbated in patients with HAAF. Following one or more episodes of recent, antecedent hypoglycemia, patients may develop a blunting of the normal hormonal counter-regulatory responses to hypoglycemia (ie, HAAF)[8] as well as a loss of symptoms, which when fully manifested results in hypoglycemia unawareness.[7] HAAF and hypoglycemia unawareness together lead to a vicious cycle of recurrent hypoglycemia, and a 25-fold increased risk for severe hypoglycemia.[9]

Understanding the mechanism(s) of reduced counter-regulatory responses to hypoglycemia requires new insights into the basic molecular, cellular, tissue, and whole-body pathophysiology of HAAF in experimental models.[7] To resolve this important clinical problem will require developing therapeutic approaches to enhance or normalize the pattern of counter-regulatory responses to hypoglycemia. Indeed, many different types of drugs, hormones, and nutrients have been shown to modulate counter-regulation by direct central nervous system effects. In this review, we will focus on potential therapeutic approaches that have been explored for the treatment of HAAF, a defective counter-regulatory response during hypoglycemia, in both animal models and in human subjects. This review will not address other approaches to prevention of hypoglycemia such as use of glucagon in closed-loop systems, continuous glucose monitoring and proactive insulin dose management, and adjustment of diet and lifestyle factors such as exercise and alcohol intake.

CENTRAL APPROACHES FOR MODULATING HAAF

Role of opioid receptors

Robust data points to a key role of the endogenous opioid system in the development of HAAF.[10] Many kinds of stressors, including hypoglycemia and exercise, precipitate the

To cite: Lontchi-Yimagou E, You JY, Carey M, *et al*. *J Investig Med* 2018;**66**:641–647.

release of endogenous opioids such as β-endorphin, which can mediate autonomic and sympathoadrenomedullary responses in humans and animals.[11 12] In fact, it has been proposed that HAAF may represent a form of stress habituation to recurrent hypoglycemia,[13] possibly as a defensive adaptation, particularly since most features of HAAF are reversible after a 2-week to 3-week period of scrupulous hypoglycemia avoidance[14] or cure of insulinoma.[5 6]

Opioid receptor blockade with naloxone has been shown to amplify the counter-regulatory hormonal response to hypoglycemia in healthy subjects and in subjects with T1DM.[15] During a single episode of hypoglycemia in normal subjects, naloxone increased endogenous glucose production (EGP) as well as epinephrine and cortisol responses to hypoglycemia as compared with placebo. Also, patients with well-controlled T1DM with suppressed hepatic and hormonal responses to insulin-induced hypoglycemia demonstrated greater stimulation of EGP as well as epinephrine, growth hormone, and cortisol release following treatment with naloxone.[15] Acute administration of intravenous naloxone during two episodes of hypoglycemia reversed experimentally induced HAAF in non-diabetic subjects by restoring the defective counter-regulatory responses (epinephrine, norepinephrine, and glucagon and EGP) during subsequent episodes of hypoglycemia.[16] Additionally, naloxone infusion during antecedent hypoglycemic episodes reduced HAAF in subjects with T1DM, with improvement of the epinephrine response as well as significant improvement of EGP during a subsequent episode of hypoglycemia.[17] In summary, these findings suggested that the opioid system plays a key modulatory role in hypoglycemia counter-regulation and could be manipulated pharmacologically, leading to the promise of novel alternative therapies to ameliorate HAAF.

Intriguingly, while overnight administration of oral naltrexone significantly increased epinephrine responses to hypoglycemia in T1DM,[18] administration of oral naltrexone twice daily for 4 weeks had no effect on hypoglycemic symptoms or on counter-regulatory hormone responses during experimental hypoglycemia in subjects with T1DM with impaired hypoglycemia awareness.[19] This chronic administration of naltrexone may upregulate opiate binding sites and lead to heightened sensitivity to endogenous β-endorphins.[20] This leads to intriguing questions about whether intermittent opioid receptor blockade might have therapeutic potential.

