

Review

Mechanisms of amino acid-stimulated insulin secretion in congenital hyperinsulinism

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The role of amino acids in the regulation of insulin secretion in pancreatic beta-cells is highlighted in three forms of congenital hyperinsulinism (HI), namely gain-of-function mutations of glutamate dehydrogenase (GDH), loss-of-function mutations of ATP-dependent potassium channels, and a deficiency of short-chain 3-hydroxyacyl-CoA dehydrogenase. Studies on disease mouse models of HI suggest that amino acid oxidation and signaling effects are the major mechanisms of amino acid-stimulated insulin secretion. Amino acid oxidation via GDH produces ATP and triggers insulin secretion. The signaling effect of amino acids amplifies insulin release after beta-cell depolarization and elevation of cytosolic calcium.

Keywords insulin secretion; amino acid; congenital hyperinsulinism; glutamate dehydrogenase

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Introduction

The importance of amino acid-stimulated insulin secretion (AASIS) by pancreatic islets has long been recognized [1], but its mechanisms of action remain poorly understood. Studies on perfused rat islets in the 1970s found that many amino acids can only stimulate insulin secretion in the presence of glucose [2,3]. These glucose-dependent amino acids include complete mixtures of all 20 amino acids as well as certain individual amino acids including alanine, asparagine, glycine, glutamate, phenylalanine, and tryptophan [3–8]. Leucine is a notable exception to the rule that glucose is required for AASIS, since the insulin-stimulatory effect of leucine in perfused rat pancreas is abolished in the presence of glucose [9]. In the 1980s, Sener and Malaisse [10] showed that leucine stimulates insulin secretion by allosterically stimulating glutamate dehydrogenase (GDH) activity. The recent discovery of amino acid-sensitive hypoglycemia in children with congenital hyperinsulinism (HI) highlights the important role of amino acids in the regulation of pancreatic beta-cell function, which has long been

neglected [11]. Congenital HI is a group of diseases that display dysregulated insulin secretion by pancreatic beta-cells due to genetic defects. Hyperinsulinemic hypoglycemia is a major clinical phenotype [11]. Protein-sensitive hypoglycemia has been shown to occur in three forms of HI, namely gain-of-function mutations of GDH (GDH-HI) [12,13], loss-of-function mutations of ATP-dependent potassium channels (KATP-HI) [14] and a deficiency of the mitochondrial fatty acid β -oxidation enzyme, short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD-HI) [15]. Although the clinical features of protein-induced hypoglycemia are similar, the mechanisms of AASIS in these three forms of HI are different. In this review, we discuss the mechanisms of amino acid hypersensitivity in HI.

Mechanism of AASIS in GDH-HI

Oxidation of amino acid triggers insulin release

In 1998, mutations of GDH were identified in children with a dominant form of HI [16]. GDH enzyme activity can be allosterically inhibited by ATP and GTP, but be activated by ADP, GDP, and leucine. The GDH reaction plays a key role in the regulation of the amino acid metabolism. The impairment of the GTP allosteric inhibition of GDH leads to a gain of GDH function, which has been found to have occurred in GDH-HI patients [16,17]. Children with GDH gain-of-function mutations are sensitive to protein feeding, and hyperinsulinemic hypoglycemia occurred after a mixture of amino acid loads. GDH-HI also shows exaggerated acute insulin responses to intravenous leucine stimulation [12,13], and this leucine response can be inhibited by rising blood glucose levels [13]. We hypothesize that the elevated amino acid oxidation seen by increasing the flux of glutamate through GDH into α -ketoglutarate, which leads to the augmentation of ATP production (ATP then closes the KATP channel and subsequently leads to beta-cell depolarization and calcium influx), finally activates the 'triggering' pathway for insulin release similar to glucose-stimulated insulin secretion (GSIS) [18].

'Run-down' phenomenon

In order to test the hypothesis of which GDH activation leads to increase in amino acid oxidation and ATP production, we first performed leucine-stimulated insulin secretion (LSIS) in isolated rat islets. As shown in **Fig. 1(A)**, after 3 days of culture in 10 mM glucose, islets failed to respond to LSIS after 50 min of run-down. In contrast, through prolonged run-down (up to 120 min) by depleting intracellular energy, islets became leucine sensitive [19]. This leucine sensitivity can be completely blocked by pre-exposure to high concentration of glucose. This run-down phenomenon in rat islets suggests that GDH may serve as an intracellular energy sensor; when intracellular phosphate potential is high, GDH can be inhibited by GTP or ATP, whereas after run-down, intracellular energy is depleted and GDH gains the sensitivity for leucine activation.

GDH gain-of-function mutation leads to hypoglycemia

To further examine the hypothesis of which GDH gain-of-function mutations result in leucine-sensitive hypoglycemia in GDH-HI, we generated transgenic (TG) mice in

order to express a severe form of GDH mutation (H454Y) in beta-cells following a rat insulin promoter. GDH-TG mice showed a consistent hypoglycemia phenotype [20]. The oral protein tolerant test showed GDH-TG mice to be sensitive to amino acid stimulation with rapid falls in blood glucose and increases in insulin secretion after a mixture of amino acids challenge [21], similar to GDH-HI patients [12]. GDH enzyme kinetics in isolated islets from H454Y-TG mice showed a right shift in the GTP inhibition curve, which confirmed H454Y expression in islets [17]. Dynamic insulin secretion in perfused islets isolated from GDH-TG mice was also sensitive to stimulation by amino acid mixture and leucine, even responding to glutamine alone, whereas control islets were unresponsive to either glutamine or a mixture of amino acids [20]. This study thus confirmed that GDH gain-of-function mutation is disease-causing in GDH-HI.

