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Nutrient sensing in pancreatic islets: lessons from congenital hyperinsulinism and monogenic diabetes

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Abstract

Pancreatic beta cells sense changes in nutrients during the cycles of fasting and feeding and release insulin accordingly to maintain glucose homeostasis. Abnormal beta cell nutrient sensing resulting from gene mutations leads to hypoglycemia or diabetes. Glucokinase (GCK) plays a key role in beta cell glucose sensing. As one form of congenital hyperinsulinism (CHI), activating mutations of GCK result in a decreased threshold for glucose-stimulated insulin secretion (GSIS) and hypoglycemia. In contrast, inactivating mutations of GCK result in diabetes, including a mild form (MODY2) and a severe form (permanent neonatal diabetes mellitus, PNDM). Mutations of beta cell ion channels involved in insulin secretion regulation also alter glucose sensing. Activating or inactivating mutations of ATP-dependent potassium (K_{ATP}) channels results in severe but completely opposite clinical phenotypes, including PNDM and CHI. Mutations of the other ion channels, including voltage-gated potassium channels ($K_v7.1$) and voltage-gated calcium channels, also lead to abnormal glucose sensing and CHI. Furthermore, amino acids can stimulate insulin secretion in a glucose-independent manner in some forms of CHI, including activating mutations of glutamate dehydrogenase, HDAH deficiency, and inactivating mutations of K_{ATP} channels. These genetic defects have provided insight into a better understanding of the complicated nature of beta cell fuel-sensing mechanisms.

Keywords

beta cell; insulin secretion; congenital hyperinsulinism; monogenic diabetes

Introduction

Pancreatic beta cells sense changes in nutrients during the cycles of fasting and feeding and release insulin accordingly to maintain glucose homeostasis. These nutrients, also known as

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Competing interests

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macronutrients, include glucose, amino acids and fatty acids. The processes of glucose-stimulated insulin secretion (GSIS) start with glucose transport and glucose phosphorylation and subsequently flux through glycolytic pathways and mitochondrial oxidation. The production of ATP, or the increased ratio of ATP/ADP, leads to closure of ATP-sensitive potassium (K_{ATP}) channels and elevation of beta cell membrane potential. Membrane potential, when elevated to about -40 mV, triggers voltage-dependent calcium channels^{1–3}. High intracellular calcium activates certain pathways, including protein kinase A and C, finally leading to exocytosis of insulin granules, which is generally accepted as the triggering pathway of insulin secretion⁴. During glucose oxidation, metabolic coupling factors are generated, including cAMP, ATP, diacylglycerol, inositol trisphosphate (IP3), alpha-ketoglutarate, glutamate, glutamine, NAD(P)H, malonyl-CoA, GTP, citrate, and malonyl-CoA.^{3–11} These metabolic coupling factors potentiate the action of calcium on insulin secretion which has been termed the *amplification pathway* of insulin secretion⁴. Numerous efforts have been conducted in the past trying to explore the mechanisms involved in the process of insulin secretion regulation. This review tries to summarize the current understanding of nutrient or fuel sensing in beta cells, mainly focusing on abnormal insulin secretion occurring in the human monogenic disorders of diabetes and hypoglycemia. We attempt to focus on the concept of *islet nutrient sensing* as a functional consequence of insulin secretion, rather than discussing the role of nutrient sensing on the regulation of gene transcription in this review, including genes of insulin (INS), Pancreas/duodenum homeobox protein 1 (PDX1), Wolframin ER transmembrane glycoprotein (WFS1), etc., since those topics have been intensively discussed^{12–14}.

Congenital hyperinsulinism and monogenic diabetes

Congenital hyperinsulinism (CHI), the most common cause of permanent disorders of hypoglycemia in children, results from genetic mutations in the key steps of insulin secretion^{15–17}. This disease is in the orphan disease category and is estimated to affect 1 in 30,000 to 40,000 live births, but the prevalence of CHI may be as high as 1 in 2,500 in communities where significant consanguinity exists^{15, 17}. Currently, 11 gene loci have been identified to cause CHI^{16, 17}. Diazoxide, a K_{ATP} channel agonist, has served as the primary drug to treat CHI by inhibiting insulin secretion¹⁸. However, diazoxide is ineffective in most cases of CHI with mutations of K_{ATP} channels^{16, 17}.

Like hypoglycemia in CHI, hyperglycemia in monogenic diabetes, including maturity-onset diabetes of the young (MODY) and permanent neonatal diabetes mellitus (PNDM), is also a result of genetic mutations. Sometimes, inactivating or activating mutations of the same gene can lead to either CHI or monogenic diabetes.

Glucose sensing

The primary function of the pancreatic beta cell is to sense changes in blood glucose levels and release insulin. Therefore, beta cell glucose sensing and subsequent insulin secretion are tightly coupled with blood glucose levels. Hyperinsulinemic hypoglycemia and hyperglycemia due to imbalanced insulin secretion and insulin sensitivity are normally caused by an impaired or broken link between blood glucose and beta cell glucose sensing

and the subsequent insulin secretion regulation. Studies of CHI and monogenic diabetes have provided pathophysiological examples to support the concept that beta cells play a central role in maintaining glucose homeostasis.