Role of ATP-sensitive K+ (K_{ATP}) channels

It has been demonstrated in numerous rodent studies that hypothalamic K_{ATP} channels play an important role in sensing hypoglycemia.[21] Specifically, pro-opiomelanocortin (POMC) neurons in the ventromedial hypothalamus (VMH) express a unique complement of inwardly rectifying potassium channels (Kir6.2) that allow them to respond to changes in ambient glucose concentrations. Diazoxide activates Kir6.2 channels in glucose-responsive neurons of the VMH, leading to neuronal hyperpolarization.[22] McCrimmon *et al* studied the effect of K_{ATP} channel activators (diazoxide or NN414) via microinjection into the VMH of non-diabetic rats. Both during a single episode and following recurrent episodes of hypoglycemia, the rats showed increased counter-regulatory hormonal responses (epinephrine and glucagon) and decreased glucose infusion rate (GIR), indicative of increased EGP.[21] The same group subsequently studied the contrasting effects of activating versus inhibiting K_{ATP} channels. Intravenous injection of NN414 increased plasma epinephrine levels and reduced GIR, and this effect was blocked by glibenclamide microinjection into the VMH, indicating that these effects of the K_{ATP} channel activator were centrally mediated.[23] These findings were duplicated in both non-diabetic and diabetic rats during a single episode and after recurrent episodes of hypoglycemia.

In contrast, other studies did not report a significant difference in counter-regulation during a single episode of hypoglycemia when either diazoxide or glyburide were orally administered to non-diabetic human subjects.[24 25] However, patients with T1DM who received oral diazoxide over a 12-hour period prior to a hypoglycemic clamp had increased hormonal responses to hypoglycemia. Furthermore, the subjects whose hormonal responses were most enhanced by diazoxide had an activating E23K polymorphism of the K_{ATP} channel, collectively suggesting that activation of K_{ATP} channels improves counter-regulatory responses in patients with T1DM with established HAAF.[26] Intriguingly, in addition to Kir6.2 channels, other subtypes of POMC neurons express Kir3.1–3.4 channels that are complexed to the μ-opioid receptor in these cells. Indeed, some POMC neurons respond to both μ-opioid receptor activation and K_{ATP} channel activation by diazoxide, while others respond either to μ-opioid activation alone or to diazoxide alone.[22] These complementary neuronal response patterns raise the exciting possibility that μ-opioid receptor antagonism together with activation of K_{ATP} channels could have synergistic effects in patients with HAAF. In conclusion, both rat and human studies have suggested that systemically administered K_{ATP} channel activators work centrally to increase counter-regulatory responses to hypoglycemia, despite limited results in non-diabetic subjects.

Role of adrenergic receptors

Initial seminal studies reporting elevated levels of catecholamines in response to insulin-induced hypoglycemia in non-diabetic subjects suggested that epinephrine and norepinephrine play an important role in mediating normal counter-regulatory responses to hypoglycemia,[27] and highlighted the importance of the adrenergic system. Administration of a β-agonist (terbutaline) has been shown to decrease the risk of nocturnal hypoglycemia.[28] Administration of β-antagonists (eg, propranolol, metoprolol) during hypoglycemia slowed glucose recovery times in many studies.[29 30] When subjects with insulin-induced hypoglycemia were given somatostatin together with α-antagonist and β-antagonist, glucose recovery was slower than in controls, leading to the conclusion that when glucagon secretion is inhibited by somatostatin, sympathetic stimulation is upregulated to compensate for low plasma glucose levels.[31] This study and others support a counter-regulatory role of sympathetic stimulation in glucose restoration during hypoglycemia, especially when glucagon secretion is defective in patients with diabetes.[32–35] Additionally, dogs with experimentally-induced hypoglycemia had

reduced hepatic and renal glucose production after being given β-antagonists.[36] Furthermore, the sensitivity of both β-1-receptors and β-2-receptors, measured as the dose of intravenous isoproterenol that increased the heart rate by 25 beats/min, was lower in subjects with insulin-induced hypoglycemia.[37 38] In summary, epinephrine seems to exert its glucose-elevating effects through both α-adrenergic and β-adrenergic receptor stimulation.[39]