H454Y GDH transgene results in increased GDH fluxes and ATP production

To examine the effects of the H454Y GDH mutation on flux through GDH, the metabolomic approach was applied

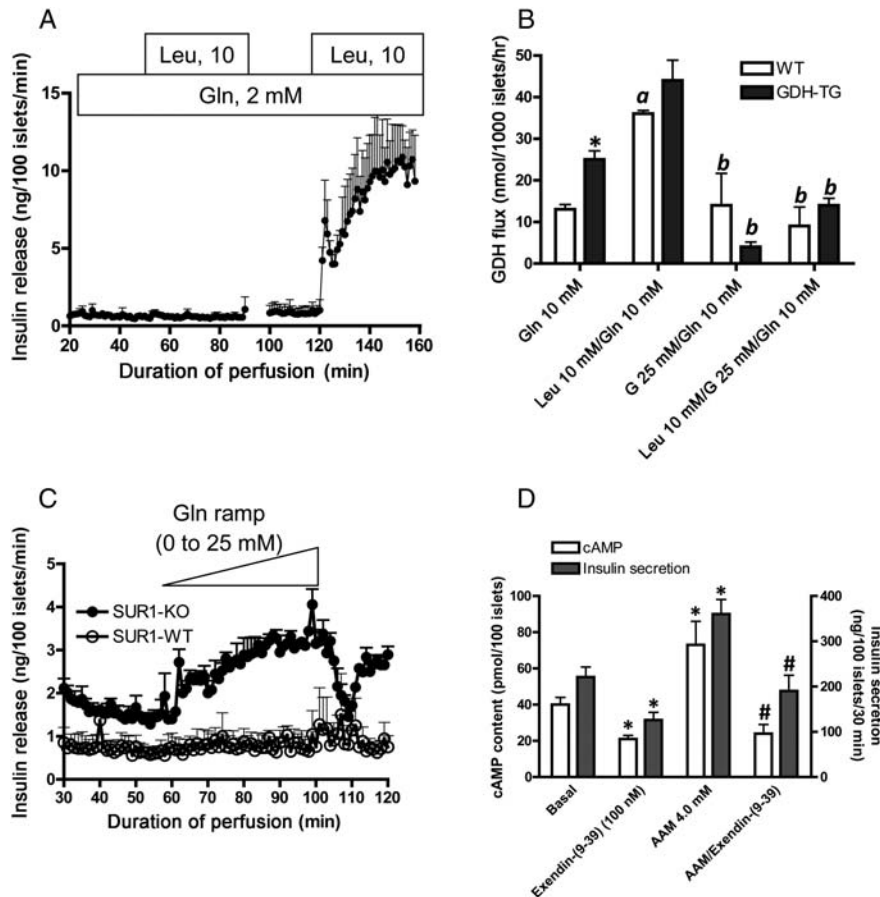


Figure 1 Amino acid stimulated insulin secretion in islets from rat, GDH transgenic mice, and SUR1 knockout mice (A) Leucine stimulated insulin secretion and run-down in rat islets. (B) GDH fluxes in GDH-transgenic mouse islets, vs. wild-type controls, $^*P < 0.05$; vs. Gln 10 mM, $^aP < 0.05$; vs. Gln 10 mM/Leu 10 mM, $^bP < 0.05$ ($n = 3$). (C) Glutamine-stimulated insulin secretion in SUR1 knockout mouse islets. (D) Amino acid mixture-stimulated insulin secretion and cAMP production in SUR1 knockout islets and the effects of Exendin-(9–39), vs. basal, $^*P < 0.05$; vs. AAM, $^{\#}P < 0.05$ ($n = 4–8$).

in isolated islets using a stable isotope tracing technique with gas chromatography/mass spectrometry to trace ^{15}N flux from $[2\text{-}^{15}\text{N}]\text{glutamine}$ to $[2\text{-}^{15}\text{N}]\text{glutamate}$ and subsequently to $[^{15}\text{N}]\text{ammonia}$. These data could then be used to calculate the specific flux *via* the GDH reaction. Compared with wild-type control littermates, in response to 10 mM $[2\text{-}^{15}\text{N}]\text{glutamine}$, H454Y-TG islets have greater insulin secretion, ammonia production, $[^{15}\text{N}]\text{ammonia}$ enrichment, and GDH flux as shown in **Fig. 1(B)**. In control islets, the activation of GDH with 10 mM leucine leads to an increase in insulin release, accompanied by increased rates of ammonia production and a three-fold stimulation of flux through GDH. The effect of leucine on GDH flux in control islets was similar to the rates seen in TG islets in the absence of leucine, suggesting that GDH flux in TG islets was close to maximal even without leucine activation [20]. The increased flux of GDH in TG islets results in the elevation of the ATP/ADP ratio, which is responsible for the activation of a triggering pathway of insulin release. The glucose inhibition of LSIS observed in GDH-HI patients can be explained by the almost complete inhibition of ammonia production, ^{15}N -enrichment of ammonia, and GDH flux in normal and TG islets by a high level of glucose; leucine was unable to override this inhibitory effect of glucose [13,20]. This glucose inhibition of GDH fluxes reflects the elevated phosphate potential generated during glucose oxidation and its strong inhibitory effect on GDH. This study thus provides strong evidence that GDH serves as an intracellular energy sensor that can sense the rise and fall of glucose metabolism. In response to the changes in phosphate potential lead by the glucose metabolism, an ‘on and off’ switch of GDH will lead to an increase or a decrease of amino acid oxidation in order to maintain intracellular energy potential.