Glucose sensing and the threshold of GSIS in isolated islets

Maintaining inter-prandial blood glucose levels at around 5 mM in individuals with normal insulin sensitivity is critical for general health. The threshold or set point of GSIS in pancreatic islets is, therefore, around 5 mM¹⁹. Increased sensitivity to glucose leads to a lower threshold of GSIS and results in hypoglycemia, while decreased sensitivity leads to hyperglycemia. Although the threshold of GSIS is not solely determined by beta cell glucokinase (GCK), the concept of GCK as a master regulator or glucose sensor of GSIS is widely accepted^{3, 19}. Figure 1 shows glucose-ramp stimulated insulin secretion in isolated and cultured mouse, rat and human islets. Despite species differences, the glucose threshold is remarkably similar. The advantage of studying insulin secretion in isolated islets by a ramp protocol is that the ramp stimulation will help us determine the threshold as well as the maximum insulin secretion response to gradually increased concentrations of glucose.

Glucose metabolism in normal human islets

Investigating glucose metabolism in human islets will help us establish the foundation for understanding glucose sensing and regulation of insulin secretion, especially in providing biochemical bases to explain the pathophysiological abnormalities in CHI and diabetes. There are several ways to evaluate glucose metabolism in isolated islets, including measurement of the production of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]\text{glucose}$, measurement of glucose usage determined by the production of tritiated water from $[\text{5-}^3\text{H}]\text{glucose}$, and metabolomic approaches using stable isotope tracing and gas chromatography–mass spectrometry (GC/MS) or nuclear magnetic resonance spectroscopy (NMR) to determine the intermediate metabolites of glucose oxidation^{20–23}. In addition, oxygen consumption rates (OCR) in perfused islets can be measured to directly evaluate not only the dynamics of glucose oxidation and mitochondrial function but also the correlation of these dynamics with insulin secretion^{24–26}. Measurement of OCR in isolated human islets from non-diabetic organ donors using the phosphorescence quenching method showed that OCR increased as a function of the stepwise rise of glucose concentrations²⁶. The glucose threshold of the significant increase of OCR was about 4 to 5 mM, similar to the insulin secretion threshold obtained by glucose-ramp stimulated insulin secretion in perfused human islets (Fig. 1). ATP production can be calculated based on measurements of OCR, glucose usage, and glucose oxidation. Insulin secretion correlated with ATP production and the correlation was fitted to a sigmoidal curve²⁶. Interestingly, the threshold of insulin secretion and half-maximum of ATP production of human islets was very similar with those for mouse and rat islets²⁶. These data provide a plausible explanation for the similar thresholds for GSIS in mouse, rat, and human islets shown in Figure 1, since the effective rate of ATP production in mouse, rat, and human islets is very similar.

GCK as a master regulator of GSIS

The initial step of glucose metabolism is ATP-dependent phosphorylation, which produces glucose-6-phosphate in a reaction catalyzed by hexokinases (HK). GCK, or HK 4, is one of

those HKs. The protein structure and enzyme kinetics of HK 1, 2, 3 and 4 (GCK) are quite different²⁷. The HKs (1 to 3) have a high affinity for glucose (submillimolar K_m) binding and are sensitive to end-product feedback inhibition by glucose-6-phosphate, in contrast to GCK's low glucose affinity and lack of significant end-product feedback inhibition^{19, 28, 29}. Past studies have firmly established the concept that GCK is probably the only hexokinase expressed in mature beta cells^{19, 30–32}. From an evolutionary point of view, why GCK, but not the other low K_m HKs, governs glucose phosphorylation in beta cells is an interesting question. The advantage of having exclusive GCK expression in beta cells is clear: the threshold for GSIS governed by GCK increases to around 5 mM, ensuring that glucose levels are maintained within their physiological range. However, recent studies have shown that HKs, especially HK1, are expressed in beta cells in the neonatal period^{31, 33}. The expression of HK1 is switched off after birth in the process of beta cell maturation. HK1 gene expression, therefore, is downregulated in mature beta cells^{31, 33}. If HK1 were present in mature beta cells, one would expect the threshold for GSIS to be lower. A transgenic mouse model with a beta cell over-expression of HK1 has provided evidence to support this hypothesis, as model demonstrated a hypoglycemia phenotype and a low threshold of GSIS³⁴. The recent discovery of HK1-associated CHI has provided additional evidence to support the concept that HK1 expression is normally absent in mature beta cells.

HK1-CHI

A dominant form of diazoxide-responsive CHI has recently been mapped to the *HK1* locus³⁵. In general, if presenting early in infancy, resulting hypoglycemia is mild and responds to diazoxide therapy. However, hypoglycemia in some cases is severe enough to cause seizures and permanent brain damage. In affected individuals, three novel variants have been identified in noncoding regions of *HK1* that might potentially interfere with the normal suppression of *HK1* expression in beta cells after birth. Patients carrying these *HK1* variants have hypoglycemia during prolonged fasting, as shown by the fall of glucose levels to around 3 mM. During prolonged fasting, islets still secrete significant amounts of insulin, leading to the suppression of ketone body production and hypoglycemia. Administration of oral glucose tolerance tests (OGTT) resulted in a similar phenomenon, including the over-secretion of insulin in response to the glucose challenge and failure to suppress insulin secretion when glucose was decreased. These CHI cases suggest that abnormal expression of HK1 in beta cells leads to a lower glucose threshold for GSIS, similar to gain-of-function mutations of GCK.