However, despite the above evidence that β-antagonists reduce the response to an individual episode of hypoglycemia, there is evidence suggesting that administering certain β-blockers to subjects with insulin-induced hypoglycemia causes increased counter-regulatory hormone levels, such as epinephrine, growth hormone, ACTH and cortisol.[40] Administering adrenergic antagonists during one episode of hypoglycemia prevented subjects from becoming desensitized to the effects of catecholamines during subsequent bouts of hypoglycemia.[41] This introduces an intriguing paradox, in that the use of β-antagonists can be detrimental to the responses to an individual episode of hypoglycemia yet helpful in the treatment of HAAF. Furthermore, data from rodent models are mixed: while one study in rats showed that administration of β-2-agonists into the VMH caused a greater release of epinephrine during hypoglycemia,[37] another study found that blocking adrenergic receptors in rats did not affect the VMH's ability to sense hypoglycemia, concluding that adrenergic sympathetic responses may not be critical for the recognition of hypoglycemia in rats.[38] In patients with T2DM with impaired β-cell function, C peptide levels were markedly reduced in response to administration of β-antagonists, and non-significant but elevated epinephrine and norepinephrine responses were shown as compared with healthy control subjects.[42] In conclusion, since increased epinephrine release during hypoglycemic stress has been shown to reduce acute counter-regulatory responses to subsequent episodes of hypoglycemia, β-blockers may play a role in preventing HAAF.

Role of serotonin selective receptor inhibitors

Serotonin selective receptor inhibitors (SSRIs), used as antidepressant medications, have been studied as potential agents for preventing the development of HAAF. Serotonergic pathways are known to modulate neuroendocrine responses, and serotonin neurons in the caudal hindbrain are sensitive to glucose. Taken together, the evidence suggests serotonergic pathways may play a crucial role in counter-regulation during hypoglycemia. Several studies have been reported in rodents, healthy individuals and patients with T1DM. Sanders *et al* studied 6-day or 21-day sertraline-treated non-diabetic rodents for their responses to both a single episode and recurrent episodes of hypoglycemia. Epinephrine and some glucagon responses to hypoglycemia were restored in these rodents after sertraline treatment for both 6 days and 21 days, with a more robust response seen in the 6-day treated group.[43]

Briscoe *et al* studied the effect of 6 weeks' administration of fluoxetine on response to a single episode of hypoglycemia in healthy individuals; treated subjects demonstrated restored counter-regulatory hormonal responses (epinephrine, norepinephrine, and cortisol), muscle sympathetic nerve activity, and metabolic responses (endogenous glucose production, glycogenolysis, and lipolysis), but did not have restoration of hypoglycemic symptoms despite significantly increased heart rate and systolic blood pressure.[44] Similar results were shown in a study in subjects with T1DM. In these studies, subjects' weight remained stable during both fluoxetine and placebo administration. Based on these data, it appears unlikely that SSRIs' HAAF-reducing effects are mediated via weight gain, although it is certainly possible that these effects are mediated via hyperglycemia as a medication side effect.[44] Furthermore, additional data are accumulating regarding possible mechanisms for SSRIs' enhancement of both autonomic nervous system and hypothalamic-pituitary-adrenal axis responses during hypoglycemia. For example, activation of a number of serotonergic receptors has been demonstrated to increase sympathetic nervous system outflow.[45] These findings suggest that the etiology of SSRIs' HAAF-reducing effects is likely multifactorial. Although SSRI treatment did not significantly improve hypoglycemic symptoms, it was able to restore hormonal and metabolic responses to hypoglycemia, suggesting that these agents may play an important role in the treatment of HAAF.

Role of γ-aminobuyric acid receptors

Rat studies have shown that recurrent bouts of hypoglycemia are associated with an increase in GABAergic tone in the VMH, which is responsible for sensing glucose levels, possibly resulting in HAAF.[46 47] Administering a γ-aminobuyric acid (GABA) agonist, alprazolam, to healthy adults before a hypoglycemic episode reduced adrenergic (epinephrine, norepinephrine) and hormonal (glucagon and growth hormone) activity in subsequent states of hypoglycemia.[48] When injected with a GABA antagonist, bicuculline methiodide, in the VMH, prior to hypoglycemia, rats showed significant increases in glucagon and epinephrine responses when compared with hypoglycemic rats injected with a GABA agonist.[49] Conversely, when given a GABA antagonist, modafinil, before a state of induced hypoglycemia, release of norepinephrine, but not of ACTH, cortisol or growth hormone, was increased in healthy adults.[50] Similarly, modafinil was shown to improve 'adrenergic sensitivity' and cognitive function in hypoglycemic adults who had been injected with the GABA antagonist prior to hypoglycemia.[51] In summary, GABA agonists reduce counter-regulation, and GABA antagonists were able to partially improve counter-regulation during hypoglycemia.