Epigallocatechin gallate has the therapeutic potential to treat GDH-HI

GDH-HI caused by increased GDH fluxes and thus the inhibition of the mutant form of GDH have therapeutic potential. Green tea is often emphasized as a rich source of polyphenols. Among the four major polyphenols in green tea, namely epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate (ECG), and epicatechin, EGCG and ECG have a strong inhibitory effect on GDH activity with nanomolar ED_{50} [22]. The co-crystallization of GDH protein with ECG shows that ECG binds to and hijacks the ADP activation site, resulting in the inhibition of GDH enzyme activity [21]. Mutations of the ADP-binding site (R90S, D123A, and S397I) disrupt ADP binding as well as EGCG inhibition, strongly supporting the notion that EGCG binds to the ADP site. Interestingly, EGCG also inhibits several disease-causing GTP-insensitive GDH mutations, including H454Y, since it binds to the ADP site

rather than to the GTP site. This special feature of the EGCG inhibition of GTP-insensitive GDH mutations provides a therapeutic potential to treat GDH-HI. The experiments on GDH-TG mice indeed showed that pre-exposure to oral EGCG (100 mg/kg of body weight) diminished amino acid-induced hypoglycemia in TG mice. EGCG also blocked glutamine-stimulated calcium influx and insulin secretion in GDH-TG islets, suggesting that the action of EGCG in GDH-TG mice is on beta-cell insulin secretion [21]. This study provides the ‘proof of concept’ that GDH inhibition by small molecules leads to the novel treatment of GDH-HI, since current treatment that only targets dysregulated insulin secretion using diazoxide, hyperammonemia, and neurological abnormalities certainly requires attention for clinical management [16,23]. Early attempts have already identified several GDH inhibitors, but further study is certainly required to pursue this concept [24].

Mechanism of AASIS in SCHAD-HI: SCHAD–GDH interaction

Recently, a new form of HI has been described that is associated with a deficiency in SCHAD [25,26]. SCHAD catalyzes the beta-oxidation cycle for medium and short-chain 3-hydroxy fatty acyl-CoAs (C4–C10). Children with SCHAD deficiency have hypoglycemia and accumulations of fatty acid metabolites, including plasma 3-hydroxy-butyrylcarnitine and urinary 3-hydroxyglutaric acid. Unlike ketotic hypoglycemia that occurs in the other genetic defects of fatty acid oxidation, hypoglycemia in SCHAD deficiency results from HI [27]. In order to investigate the mechanisms responsible for hypoglycemia in SCHAD deficiency SCHAD-knockout (KO) mice were generated [28]. SCHAD-KO mice have lower plasma glucose levels than controls in both fed and fasting states. The plasma acylcarnitine profiles of SCHAD-KO mice showed a four-fold elevation in plasma 3-hydroxy-butyrylcarnitine compared with controls and they were similar to those values reported in children with SCHAD deficiency. Compared with wild-type mice, SCHAD-KO mice showed a rapid decline in blood glucose after receiving oral amino acids, which was due to the stimulation of insulin release in response to amino acids in KO mice, similar to the protein-induced hypoglycemia seen in patients with SCHAD deficiency. Moreover, SCHAD-KO mice have comparable glucose tolerance test results compared with control littermates. Studies of isolated islets have confirmed these *in vivo* findings. SCHAD-KO islets are also very sensitive to stimulation with a physiological mixture of amino acids similar to islets from GDH-TG mice, but they have a higher threshold of AASIS. When glutamine and leucine were both removed from the amino acid mixture, KO islets were completely unresponsive, indicating that GDH activation was essential for abnormal insulin