Activating mutations of GCK and hypoglycemia

Additional evidence to support the concept that GCK is a master regulator for glucose sensing and GSIS comes from human diseases associated with either activating or inactivating mutations of GCK. The activating mutations of GCK result in hypoglycemia phenotypes, the clinical presentations of which vary from severe neonatal hypoglycemia to mild hypoglycemia only recognized later in life^{36–38}. The enzyme kinetics of the activating GCK mutant protein showed a left-shift of the glucose dose dependency curve with reduced glucose $S_{0.5}$. ATP K_m was similar to that of wild-type proteins and the calculated activity index was increased^{36, 38}. Clinical investigation has confirmed the role of GCK activating mutations in the regulation of insulin secretion. GCK-CHI patients show typical features of

hyperinsulinism, including abnormal insulin secretion, suppressed ketone production during fasting, and an inappropriate positive response to glucagon-stimulated blood glucose elevation after prolonged fasting.^{17, 36, 38–40} A mouse disease model has been generated to express one of the GCK activating mutants (A456V)⁴¹. As we expected, the threshold for glucose ramp-stimulated insulin secretion in isolated islets was left-shifted in heterozygous mouse islets, and even more left-shifted for islets isolated from the homozygous A456V GCK mutation⁴¹.

Since diazoxide inhibits insulin secretion downstream of GCK by opening K_{ATP} channels, it would be logical to inhibit insulin secretion and control hypoglycemia in GCK-CHI patients. However, diazoxide has not been very effective in many GCK-CHI patients^{36, 38}. Some patients with severe hypoglycemia and no response to diazoxide therapy required near-total pancreatectomy⁴². The plausible explanation for the ineffective response to diazoxide is that activating GCK mutations will override any effort to raise blood glucose above the threshold or the set-point for GSIS. The concept of GCK activation in lowering the threshold for GSIS and improving insulin secretion has guided drug discovery for type 2 diabetes.

Inactivating mutations of GCK and diabetes

Inactivating mutations of GCK, a form of monogenic diabetes also called MODY2, leads to a right-shift of the GSIS threshold⁴³. The set point for GSIS in MODY2 is higher than normal. Several hundred GCK mutations have been identified to cause diabetes^{44, 45}. Enzyme kinetics studies of those mutant GCK showed increased $S_{0.5}$ for glucose when the mutations were located in glucose binding sites and increased K_m for ATP for ATP binding site mutations. Some mutations led to unstable GCK proteins, while some mutations only showed decreased K_{cat} , etc.⁴⁶. Considering all the factors involved in enzyme kinetics, including $S_{0.5}$ for glucose, K_m for ATP, the Hill coefficient for glucose, and K_{cat} for enzyme turnover number, the calculated GCK activity index in MODY2 GCK inactivating mutations was lower than normal^{19, 46}. In patients with inactivating GCK mutations, a key and common clinical feature is a lifelong mild fasting hyperglycemia, with glucose levels normally ranging from 5.5 to 8 mmol/L^{43, 45}. Despite the wide range of differences in the enzyme kinetics of mutant GCK proteins, the clinical presentation among those MODY2 patients is very similar. However, if patients have almost completely inactive GCK mutations, normally homozygous or compound heterozygous forms of the inactivating mutations will result in permanent neonatal diabetes mellitus (PNDM)⁴⁷. GCK-associated PNDM causes severe hyperglycemia occurring within the first month of life. These patients require life-long insulin therapy to control hyperglycemia. GCK-associated PNDM is rare; at least a limited number of cases have been reported^{47–49}. Enzyme kinetics of mutant GCK from PNDM were either very low or indicative of unstable proteins, suggesting that glucose phosphorylation was markedly reduced or completely blocked in beta cells⁴⁴.

Ion channels mutations alter glucose sensing

As discussed above, GCK and glucose-phosphorylation play very important roles in determining the threshold of GSIS. However, studies have shown that pathways downstream of GCK can alter glucose sensing, especially in beta cells with abnormal cell membrane ion

channels. Insulin release is finely regulated by the electrical excitability of beta cells encompassing multiple ion channels, such as K_{ATP} and voltage-dependent Ca^{2+} channels. Figure 3 shows the expression of different K^+ , Cl^- , and Ca^{2+} channel genes, as well as Na^+ channel or transporter genes associated with Na^+ trafficking in purified human beta cells⁵⁰. Several channels have been shown to associate with monogenetic diabetes or CHI, including K_{ATP} , voltage-gated potassium channels ($K_v7.1$), and voltage-dependent Ca^{2+} channels.

Glucose “blindness” in K_{ATP} -CHI

Mutations of sulfonylurea receptor 1 (SUR1) and potassium inward rectifier 6.2 (Kir6.2), the two adjacent subunits that comprise the beta cell membrane K_{ATP} channels, are responsible for the most common form of CHI in children^{15–17}. Many studies have shown that K_{ATP} -CHI patients have decreased insulin responses to oral and intravenous glucose challenges^{51–54}. SUR1 knockout mice demonstrated a similar phenomenon that is likely due to the impaired response to GSIS in perfused islets⁵⁵. As shown in Figure 4, islets isolated from SUR1 gene (*Abcc8*) knockout mice presented a higher basal insulin secretion and impaired GSIS. Chronic treatment of wildtype mice with sulfonylureas also resulted in glucose “blindness”^{56, 57}, similar to *Abcc8* knockout mice^{5, 58, 59}. Based on those data, we can postulate that the chronic beta cell depolarization and elevation of cytosolic calcium, as consequences of the closure of K_{ATP} channels by either knockout of *Abcc8* or sulfonylureas administration, will affect glucose metabolism and lead to impaired GSIS or glucose blindness. In order to test this hypothesis, a metabolomic approach was used to trace ^{13}C flux from [U- ^{13}C]glucose to amino acids in islets isolated from SUR1 knockout and wild-type mice⁵⁹. Similar to ^{13}C fluxes from [U- ^{13}C]glucose in human islets, ^{13}C enrichments of amino acids in mouse islets were detected in alanine, aspartate, glutamate, GABA, and glutamine, indicating the incorporation of glucose carbon into those amino acids^{22, 59}. Compared to wild-type islets, *Abcc8* knockout islets showed alteration of metabolic pathways of [U- ^{13}C]glucose oxidation⁵⁹. The altered glucose metabolism in *Abcc8* knockout islets may contribute to the mechanism causing impaired glucose sensing or glucose blindness. Glucose blindness was also observed in islets isolated from the pancreases of children with inactivating mutations of K_{ATP} channels who required pancreatectomy⁶⁰.