Role of N-methyl D-aspartate receptors

N-methyl D-aspartate (NMDA) is an excitatory glutamate receptor. It has been implicated in long-term potentiation of memory formation and learning.[52] A study in dogs treated with an NMDA antagonist before undergoing insulin- induced hypoglycemia found a reduction in plasma epinephrine and cortisol levels, suggesting that glutamate stimulates the hypothalamic-pituitary-adrenal and the sympathetic-adrenal axis via NMDA channels.[53] When given an NMDA antagonist for 4 days prior to undergoing hypoglycemic studies, healthy human subjects demonstrated reduced cortisol, ACTH, epinephrine, norepinephrine, growth hormone, and glucagon secretion, which led

the authors to conclude that NMDA antagonists are not effective in preventing the hypoglycemic counter-regulatory response.[52] However, a study found that memantine, an NMDA antagonist, caused an increase in counter-regulatory hormones as well as neuroglycopenic symptoms in healthy adults.[54] Thus, the role of NMDA in HAAF warrants further study.

HORMONES

Role of cortisol

An intriguing pair of studies suggested that hypercortisolemia induced by hypoglycemia could contribute to the development of HAAF. When cortisol was infused twice to match hypoglycemia-induced rises in cortisol levels in healthy males, counter-regulatory hormonal responses to hypoglycemia on the following day were suppressed to a degree similar to antecedent hypoglycemia.[55] These investigators then performed a similar study in patients with primary adrenocortical failure. In the absence of normal cortisol secretion, recurrent episodes of hypoglycemia failed to induce HAAF in these patients, although baseline epinephrine responses were also profoundly suppressed in these patients.[56] Another study supported these findings by demonstrating reduced autonomic hormonal responses to hypoglycemia and reduced hypoglycemic symptoms after infusion of α-(1-24)-ACTH, which raised cortisol levels.[57] However, two subsequent studies did not report an effect of antecedent cortisol elevation on the development of HAAF in healthy subjects. Antecedent cortisol infusion on the previous day did not reduce counter-regulatory hormonal responses to a single episode of hypoglycemia in healthy non-diabetic subjects.[58] In addition, Goldberg *et al* studied the effect of both antecedent cortisol and metyrapone, a blocker of endogenous cortisol production. Neither cortisol nor metyrapone had a significant effect on counter-regulatory hormonal responses to hypoglycemia, compared with control.[59] Given differences in study design and subject populations among the above studies, the role of cortisol in the development of HAAF remains to be further clarified.

Role of sex hormones

Sex hormones such as estrogen and dehydroepiandrosterone (DHEA) or DHEA sulfate (DHEAS) have been studied to assess their roles in the development of HAAF. Because women have been shown to have reduced neuroendocrine and sympathetic nervous system responses to physical and cognitive stress, investigators have hypothesized that estrogen may affect counter-regulation during hypoglycemia.[60] During a hypoglycemic clamp, counter-regulatory hormonal responses were significantly reduced in estrogen-receiving postmenopausal women compared with either postmenopausal women without estrogen replacement or to men.[60]

Patients with rheumatoid arthritis were found to have lower levels of DHEAS, which was associated with reduced counter-regulatory hormonal responses during hypoglycemia.[61] Recently, another group studied the effect of DHEA administration on development of HAAF, suggesting that DHEA and DHEAS have anti-GABA, anticorticosteroid, stimulatory nitric oxide, and NMDA agonist effects, all of which could potentially improve counter-regulatory responses during recurrent hypoglycemia.[62] After antecedent hypoglycemia, subjects receiving placebo had a reduced counter-regulatory hormonal response, but those who received DHEA had a preserved counter-regulatory response. Overall, despite the limited amount of studies, male and female sex hormones seem to have opposite effects on counter-regulation during hypoglycemia, with DHEA or DHEAS improving the counter-regulatory hormonal response while estrogen impairs the response.