release. KO islets were also more sensitive to leucine stimulation but unlike islets from GDH-TG, failed to respond to glutamine alone. The insulin secretion results indicated an activation of GDH in SCHAD-KO islets, but not as active as GDH gain-of-function mutations. GSIS was similar between KO and wild-type islets, suggesting that HI in SCHAD-KO mice is not due to altered GSIS as hypothesized [29]. The measurement of GDH enzyme kinetics indicated that KO islets show a normal response to GTP inhibition, but have a reduced affinity for the GDH of α -ketoglutarate, but not ammonia, suggesting that the presence of SCHAD protein affects the binding of the substrate in the catalytic site. The effect was limited to islet GDH, perhaps reflecting the high levels of SCHAD and high ratio of SCHAD to GDH in islet tissue [28,30]. We further examined the possibility of protein–protein interactions between the two enzymes. When an anti-SCHAD antibody was used as bait, GDH was co-precipitated with SCHAD in wild-type mouse liver mitochondria, but not in KO mice, consistent with a GDH–SCHAD protein complex in wild-type liver mitochondria. Our findings suggest that GDH activation in SCHAD deficiency is due to the loss of a direct protein–protein interaction between the two enzymes. It is interesting to speculate that the inhibitory effect of SCHAD on GDH might be part of a mechanism for the reciprocal control of fatty acid and amino acid oxidation. Indeed, GDH may be the central regulatory step in the metabolic interaction, including glucose, fatty acid, and amino acid. Further studies have found that SCHAD not only binds to GDH, but also associates with other proteins, such as carbamoyl phosphate synthase 1, citrate synthase, glutamine synthetase, pyruvate dehydrogenase, and ATP synthase [31], which involves ureagenesis, the metabolism of glucose, amino acid, and ATP production, highlighting the complexity of SCHAD interaction.

Mechanism of AASIS in KATP-HI

The signaling effect of amino acids

The loss function mutation of KATP channels is the most common and severe form of HI, which often requires pancreatectomy to control hypoglycemia [11]. Interestingly, KATP-HI also has protein-sensitive hypoglycemia [14]. To investigate the mechanism of protein-induced hypoglycemia in KATP-HI we examined insulin secretion in islets from SUR1-KO mice [32,33]. SUR1-KO islets showed elevated basal intracellular calcium and basal insulin secretion, which is a characteristic feature of dysfunctional KATP channels. Unlike wild-type islets, SUR1-KO islets showed no response to leucine- or glucose-stimulated insulin release, but do release insulin in response to a mixture of amino acids and glutamine [Fig. 1(C)]. The paradoxical effect of glutamine and leucine on insulin secretion was unlikely to be caused by glutamine oxidation via GDH, since

the activation of GDH by leucine failed to stimulate insulin secretion and the sensitivity to glutamine was not inhibited by 6-diazo-5-oxo-L-norleucine, a glutaminase inhibitor, can block glutamine oxidation. Therefore, we hypothesize that glutamine may have specific effects on downstream amplification pathways for insulin release, distal to the elevation of cytosolic calcium. Recent studies suggest that the signaling effect of glutamine may involve cAMP-dependent pathways [34], as evidenced by the fact that amino acids stimulate cAMP production while stimulating insulin secretion in SUR1-KO mouse islets [Fig. 1(D)]. A similar effect can be obtained by using glutamine alone (unpublished observation). Additional evidence has also supported the hypothesis that the inhibition of the GLP-1 receptor by its antagonist, Exendin-(9–39), decreases baseline intracellular cAMP and blocks the stimulatory effect of amino acids on both cAMP production and insulin secretion [Fig. 1(D)]. The same phenomenon can be reproduced in control islets during GSIS, suggesting that the amino acid amplification of insulin secretion is an important pathway during GSIS [32]. This study suggests that the blockage of cAMP production by the GLP-1 receptor antagonist inhibits AASIS in KATP-HI, which may provide a novel therapeutic target to treat KATP-HI. *In vivo* experiments in SUR1-KO mice strongly support the hypothesis that fasting hypoglycemia in SUR1-KO mice can be corrected by prolonged subcutaneous infusions of Exendin-(9–39) [34]. A pilot clinical study in KATP-HI patients confirmed the findings observed in mouse models, namely that Exendin-(9–39) administration significantly increases fasting glucose levels in patients [35]. Studies of islets isolated from surgical specimens after pancreatectomy in KATP-HI patients have suggested that human islets with loss-of-function mutations of the KATP channel are indeed super-sensitive to AASIS and that Exendin-(9–39) blocks such effects [35]. These results suggest that amino acid-sensitive hypoglycemia or AASIS plays an important role in severe hypoglycemia in KATP-HI patients and that this effect can serve as a novel target for drug development to treat KATP-HI. The precise mechanism by which glutamine or amino acid exerts its effects on the amplification pathways of insulin secretion remains to be determined. However, it seems to be of great importance clinically in children with KATP-HI disorders and it may also be important as a major pathway for amplifying the stimulation of insulin secretion in normal individuals in response to glucose and other metabolic fuels.

Chronic depolarization of beta-cells alters glucose oxidation

The long-term treatment of type 2 diabetes with sulfonylureas often leads to insulin secretion failure [36]. One study in mice showed that beta-cell hyperactivity stimulated by sulfonylureas results in ‘glucose blindness’ [37], similar to