K_{ATP} -PNDM

Similar to GCK activating or inactivating mutations which have completely opposite clinical phenotypes, i.e. hypoglycemia (CHI) or hyperglycemia (MODY2 and PNDM), inactivating or activating mutations of K_{ATP} channels also have opposite clinical consequences. Activating mutations in the gene *KCNJ11*, which encodes the Kir6.2 subunit of the K_{ATP} channels in pancreatic beta cells, were found to be an important cause of PNDM⁶¹. Because the mutated K_{ATP} channels have increased the threshold for ATP to close the channel, the normal range of glucose cannot produce sufficient ATP to reach the threshold for the mutant K_{ATP} channels to function. Therefore, the beta cell membrane is constantly hyperpolarized and GSIS does not occur. All patients with K_{ATP} -PNDM have been diagnosed before six months of age, with marked hyperglycemia, ketoacidosis, and undetectable C-peptide^{61, 62}. The functional studies of the channel mutations associated with PNDM showed impairment of ATP-stimulated channel closure. Some mutations are directly located in the ATP binding

site in Kir6.2 subunit. Some are not close to the ATP-binding site but still interfere with ATP binding, channel kinetics or gating^{61, 63–65}. Since the SUR1 subunit of K_{ATP} is normal, sulfonylurea drugs can be a useful treatment to replace insulin therapy. Indeed, sulfonylurea therapy has been used very successfully in the treatment of PNDM patients. Many patients have successfully switched from insulin to sulfonylurea therapy⁶⁵.

Long-QT syndrome and CHI

The gene *KCNQ1* encodes the pore-forming alpha subunit of a voltage-gated potassium channel (K_v7.1), which enables a K⁺ current after electrical depolarization of the cell membrane^{66, 67}. *KCNQ1* is predominantly expressed in the myocardium, inner ear, stomach, intestine, and pancreas. *KCNQ1* is critical for ion homeostasis in these tissues, as demonstrated by the phenotypes of individuals harboring mutations in this gene, which include long QT syndrome, deafness, and gastrointestinal defects^{68, 69}. Interestingly, a recent report presented evidence of hyperinsulinemic hypoglycemia in individuals with long QT syndrome caused by mutations in *KCNQ1*⁷⁰. In these individuals, GSIS was increased during OGTT and plasma glucose was lower at the end of a prolonged glucose tolerance test. Continuous glucose monitoring demonstrated that hypoglycemia episodes occurred 3–5 h after meals. In addition, *Kcnq1* knockout mice showed a similar phenomenon. Compared to wildtype control mice, *Kcnq1* knockout mice have improved glucose tolerance⁷¹. The *Kcnq1* knockout mice, however, also showed increased insulin sensitivity, which may also have contributed to the hypoglycemia phenotype⁷¹. In addition, the variants in K_v7.1 have been associated with an increased risk for type 2 diabetes⁷². Although the mechanism underlining the involvement of K_v7.1 in the regulation of glucose homeostasis is not entirely clear, the evidence suggests that K_v7.1 may play a role in the regulation of insulin secretion by controlling plasma membrane repolarization.

Beckwith-Wiedemann syndrome-CHI

Beckwith-Wiedemann syndrome (BWS) is a growth regulation disorder manifesting as somatic overgrowth, congenital malformations, and tumor predisposition. BWS is associated with genetic or epigenetic abnormalities (i.e., paternal uniparental disomy, UPD) in a cluster of imprinted genes on human chromosome 11p15. Fifty percent of children with BWS have neonatal hypoglycemia due to hyperinsulinism. In most cases of BWS, the hyperinsulinism is usually transient and responsive to diazoxide, resolving within the first few months. However, 5% of BWS patients have persistent hyperinsulinism that does not respond to diazoxide and may require pancreatectomy^{73, 74}. Pancreas tissue sections from those severely hypoglycemic BWS patients demonstrated a range in the degree of islet expansion across the entire pancreas. The islet cell expansion was accompanied by a corresponding decrease in nuclear *CDKN1C* (P57) expression. It is possible that the HI is related to a combination of islet overgrowth due to the overexpression of *IGF2* and the under-expression of *H19* and *CDKN1C*, together with a lack of expression of the known maternally-imprinted *KCNQ1*. *KCNQ1* is located in the same imprinted region of 11p15. *KCNQ1* has been suggested to be exclusively expressed from the maternal allele in human fetal islets. Therefore, UPD in BWS is likely to result in the impaired expression of *KCNQ1* during islet development in the neonatal period⁷⁵. We postulate that the mechanisms of hyperinsulinism

in BWS may similar to the long-QT syndrome associated hypoglycemia. The *KCNQ1* defects in BWS may be dramatically amplified by the over-growth of islet tissue⁷⁴.