Role of glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors increase glucose-stimulated insulin secretion, inhibit glucagon release,[63] and lower glucose levels during hyperglycemia without causing severe hypoglycemia. When lixisenatide was given for 6 weeks to patients with T2DM, epinephrine and glucagon levels were significantly reduced during mild hypoglycemia but not during severe hypoglycemia.[64] Similar findings were reported when albiglutide was given to patients with T2DM as glucagon levels were not significantly different compared with placebo control.[63] When exenatide was administered to non-diabetic subjects during a hypoglycemic clamp, no significant difference in the counter-regulatory hormonal response was shown. However, another group showed that synthetic GLP-1 significantly reduced growth hormone secretion in response to hypoglycemia.[65 66] These disparate findings may reflect the slightly different actions of exenatide versus GLP-1 in their ability to modulate various elements of the hypoglycemic counter-regulatory hormonal response. In patients with T2DM, linagliptin (DPP-4 inhibitor) and liraglutide (GLP-1 agonist) reduce hyperglycemia without increasing the risk of hypoglycemia, and have no effect on the overall counter-regulatory response including glucagon secretion to hypoglycemia.[67] Oral saxagliptin (DPP-4 inhibitor) in patients with T1DM does not reduce frequency of hypoglycemia or degree of hypoglycemia awareness, and does not improve the counter-regulatory hormonal response during hypoglycemia.[68] In patients with T1DM, the addition of vildagliptin (DPP-4 inhibitor) to insulin therapy showed a significant reduction in the hemoglobin A1c, and caused reduced glucagon levels during meals without affecting glucagon secretion during hypoglycemia.[69] Improved glucose control through use of these medications could be beneficial in preventing severe hypoglycemia, thereby indirectly preventing development of HAAF, but these agents do not appear to have a direct role in the mediating the counter-regulatory hormonal response.

NUTRIENTS

Role of fructose

It is hypothesized that a 'catalytic' dose of fructose modulates glucokinase activity in glucose-sensing cells, contributing to fructose's counter-regulatory effect.[70 71] In both human and animal studies, it has been demonstrated that fructose plays an important role in counter-regulatory responses to hypoglycemia. Fructose infusion during a hypoglycemic clamp study caused a significant increase in epinephrine levels compared with placebo in both non-diabetic subjects and subjects with T1DM.[70 71] Subsequently, endogenous glucose production rose by 47% in non-diabetic participants and by 90% in participants with T1DM compared with control,

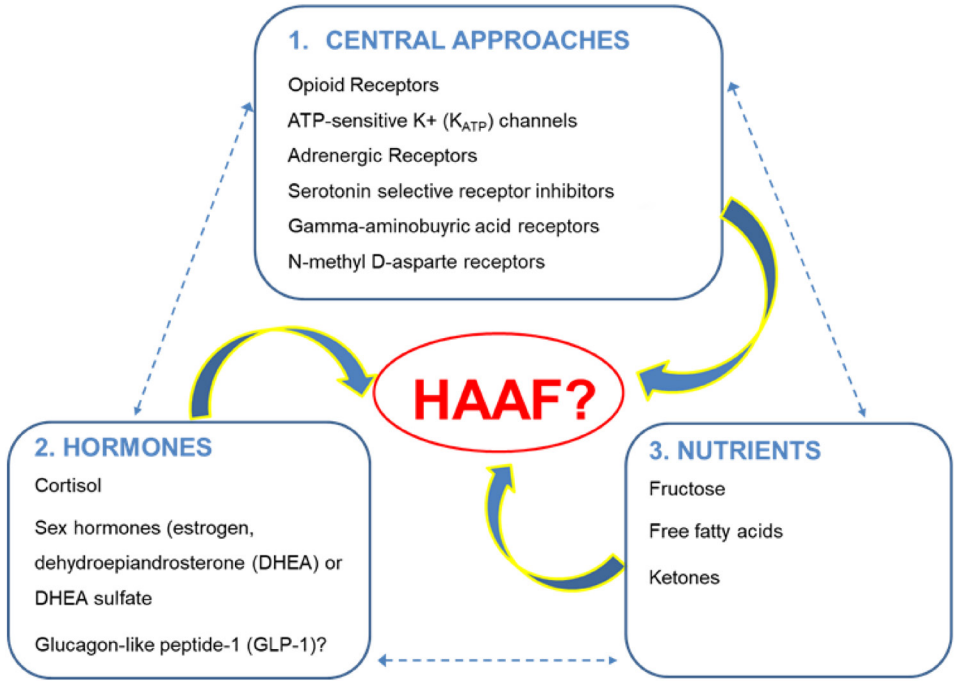

Figure 1 Potential mechanisms of hypoglycemia-associated autonomic failure (HAAF).