a lack of GSIS in islets from SUR1-KO mice [32,38]. Based on these data, we postulate that the consequence of chronic beta-cell depolarization and the elevation of cytosolic calcium affects glucose metabolism and results in impaired GSIS. In order to test this hypothesis a metabolomic approach was used to trace ^{13}C flux from $[\text{U-}^{13}\text{C}]$ glucose to amino acid in islets isolated from SUR1-KO and wild-type mice. In the presence of 4 mM physiological amino acid mixture, ^{13}C enrichments of amino acid in islets were detected in alanine, aspartate, glutamate, γ -aminobutyric acid (GABA), and glutamine, which indicates the incorporation of glucose carbon into those amino acids. By serving as metabolic indicators, those amino acids can thus be used to calculate carbon fluxes from glucose. Since the expression of lactate dehydrogenase in beta-cells is very low [39,40] and monocarboxylate transporter 1 is absent in beta-cells [41,42], changes in alanine may reflect changes in pyruvate, the end product of glycolysis. This notion is supported by the data that show that glucose indeed increases both the levels of alanine and its ^{13}C enrichment. Interestingly, the pattern of the ^{13}C enrichment of alanine showed that M+2 is about four times higher than M+3, suggesting that the M+3 pool of alanine has been diluted by an active pyruvate cycling pathway [43,44]. This conclusion is supported by the expression of the malic enzyme in mouse islets [33,45,46], a key enzyme in the pyruvate cycling pathway. Wild-type islets also have an active ‘aspartate switch’ pathway. Because glucose increases aspartate turnover by decreasing aspartate levels and increasing its ^{13}C enrichment, this pathway reflects the increased flux in the citrate acid cycle during glucose oxidation. Glucose oxidation also increases glutamine biosynthesis via glutamine synthetase using ATP and glutamate as substrates [33]. Importantly, while mouse islets operate the GABA shunt pathway, glucose lowers GABA levels and increases its ^{13}C enrichment, suggesting increased GABA shunt flux during glucose oxidation. Compared with wild-type islets, SUR1-KO islets show increased glutaminolysis, but have a 75% reduction in GABA shunt flux. The impaired GABA shunt in SUR1-KO islets can be explained by down-regulated glutamate decarboxylase gene expression, which enzymes drive the reaction to generate GABA using glutamate as a substrate [33]. Glyburide-treated wild-type islets showed similar changes in the GABA shunt compared with SUR1-KO. These alterations in the metabolism of SUR1-KO islets may lead to impaired glucose sensing but increased amino acid sensing. As illustrated in Fig. 2, ^{13}C tracing helps us outline the integrated metabolic network in pancreatic islets. Furthermore, amino acids serve as metabolic indicators, thereby providing a powerful tool to help us explore the detail of the metabolic pathway in beta-cells.

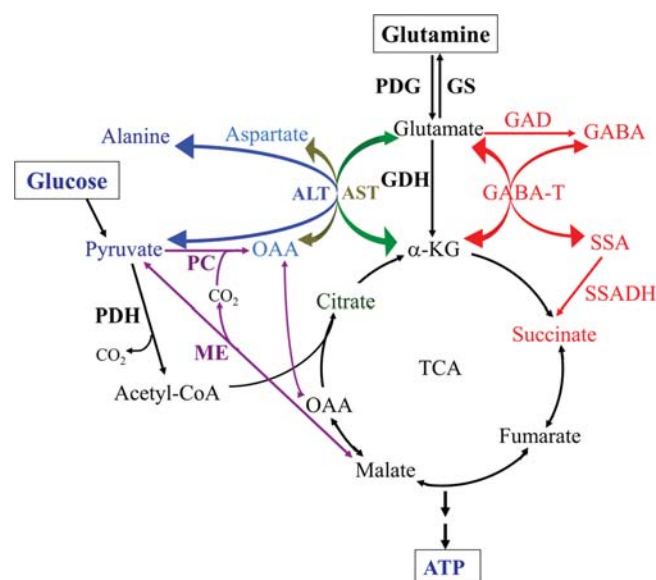


Figure 2 Integrated metabolic network of glucose and amino acid metabolism Pyruvate arising from glycolysis is in equilibrium with alanine via alanine aminotransferase (ALT). Alanine is therefore a suitable readout for pyruvate labeling. Pyruvate carbon enters the citric acid cycle through pyruvate carboxylase (PC) or with pyruvate dehydrogenase (PDH) via oxaloacetate (OAA) or acetyl-CoA, the building blocks of citrate. Pyruvate is probably also in equilibrium with malate via malic enzymes (MEs). Glucose carbon is channeled to glutamate and glutamine via transamination followed by ATP-dependent amination, respectively. Glutamate decarboxylase (GAD) is the entry point for glutamate into the GABA shunt with succinate as end product. The GABA shunt is shown in red, pyruvate cycling in purple and alanine–pyruvate transamination in blue. GABA-T, GABA transaminase; SSA, succinate semialdehyde; SSADH, SSA dehydrogenase; AST, aspartate aminotransferase; GDH, glutamate dehydrogenase; PDH, phosphate-dependent glutaminase; GS, glutamine synthetase.