Voltage-dependent calcium channel mutation and CHI

L-type voltage-dependent calcium channels (VDCC, gene: *CACNA1D*) play a key role in the process of insulin secretion. The activating mutation of VDCC (p.Gly403Asp) has been recently identified in a diazoxide-sensitive hyperinsulinism patient⁷⁶. In this case, severe hypoglycemia was recognized shortly after birth. Since the calcium channel functions in the pathways down-stream of the K_{ATP} channel, hypoglycemia of the patient can be controlled by diazoxide. This patient also has cardiac defects and neurological abnormalities, including bradycardia, mild aortic insufficiency, and seizures, suggesting that VDCC is also important for cardiac and neurological functions. While many of the patients with *CACNA1D* mutations have cardiac and neurological abnormalities, hyperinsulinism is not a common feature⁷⁷. This case may only reflect specific mutations that can cause HI. The mechanism of VDCC mutations causing HI is still unclear. We can speculate that certain VDCC mutations lower the beta cell voltage sensing threshold closer to the resting membrane potential, leading to the hypersecretion of insulin, even during fasting.

Other forms of CHI alter glucose sensing

Recently, several novel forms of CHI have been identified, including inactivating mutations of uncoupling protein 2 (UCP2) and inactivating mutations of phosphoglucosmutase 1 (PGM1)¹⁶. Patients carrying the inactivating mutations of UCP2 were diazoxide responsive⁷⁸. Hypoglycemia normally occurred after 24 hours of fasting in these patients, with suppressed ketone production. Some patients showed symptomatic hypoglycemia after OGTT, indicating a delayed turning-off of insulin secretion after the glucose tolerance test. Interestingly, some patients showed an unusual oscillation of glucose and insulin following an oral glucose challenge. The role of UCP2 in the regulation of glucose sensing and insulin secretion is still unclear. However, a recent study has hypothesized that UCP2 acts not to uncouple mitochondria, like UCP1 does, but rather to function as a carrier of four-carbon TCA cycle metabolic intermediates⁷⁹. The inactivating mutation of UCP2 may enhance glucose oxidation and increase GSIS in beta cells.

Inactivating mutations of phosphoglucosmutase 1 (PGM1) may also lead to enhanced glucose oxidation in beta cells and abnormal insulin secretion^{16, 17}. The PGM1 inactivating mutation is one of the causes of glycogen storage diseases. PGM1 catalyzes the reaction transferring the phosphate between glucose-6-phosphate and glucose-1-phosphate and is an important step for the formation and degradation of glycogen. Hypoglycemia is a common clinical feature in patients with inactivating mutations of PGM1, as exaggerated insulin secretion after glucose challenges often result in postprandial hypoglycemia^{80, 81}. The clinical studies of UCP2 and PGM1 associated CHI highlight the complexity of fuel sensing and insulin secretion regulation.

The beta cell glucose sensing and related diseases are illustrated in Figure 5. The cyclical changes of GCK and K_{ATP} result in completely opposite clinical presentations, indicating that those genes are critical for beta cell glucose sensing.

Amino acid sensing in pancreatic beta cells

Although, the role of amino acids in the regulation of insulin secretion, or amino acid sensing in beta cells, has been intensively studied in the past, the clinical and basic research has mainly focused on glucose or fatty acids as the essential determinants of insulin secretion regulation. However, a number of studies have suggested that the intake of amino acids can increase insulin secretion in both normal and diabetic subjects^{82–84}. Maximizing endogenous insulin secretion by combined ingestion of carbohydrates and amino acids/proteins can be significant for type 2 diabetes patients because amino acid or protein hydrolysate mixtures can improve insulin secretion. Those studies have suggested that the action of an amino acid in the regulation of insulin secretion is glucose-dependent; however, amino acid-stimulated insulin secretion (AASIS) can also occur in a glucose-independent manner, especially under pathophysiological conditions.

Amino acid-potentiated GSIS and glucose-dependent amino acid sensing

Experiments with perfused rat pancreas in the early 1970s confirmed that many individual amino acids could stimulate insulin release, but most were able to do so only in the presence of glucose⁸⁵. Besides complete mixtures of all 20 amino acids, certain individual amino acids including alanine, asparagine, glycine, glutamate, phenylalanine, and tryptophan can potentiate GSIS^{86–89}. As shown in Figure 6A, a ramp perfusion of a physiological mixture of 19 amino acids (AAM) strongly potentiated 10 mM glucose-stimulated insulin secretion in isolated wild-type mouse islets. Figure 6A also shows that AAM alone cannot stimulate insulin secretion in wild-type mouse islets. The role of amino acids in potentiating GSIS has also been observed in human studies. For example, when ingested with glucose gelatin strongly potentiates GSIS in type 2 diabetes patients⁸³. On a molar basis, glycine makes up ~30% of the total amino acids present in gelatin protein. When individuals received glycine instead of gelatin, it was shown that glycine also potentiates GSIS⁹⁰. Longterm administration of glycine significantly lowered A1C levels of type 2 diabetes patients⁹¹. Like glycine, many other individual amino acids, when orally ingested, also potentiated GSIS. Gannon *et al.* summarized the nutritional value of the ingested amino acid in the regulation of glucose metabolism⁹². Although the mechanisms for amino acid potentiation of GSIS are not fully understood, several studies have suggested that (1) amino acid mixtures can stimulate cAMP production in isolated islets^{55, 93}; (2) amino acids can activate the mammalian targets of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways^{94–96}; (3) amino acids can improve NADH mitochondrial shuttle activity^{97, 98}; and (4) amino acid transporters or receptors may be directly involved in the process of insulin secretion⁹⁹.