respectively. The fructose-induced rise in epinephrine was unique, as no changes in plasma norepinephrine, growth hormone, or cortisol were observed. However, while the study in subjects with T1DM demonstrated no change in glucagon levels, the study in non-diabetic subjects demonstrated marked increases in both glucagon and epinephrine levels during hypoglycemia. Similarly, a hypoglycemic clamp study in dogs showed that fructose infusion produced a large rise in hepatic glycogen, which increased epinephrine and glucagon levels during hypoglycemia and raised net hepatic glucose output.[72] Additionally fructose, although unable to cross the blood-brain barrier (BBB), acts directly on VMH of the brain, a region with prevailing systemic circulation.[71 73] It has been shown in humans that fructose inhibited prolactin secretion, slightly weakened and delayed ACTH secretion, and did not affect growth hormone secretion during hypoglycemia.[74] This suggests a role for fructose in the regulation of endogenous glucose production by a brain-liver pathway via central nervous system locations unprotected by the BBB.[72 74] Currently, there are few studies examining the relationship of fructose, hypoglycemia, and HAAF.

Role of free fatty acids and ketones

Epinephrine levels rise in response to hypoglycemia and induce increased lipolysis, demonstrated by a rise in the lipid precursors, glycerol and free fatty acids (FFA).[75 76] This relationship is supported by a study showing that α-adrenergic and β-adrenergic blockades suppressed FFA levels during recovery from hypoglycemia.[39] Epinephrine release during exercise-induced hypoglycemia was associated with an increase in FFA levels in addition to stimulating endogenous glucose production. In addition, Hussain *et al* showed that administration of IGF-1 resulted in increased FFAs and ketone bodies during hyperinsulemic-euglycemic clamp studies in healthy humans.[77] In another hypoglycemic clamp study, Haywood *et al* concluded that infusion of intralipids into the brain can augment the sympathoadrenal response during a single episode of hypoglycemia in rats, whereas systemic infusion of intralipids did not improve the counter-regulatory response.[78] Also, following recurrent hypoglycemia, rats with intracerebroventricular infusion of intralipids demonstrated a restored sympathoadrenal response to hypoglycemia.

In normal physiology, glucose is the main energy source for the brain, but during prolonged fasting, ketones can be used as an alternative energy source. Amiel *et al* examined the effect of hyperketonemia on the counter-regulatory hormone response to hypoglycemia in healthy humans.[79] During ketone infusion, the glycemic threshold for stimulating an epinephrine response, and the peak epinephrine response, were both reduced. In addition, ketone infusion resulted in reduced peak norepinephrine, cortisol, and growth hormone responses. Ketones, an alternative energy source, weaken the neurohormonal responses to hypoglycemia as the brain acknowledges the presence of another source of energy. More research should be done to expand these important observations.

CONCLUSIONS

Extensive amount of studies has been done to understand the mechanism of HAAF. Various agents have been identified as important in the underlying mechanisms of HAAF, including: 1) central pathways (modulation of opioid receptors, K_{ATP} channels, adrenergic receptors, SSRI, GABA receptors, NMDA receptors); 2) hormones (cortisol, estrogen, DHEA/DHEAS, GLP-1); and 3) nutrients (fructose, FFA, ketones). Currently, it is apparent that the mechanisms underlying the development of HAAF are not dependent on a single pathway (as presented in figure 1). As researchers

further study the mechanism of HAAF with more sophisticated study designs and different routes of administration of various agents, there is great potential for studies involving combinations of medications that could offer additive or synergistic effects in the treatment of HAAF.

Contributors ELY conceived of the idea for this review paper, and ELY and JYY did the majority of the literature review and writing. MC, HS, and MH contributed to the literature review and analysis, critical revision, and editing. IG contributed ideas and inspiration.

Funding This work was supported by grants from the National Institute of Health (DK069861 and DK48321), by the Einstein-Montefiore NIH CTSA Grant UL1TR001073 from the National Center for Research Resources (NCRR) and the Einstein-Mt. Sinai Diabetes Research Center (5P30DK020541-41). Its contents are solely the responsibility of the authors and do not necessarily represent the official views or policies of the FDA, NCRR, or NIH.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

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