Signaling Effects of Amino Acid beyond Acute Insulin Secretion in HI

As we discuss here, the acute effects of amino acids on beta-cell insulin secretion in these three forms of HI are responsible for hypoglycemia after protein meal via different mechanisms. However, the long-term effect of amino acids in maintaining chronic hyper-functional beta-cells in HI is unclear. Since amino acids, especially leucine, also regulate the mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways [47–49], we can speculate that the alteration of these pathways by amino acids may be responsible for supporting hyper-functional beta-cells in HI in the long-term, such as by increasing insulin biosynthesis in order to maintain a high secretion of insulin. Leucine alone or via the activation of GDH may regulate protein synthesis, protein phosphorylation, and gene expression in beta-cells via the mTOR and AMPK pathways, which leads to complicated and long-term changes in cellular metabolism, function, and growth [50]. Although the effects of leucine on the regulation of

the mTOR and AMPK pathways in beta-cells have been intensively investigated [50,51], the role of these pathways in the regulation of beta-cell function in HI remains largely unknown. Studies of amino acid sensitivity in HI provide insights into how amino acids regulate insulin secretion, which may further our understanding of the complex features of amino acids in the regulation of metabolism. For instance, amino acid ingestion enhances insulin secretion in type 2 diabetes patients [52].

Summary and Perspectives

The importance of AASIS is emphasized in three forms of HI. Amino acid sensitivity in GDH-HI and SCHAD-HI occur due to GDH gain of function by either GDH mutation or a lack of inhibition by SCHAD via protein–protein interactions. GDH gain of function leads to increased amino acid oxidation and ATP production, which induces insulin secretion *via* the triggering pathway. In contrast, glucose oxidation increases intracellular phosphate potential, leading to beta-cell depolarization. Under this condition, amino acids, especially glutamine, serve as a signaling molecule to amplify insulin secretion, similar to that in SUR1-KO islets. Therefore, the sensitivity of AASIS may reflect changes in the intracellular phosphate potential of pancreatic islets. When intracellular phosphate potential is low, islets become sensitive to leucine stimulation due to the release of GDH inhibition. In contrast, glucose oxidation increases phosphate potential, resulting in the inhibition of GDH but the activation of glutamine synthesis and leading to the amplification of GSIS. Understanding the mechanisms of amino acid hypersensitivity in HI will help us identify the metabolic pathways that are important for insulin secretion in order to provide potential targets for drug development to treat HI and eventually diabetes.

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References

- McQuarrie I. Idiopathic spontaneously occurring hypoglycemia in infants. Clinical significance of problem and treatment. *Am J Dis Child* 1954, 87: 399.
- Pace CS, Stillings SN, Hover BA and Matschinsky FM. Electrical and secretory manifestations of glucose and amino acid interactions in rat pancreatic islets. *Diabetes* 1975, 24: 489–496.
- Pagliara AS, Stillings SN, Hover B, Martin DM and Matschinsky FM. Glucose modulation of amino acid-induced glucagon and insulin release in the isolated perfused rat pancreas. *J Clin Invest* 1974, 54: 819–832.
- Brennan L, Shine A, Hewage C, Malthouse JP, Brindle KM, McClenaghan N and Flatt PR, *et al.* A nuclear magnetic resonance-based demonstration of substantial oxidative L-alanine metabolism and L-alanine-enhanced glucose metabolism in a clonal pancreatic beta-cell line: metabolism of L-alanine is important to the regulation of insulin secretion. *Diabetes* 2002, 51: 1714–1721.
- Malaisse WJ, Sener A, Carpinelli AR, Anjaneyulu K, Lebrun P, Herchuelz A and Christophe J. The stimulus-secretion coupling of glucose-induced insulin release. XLVI. Physiological role of L-glutamine as a fuel for pancreatic islets. *Mol Cell Endocrinol* 1980, 20: 171–189.
- Sener A, Dunlop ME, Gomis R, Mathias PC, Malaisse-Lagae F and Malaisse WJ. Role of transglutaminase in insulin release. Study with glycine and sarcosine methylesters. *Endocrinology* 1985, 117: 237–242.
- Sener A and Malaisse WJ. The stimulus-secretion coupling of amino acid-induced insulin release. Insulinotropic action of L-alanine. *Biochim Biophys Acta* 2002, 1573: 100–104.
- Malaisse WJ, Malaisse-Lagae F and Sener A. The stimulus-secretion coupling of amino acid-induced insulin release metabolic interaction of L-asparagine and L-leucine in pancreatic islets. *Biochim Biophys Acta* 1984, 797: 194–202.
- Matschinsky F, Fertel R, Brajtburg J, Stillings S, Ellerman J, Raybaud F and Thurston J. Factors governing the action of small calorogenic molecules on the islets of langerhans. The 8th Midwest Conference on Endocrinology and Metabolism, University of Missouri-Columbia, 1972: p. 63–87.
- Sener A and Malaisse WJ. L-leucine and a nonmetabolized analogue activate pancreatic islet glutamate dehydrogenase. *Nature* 1980, 288: 187–189.
- De Leon DD and Stanley CA. Mechanisms of disease: advances in diagnosis and treatment of hyperinsulinism in neonates. *Nat Clin Pract Endocrinol Metab* 2007, 3: 57–68.
- Hsu BY, Kelly A, Thornton PS, Greenberg CR, Dilling LA and Stanley CA. Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr* 2001, 138: 383–389.
- Kelly A, Ng D, Ferry RJ Jr, Grimberg A, Koo-McCoy S, Thornton PS and Stanley CA. Acute insulin responses to leucine in children with the hyperinsulinism/hyperammonemia syndrome. *J Clin Endocrinol Metab* 2001, 86: 3724–3728.
- Fourtner SH, Stanley CA and Kelly A. Protein-sensitive hypoglycemia without leucine sensitivity in hyperinsulinism caused by K(ATP) channel mutations. *J Pediatr* 2006, 149: 47–52.
- Kapoor RR, James C, Flanagan SE, Ellard S, Eaton S and Hussain K. 3-Hydroxyacyl-coenzyme A dehydrogenase deficiency and