Glucose-independent amino acid sensing

The importance of amino acids as regulators of insulin secretion independent from glucose was first appreciated in the 1950s when familial protein-sensitive hypoglycemia was described^{100, 101}. Leucine is a notable exception to the rule that glucose is required for amino acid-stimulated insulin secretion, since the stimulatory effect of leucine in perfused rat pancreas is abolished in the presence of glucose¹⁰². On the other hand, leucine-induced hypoglycemia can be produced in normal individuals with lower glucose by the prior

administration of sulfonylureas^{86, 103}. Insulin release can also be stimulated by the non-metabolizable analog of leucine, 2-aminobicyclo-(2,2,1)heptane-2-carboxylic acid (BCH), indicating that, unlike glucose, the effect of leucine does not depend on its utilization as a substrate for oxidation. In the 1980s, Sener and Malaise¹⁰⁴ showed that leucine and BCH act to trigger insulin secretion by allosterically stimulating glutamate dehydrogenase (GDH) activity.

GDH-CHI and AASIS

In 1998, when mutations of GDH were identified in children with hyperinsulinism and hyperammonemia (HI/HA) syndrome, the mutations were characterized by a lack of sensitivity to GTP allosteric inhibition, which therefore led to gain of function¹⁰⁵. GDH catalyzes the reversible oxidative deamination of L-glutamate to α -ketoglutarate using NAD(P)⁺ as a coenzyme. GDH can be allosterically activated by ADP and leucine, and inhibited by GTP and ATP^{106–108}. GDH linked hyperinsulinism (GDH-CHI) patients are sensitive to protein feeding, as hyperinsulinemic hypoglycemia occurred after a protein rich meal or a mixture of amino acid load^{109, 110}. GDH-CHI also causes exaggerated acute insulin responses to intravenous leucine stimulation, though this response can be blocked by increasing blood glucose levels¹¹⁰. To further examine the role of GDH activating mutations in the regulation of insulin secretion and to confirm that the GDH mutation is disease-causing, transgenic mice with beta cell-specific expression of GDH gene mutation (resulting in H454Y) were generated; the GDH-transgenic mice (GDH-TG) showed a consistent hypoglycemia phenotype that reproduced the clinical hypoglycemia phenotype¹¹¹. An oral amino acid tolerance test showed that GDH-TG mice are sensitive to stimulation by physiological amino acid mixtures, reacting with rapid fall of blood glucose and increases of insulin secretion¹¹². This hypersensitivity to AASIS was also found in GDH-CHI patients¹⁰⁹. GDH enzyme kinetics in isolated islets from GDH-TG mice showed a right shift of the GTP inhibition curve, confirming the H454Y expression in islets. Insulin secretion in perfused islets isolated from GDH-TG mice was also sensitive to the amino acid mixture and leucine stimulation, even in response to glutamine alone, whereas control islets were unresponsive to either glutamine or a mixture of amino acids. This study confirmed that the GDH activating mutation is disease causing in GDH-CHI¹¹¹. To directly examine the effects of the GDH activating mutation on GDH metabolic flux, ¹⁵N flux was traced from [2-¹⁵N]glutamine to [2-¹⁵N]glutamate and subsequently to [¹⁵N]ammonia, which is specifically produced via GDH step. As we expected, islets from GDH-TG mice had higher insulin secretion and elevated GDH fluxes in response to [2-¹⁵N]glutamine stimulation. Such an effect can be inhibited by high glucose, likely via production of GTP during glucose oxidation. GTP serves as a major allosteric inhibitor of GDH regulation. Mitochondrial GTP is produced via the GTP-specific isoform of succinyl-CoA synthetase¹⁰.

GDH inhibitor: treatment of GDH-CHI

In 2006, we found that epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), polyphenols derived from green tea extracts, have strong inhibitory effects on GDH activity with nanomolar LD₅₀¹¹³. EGCG also strongly inhibits several mutant forms of GDH found in GDH-CHI patients, including H454Y^{112, 113}. Further studies showed that EGCG blocked the hypersensitivity to AASIS in islets isolated from GDH-TG mice. Proof-of-concept *in*

vivo experiments showed that oral administration of high-dose EGCG to GDH-TG mice elevated fasting glucose levels and inhibited amino acid–induced hypoglycemia¹¹². However, due to its low rate of absorption and high rate of protein binding, EGCG has limited bioavailability and bioactivity in an acceptable dose range^{114–116}, which makes it an unlikely clinical drug to treat GDH-CHI. Therefore, more potent GDH inhibitors are needed for successful treatment of CHI. An attempt at using a high throughput drug screening approach has identified about 20 new GDH inhibitors from a chemical library, as well as one GDH activator^{117, 118}. These findings suggest that the approach of identifying a small molecule that directly targets the mutant GDH to normalize the hyperactive enzyme is feasible. The findings also indicate that inhibition of activating mutations of GDH can block the abnormal amino acid sensing in GDH-CHI.