- hyperinsulinemic hypoglycemia: characterization of a novel mutation and severe dietary protein sensitivity. *J Clin Endocrinol Metab* 2009, 94: 2221–2225.
- 16 Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ and Perlman K, *et al.* Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med* 1998, 338: 1352–1357.
 - 17 Fang J, Hsu BY, MacMullen CM, Poncz M, Smith TJ and Stanley CA. Expression, purification and characterization of human glutamate dehydrogenase (GDH) allosteric regulatory mutations. *Biochem J* 2002, 363: 81–87.
 - 18 Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 2000, 49: 1751–1760.
 - 19 Li C, Najafi H, Daikhin Y, Nissim IB, Collins HW, Yudkoff M and Matschinsky FM, *et al.* Regulation of leucine-stimulated insulin secretion and glutamine metabolism in isolated rat islets. *J Biol Chem* 2003, 278: 2853–2858.
 - 20 Li C, Matter A, Kelly A, Petty TJ, Najafi H, MacMullen C and Daikhin Y, *et al.* Effects of a GTP-insensitive mutation of glutamate dehydrogenase on insulin secretion in transgenic mice. *J Biol Chem* 2006, 281: 15064–15072.
 - 21 Li C, Li M, Chen P, Narayan S, Matschinsky FM, Bennett MJ and Stanley CA, *et al.* Green tea polyphenols control dysregulated glutamate dehydrogenase in transgenic mice by hijacking the ADP activation site. *J Biol Chem* 2011, 286: 34164–34174.
 - 22 Li C, Allen A, Kwagh J, Doliba NM, Qin W, Najafi H and Collins HW, *et al.* Green tea polyphenols modulate insulin secretion by inhibiting glutamate dehydrogenase. *J Biol Chem* 2006, 281: 10214–10221.
 - 23 Bahi-Buisson N, Roze E, Dionisi C, Escande F, Valayannopoulos V, Feillet F and Heinrichs C, *et al.* Neurological aspects of hyperinsulinism-hyperammonemia syndrome. *Dev Med Child Neurol* 2008, 50: 945–949.
 - 24 Li M, Smith CJ, Walker MT and Smith TJ. Novel inhibitors complexed with glutamate dehydrogenase: allosteric regulation by control of protein dynamics. *J Biol Chem* 2009, 284: 22988–23000.
 - 25 Hussain K, Clayton PT, Krywawych S, Chatziandreou I, Mills P, Ginbey DW and Geboers AJ, *et al.* Hyperinsulinism of infancy associated with a novel splice site mutation in the SCHAD gene. *J Pediatr* 2005, 146: 706–708.
 - 26 Molven A, Matre GE, Duran M, Wanders RJ, Rishaug U, Njolstad PR and Jellum E, *et al.* Familial hyperinsulinemic hypoglycemia caused by a defect in the SCHAD enzyme of mitochondrial fatty acid oxidation. *Diabetes* 2004, 53: 221–227.
 - 27 Stanley CA, Bennett MJ and Mayatepek E. Disorders of Mitochondrial Fatty Acid Oxidation and Related Metabolic Pathways. Heidelberg: Springer, 2006.
 - 28 Li C, Chen P, Palladino A, Narayan S, Russell LK, Sayed S and Xiong G, *et al.* Mechanism of hyperinsulinism in short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency involves activation of glutamate dehydrogenase. *J Biol Chem* 2010, 285: 31806–31818.
 - 29 Eaton S, Chatziandreou I, Krywawych S, Pen S, Clayton PT and Hussain K. Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with hyperinsulinism: a novel glucose-fatty acid cycle? *Biochem Soc Trans* 2003, 31: 1137–1139.
 - 30 Martens GA, Vervoort A, Van de Castele M, Stange G, Hellemans K, Van Thi HV and Schuit F, *et al.* Specificity in beta cell expression of L-3-hydroxyacyl-CoA dehydrogenase, short chain, and potential role in down-regulating insulin release. *J Biol Chem* 2007, 282: 21134–21144.
 - 31 Narayan SB, Master SR, Sireci AN, Bierl C, Stanley PE, Li C and Stanley CA, *et al.* Short-chain 3-hydroxyacyl-coenzyme A dehydrogenase associates with a protein super-complex integrating multiple metabolic pathways. *PLoS One* 2012, 7: e35048.
 - 32 Li C, Buettger C, Kwagh J, Matter A, Daikhin Y, Nissim IB and Collins HW, *et al.* A signaling role of glutamine in insulin secretion. *J Biol Chem* 2004, 279: 13393–13401.
 - 33 Li C, Nissim I, Chen P, Buettger C, Najafi H, Daikhin Y and Collins HW, *et al.* Elimination of KATP channels in mouse islets results in elevated [U-13C]glucose metabolism, glutaminolysis, and pyruvate cycling but a decreased gamma-aminobutyric acid shunt. *J Biol Chem* 2008, 283: 17238–17249.