Fatty acid oxidation defect and AASIS

Children with 3-hydroxyacyl-Co A dehydrogenase (HADH; aka SCHAD) deficiency, or an inactivating mutation of the gene *HADH*, have an accumulation of certain fatty acid metabolites, like patients with other fatty acid oxidation defects. Besides the common features of fatty acid oxidation defects, patients with HADH deficiency also have hyperinsulinemic hypoglycemia^{119, 120}. HADH catalyzes the beta-oxidation cycle for medium and short-chain 3-hydroxy fatty acyl-CoAs. 3-Hydroxyacyl-Co A dehydrogenase gene (*Hadh*) knockout mice have the hypoglycemia phenotype and oral amino acids-induced hypoglycemia, similar to the protein-induced hypoglycemia seen in patients with HADH deficiency^{121, 122}. Islets isolated from *Hadh* knockout mice have increased AASIS and higher sensitivity to leucine-stimulated insulin secretion, which is similar to the islets phenotype observed in GDH transgenic mice. Indeed, GDH enzyme kinetics in *Hadh* knockout islets have reduced the affinity of GDH for alpha-ketoglutarate and increased enzyme efficiency, but GDH enzymes have a normal response to GTP inhibition. Further studies confirmed that HADH inhibits GDH via a protein-protein interaction mechanism. The findings suggest that GDH activation in HADH deficiency is due to the loss of direct protein-protein interaction between the two enzymes^{122–124}.

K_{ATP}-CHI and AASIS

As we have discussed, patients with inactivating mutations of K_{ATP} channel have impaired glucose tolerance, but have hypoglycemia induced not by leucine, but by amino acid mixtures¹²⁵. Similar to those clinical observations, islets from SUR1 knockout mice have an impaired response to leucine- or glucose-stimulated insulin release, but have increased insulin responses to a mixture of amino acids and glutamine stimulation^{5, 59}. The sensitivity to glutamine-stimulated insulin secretion in SUR1 knockout islets was not inhibited by 6-diazo-5-oxo-L-norleucine, a glutaminase inhibitor. Therefore, the action of glutamine is likely through downstream amplification pathways for insulin release, distal to the elevation of cytosolic calcium⁵. 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 in SUR1 gene knockout islets⁵⁵. A pilot clinical study in K_{ATP}-CHI patients showed that Exendin-(9–39) administration significantly increases fasting glucose levels⁹³. Studies of islets isolated from surgical specimens after pancreatectomy in K_{ATP}-CHI patients have suggested that human islets with inactivating

mutations of the K_{ATP} channel are indeed hypersensitive to AASIS and that Exendin-(9–39) blocks such effects^{60, 93}. Figure 6B shows the AAM ramp stimulated insulin secretion in perfused islets isolated from SCHAD knockout mice, SUR1 gene knockout mice, and GDH transgenic (*Glud1*) mice.

In summary, amino acid potentiation of GSIS is likely an important mechanism of insulin secretion after the ingestion of a mixed meal. In contrast, glucose-independent AASIS contributes to protein-induced hypoglycemia in 3 forms of CHI: inactivating mutations of K_{ATP} channel, HDAH deficiency and GDH activating mutations (Figure 7).

Fatty acid sensing

The roles of fatty acids (FAs) on insulin secretion regulation have been intensively studied in the past. The phenomenon of acute elevation of FA levels potentiating GSIS has been repeatedly observed^{126, 127}. In contrast, chronic elevation of blood fatty acid levels, particularly in association with increased glucose, leads to glucolipotoxicity, including reduced insulin biosynthesis, impaired insulin secretion, and increased β -cell apoptosis^{128–130}. Glucolipotoxicity has been proposed as one of the mechanisms causing beta cell function failure and mass reduction in type 2 diabetes³.

Many inborn errors of fatty acid oxidation are associated with hypoglycemia, though the cause is a failure of ketogenesis rather than inappropriate insulin secretion when blood glucose is low. As we have discussed, the clinical phenotype associated with deficiency of HADH is uniquely different. The mechanism of SCHAD-CHI is caused by increased amino acid oxidation rather than a direct result of defects of fatty acid oxidation^{122, 123}. Wild-type mice that received *Hadh* knockout islets transplantation also developed hypoglycemia, strongly supporting that SCHAD-CHI is not likely a result of defects of fatty acid oxidation¹³¹. Recently, a study showed that the acyl-CoA synthetase long-chain family member 5 (ACSL5), a key player in lipid biosynthesis and fatty acid degradation, is associated with the gene *TCF7L2*¹³², which was identified from genome-wide association studies and strongly associated with type 2 diabetes loci¹³³. However, monogenetic mutations in the key steps of fatty acid oxidation pathways which directly result in CHI or diabetes still need to be identified.

In the past decade, studies of FA sensing have been largely focused on G protein-coupled receptors (GPCR), including GPR40, GPR119, and GPR120. GPR40 activation by long-chain FAs amplifies GSIS by enhancing the effects of the intracellular second messenger, phospholipase C (PLC) and subsequently, Inositol trisphosphate (IP_3) and diacylglycerol¹³⁴. Activation of GPR119 by FAs also augments insulin release via cAMP pathways¹³⁵. GPR120 stimulated by FAs increases circulating insulin by an indirect mechanism that promotes the secretion of Glucagon-like peptide-1 (GLP-1)¹³⁶. GPR40, GPR120, and GPR119 have served as new drug targets for the treatment of type 2 diabetes; it has been reported that some small molecules targeting several GPCRs in beta cells are currently undergoing clinical evaluation,¹³⁷ for example, TAK-875 has entered phase III clinical trials as a small-molecule agonist of GPR40¹³⁸.

Concluding remarks

In summary, under physiological conditions, beta cells sense the changes in glucose, amino acids and fatty acids during the fasting and feeding cycle and release insulin accordingly to maintain glucose homeostasis. However, under pathophysiological conditions like congenital hyperinsulinism and monogenic diabetes, a single defect in the beta cell sensing pathways can result in profound clinical outcomes. Serving as experiments in nature, genetic human defects with CHI or monogenic diabetes have provided important information that is helping to dissect the complexity of beta cell fuel-sensing mechanisms. Genetically-modified mouse models have provided insights into the pathophysiology of CHI and monogenic diabetes. However, the phenotype of mouse models does not fully reproduce the human phenotype, suggesting that mechanisms of glucose homeostasis are not identical in rodents and humans^{5, 55, 59, 139, 140}. Furthermore, even in normal islets, studies have suggested differences in metabolic pathways between islets of humans and rodents¹⁴¹. Therefore, performing studies in human islets, especially in islets isolated from surgical specimens via pancreatectomy or from the pancreases of cadaver organ donors with different disease conditions, is critical for understanding the pathophysiology of human disease. For example, the hypoglycemia phenotype in mouse models with reduced or absent K_{ATP} channels is much milder than in humans with K_{ATP} -CHI. In addition, a progression from K_{ATP} -CHI to diabetes later in life has been described, even in individuals who have not undergone a pancreatectomy^{142, 143}. It has been proposed that increased rates of apoptosis, resulting from chronic elevation in Ca^{2+} , are responsible for progression to diabetes, though this remains to be proven^{144, 145}. It is likely that the mechanism causing CHI in early life to develop into diabetes in later life involves the effects of chronic derangements on fuel metabolism and fuel sensing⁶⁰. Thus, understanding these metabolic abnormalities may facilitate the prevention of diabetes in CHI patients in the long-term. The studies of CHI and monogenic diabetes have the potential to improve our understanding of the mechanisms mediating the triggering and the amplifying pathway of insulin secretion, which in turn, will be helpful for understanding the mechanisms implicated in the progressive beta cell failure that leads to type 2 diabetes. Thus, these studies may lead to the identification of novel targets for therapy not only for congenital hyperinsulinism but also for diabetes.

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Figure 1. Glucose-ramp stimulated insulin secretion in isolated islets

Rat, mouse and human islets were perifused with a glucose ramp (0 to 25 mM) stimulation with 0.625 mM/min increment. The threshold of glucose-stimulated insulin secretion is around 5 mM. N = 3 for each species, insulin was determined by homogeneous time resolved fluorescence assays. Data were adapted from Refs. 5, 22, 60, 122, 146.

Figure 2. Glucose metabolic pathways in beta cells

During glucose oxidation, different metabolic pathways are synchronized and integrated, ensuring that GSIS is tightly coupled with glucose oxidation. Glucose is first phosphorylated via glucokinase (GCK), then produce pyruvate through glycolysis. Pyruvate converts (1) to alanine via transamination (alanine aminotransferase, ALT); (2) to oxaloacetate (OAA) via pyruvate carboxylase (PC); (3) to Acetyl-CoA via pyruvate dehydrogenase (PDH). OAA and Acetyl-CoA enter tricarboxylic acid (TCA) cycle via citrate. Pyruvate cycling involves malate enzyme (ME) and PC. Aspartate switch represents the interexchange between OAA and aspartate via aspartate aminotransferase (AST). GABA shunt starts from glutamate decarboxylase (GAD) using glutamate as a substrate to produce GABA, GABA is then converted to succinate semialdehyde (SSA) via GABA transaminase (GABA-T) and subsequently to succinate via SSA dehydrogenase (SSADH). During glucose oxidation, phosphate-dependent glutaminase (PDG) and glutamate dehydrogenase (GDH) are inhibited by high phosphate potential, leads to inhibition of glutaminolysis. In contrast, glucose stimulates glutamine synthesis via glutamine synthetase (GS).

Figure 3. Gene expression of ion channels in human beta cells

Based on RNA sequence data obtained from purified human beta cells⁵⁰, numbers of potassium channels (A), chloride channels (B), calcium channels (C), and sodium channels or transporters associate with sodium trafficking (D) are significantly expressed. Data were adapted from previously published RNA sequences.⁵⁰

Figure 4. Glucose “blindness” in SUR1 gene (*Abcc8*) knockout mice

The glucose-ramp stimulated insulin secretion in isolated and cultured islets from *Abcc8* knockout and wild-type mice showed that *Abcc8* knockout islets have higher basal insulin secretion but blinded to GSIS. Data were adapted from previously Ref. 5.

Figure 5. Glucose sensing in congenital hyperinsulinism or monogenic diabetes

Genetic disorders, including congenital hyperinsulinism (CHI), maturity-onset diabetes of the young (MODY) and permanent neonatal diabetes mellitus (PNDM), lead to abnormal glucose sensing in beta cell. Mutations in key steps of glucose oxidation or ion channels involve insulin secretion regulation result increased or decreased insulin secretion.

Figure 6. Amino acids-stimulated insulin secretion in mouse islets

Panel A shows insulin secretion from isolated and cultured wildtype mouse islets perfusions. Islets were perfused with a ramp of amino acid mixture (AAM) (0 to 12 mM) with (black circles) or without (gray triangles) 10 mM glucose pre-exposure. AAM ramp alone failed to stimulate insulin secretion, however, AAM potentiates glucose-stimulated insulin secretion. Panel B shows glucose independent AAM-stimulated insulin secretion in islets isolated from wild-type mice (blue), *Hadh* knockout mice (purple), *Abcc8* knockout mice (red) and GDH transgenic mice (brown). Data were adapted from Refs. 5, 111, 122.

Figure 7. Amino acid sensing in congenital hyperinsulinism

Glucose-independent amino acid-stimulated insulin secretion or increased amino acid sensing in the beta cells lead to protein-induced hypoglycemia in 3 forms of congenital hyperinsulinism, including SCHAD deficiency, inactivating mutations of K_{ATP} channels and activating mutations of GDH.