 - 34 De Leon DD, Li C, Delson MI, Matschinsky FM, Stanley CA and Stoffers DA. Exendin-(9–39) corrects fasting hypoglycemia in SUR-1^{-/-} mice by lowering cAMP in pancreatic beta-cells and inhibiting insulin secretion. *J Biol Chem* 2008, 283: 25786–25793.
 - 35 Calabria AC, Li C, Gallagher PR, Stanley CA and De Leon DD. GLP-1 receptor antagonist Exendin-(9–39) elevates fasting blood glucose levels in congenital hyperinsulinism owing to inactivating mutations in the ATP-sensitive K⁺ channel. *Diabetes* 2012, 61: 2585–2591.
 - 36 Rendell M. The role of sulphonylureas in the management of type 2 diabetes mellitus. *Drugs* 2004, 64: 1339–1358.
 - 37 Remedi MS and Nichols CG. Chronic antidiabetic sulfonylureas in vivo: reversible effects on mouse pancreatic beta-cells. *PLoS Med* 2008, 5: e206.
 - 38 Doliba NM, Qin W, Vatamaniuk MZ, Li C, Zelent D, Najafi H and Buettger CW, *et al.* Restitution of defective glucose-stimulated insulin release of sulfonylurea type 1 receptor knockout mice by acetylcholine. *Am J Physiol Endocrinol Metab* 2004, 286: E834–843.
 - 39 Sekine N, Cirulli V, Regazzi R, Brown LJ, Gine E, Tamarit-Rodriguez J and Girotti M, *et al.* Low lactate dehydrogenase and high mitochondrial glycerol phosphate dehydrogenase in pancreatic beta-cells. Potential role in nutrient sensing. *J Biol Chem* 1994, 269: 4895–4902.
 - 40 Ainscow EK, Zhao C and Rutter GA. Acute overexpression of lactate dehydrogenase-A perturbs beta-cell mitochondrial metabolism and insulin secretion. *Diabetes* 2000, 49: 1149–1155.
 - 41 Quintens R, Hendrickx N, Lemaire K and Schuit F. Why expression of some genes is disallowed in beta-cells. *Biochem Soc Trans* 2008, 36: 300–305.
 - 42 Otonkoski T, Jiao H, Kaminen-Ahola N, Tapia-Paez I, Ullah MS, Parton LE and Schuit F, *et al.* Physical exercise-induced hypoglycemia caused by failed silencing of monocarboxylate transporter 1 in pancreatic beta cells. *Am J Hum Genet* 2007, 81: 467–474.
 - 43 Lu D, Mulder H, Zhao P, Burgess SC, Jensen MV, Kamzolova S and Newgard CB, *et al.* 13C NMR isotopomer analysis reveals a connection between pyruvate cycling and glucose-stimulated insulin secretion (GSIS). *Proc Natl Acad Sci USA* 2002, 99: 2708–2713.
 - 44 Newgard CB, Lu D, Jensen MV, Schissler J, Boucher A, Burgess S and Sherry AD. Stimulus/secretion coupling factors in glucose-stimulated insulin secretion: insights gained from a multidisciplinary approach. *Diabetes* 2002, 51(Suppl 3): S389–393.
 - 45 Xu J, Han J, Long YS, Lock J, Weir GC, Epstein PN and Liu YQ. Malic enzyme is present in mouse islets and modulates insulin secretion. *Diabetologia* 2008, 51: 2281–2289.
 - 46 MacDonald MJ, Longacre MJ and Kendrick MA. Mitochondrial malic enzyme (ME2) in pancreatic islets of the human, rat and mouse and clonal insulinoma cells. *Arch Biochem Biophys* 2009, 488: 100–104.
 - 47 Xu G, Kwon G, Cruz WS, Marshall CA and McDaniel ML. Metabolic regulation by leucine of translation initiation through the mTOR-signaling pathway by pancreatic beta-cells. *Diabetes* 2001, 50: 353–360.
 - 48 Gleason CE, Lu D, Witters LA, Newgard CB and Birnbaum MJ. The role of AMPK and mTOR in nutrient sensing in pancreatic beta-cells. *J Biol Chem* 2007, 282: 10341–10351.
 - 49 Yang J, Dolinger M, Ritaccio G, Mazurkiewicz J, Conti D, Zhu X and Huang Y. Leucine stimulates insulin secretion via down-regulation of surface expression of adrenergic alpha2A receptor through the

- mTOR (mammalian target of rapamycin) pathway: implication in new-onset diabetes in renal transplantation. *J Biol Chem* 2012, 287: 24795–24806.
- 50 Newsholme P, Brennan L, Rubi B and Maechler P. New insights into amino acid metabolism, beta-cell function and diabetes. *Clin Sci (Lond)* 2005, 108: 185–194.
- 51 Yang J, Chi Y, Burkhardt BR, Guan Y and Wolf BA. Leucine metabolism in regulation of insulin secretion from pancreatic beta cells. *Nutr Rev* 2010, 68: 270–279.
- 52 van Loon LJ, Kruijshoop M, Menheere PP, Wagenmakers AJ, Saris WH and Keizer HA. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. *Diabetes Care* 2003, 26: 625–630.