Amino Acid Metabolism, β-Cell Function, and Diabetes

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Specific amino acids are known to acutely and chronically regulate insulin secretion from pancreatic β-cells in vivo and in vitro. Mitochondrial metabolism is crucial for the coupling of amino acid and glucose recognition to exocytosis of insulin granules. This is illustrated by in vitro and in vivo observations discussed in the present review. Mitochondria generate ATP, which is the main coupling messenger in insulin secretion, and other coupling factors, which serve as sensors for the control of the exocytotic process. Numerous studies have sought to identify the factors that mediate the key amplifying pathway over the Ca^{2+} signal in nutrient-stimulated insulin secretion. Predominantly, these factors are nucleotides (ATP, GTP, cAMP, and NADPH), although metabolites have also been proposed, such as long-chain acyl-CoA derivatives and glutamate. This scenario further highlights the importance of the key enzymes or transporters, e.g., glutamate dehydrogenase, the aspartate and alanine aminotransferases, and the malate-aspartate shuttle in the control of insulin secretion. In addition, after chronic exposure, amino acids may influence gene expression in the β-cell, which subsequently alters levels of insulin secretion. Therefore, amino acids may play a direct or indirect (via generation of putative messengers of mitochondrial origin) role in insulin secretion. *Diabetes* 55 (Suppl. 2):S39–S47, 2006

Amino acids can, under appropriate conditions, enhance insulin secretion from primary islet cells and β-cell lines (1–5). In vivo, L-glutamine and L-alanine are quantitatively the most abundant amino acids in the blood and extracellular fluids followed closely by the branched chain amino acids (6). However, unlike glucose, individual amino acids do not provoke insulin secretion in vitro when added at physiological concentrations. Combinations of amino acids at physiological concentrations or high concentrations of individual amino acids are much more effective. In vivo, amino acids derived from dietary proteins and those released from intestinal epithelial cells, in combination with glucose, stimulate insulin secretion, thereby leading to protein synthesis and amino acid transport in target tissues such as skeletal muscle (7). These effects occur independently of the well-characterized effects of insulin on GLUT4 translocation and glucose uptake and storage. In periods of fasting or starvation, amino acid release from skeletal muscle (primarily L-glutamine and L-alanine [8]) may modulate glucagon release from pancreatic α-cells, which subsequently may influence insulin secretion from β-cells. Dietary amino acids may also stimulate incretin release, e.g., GLP-1, from intestinal L-cells (9,10) and therefore stimulate insulin secretion via indirect mechanisms. The positive effect of administration of two amino acids to insulin secretion in vivo was reported in a recent clinical assessment of the effect of leucine and phenylalanine administered in the presence of a protein hydrolysate to type 2 diabetic patients and suitable control subjects, which resulted in a threefold increase in insulin secretion compared with carbohydrate alone (11). Because in vivo insulin secretion is normally determined by administration of an oral or intravenous glucose load, it is probable that in vivo insulin secretion measurements are an underestimate of that possible from a mixed nutritional load. Using in vitro mouse islet incubations with specific amino acid mixtures at physiological concentrations, insulin secretion was robustly stimulated (12). Four amino acids were found to be particularly important for stimulating β-cell electrical activity, essential for insulin secretion (leucine, isoleucine, alanine, and arginine).

Only a relatively small number of amino acids promote or synergistically enhance insulin release from pancreatic β-cells (13,14). The mechanisms by which amino acids enhance insulin secretion are varied. The cationically charged amino acid, L-arginine, does so by direct depolarization of the plasma membrane at neutral pH but only in the presence of glucose, whereas other amino acids, which are co-transported with Na^+, can also depolarize the cell membrane as a consequence of Na^+ transport and thus induce insulin secretion by activating voltage-dependent calcium channels. Metabolism, resulting in partial oxidation, e.g., L-alanine (3), may initially increase the cellular content of ATP, leading to closure of the ATP-sensitive K^+ (K_{ATP}) channel, depolarization of the plasma membrane, activation of the voltage-activated Ca^{2+} channel, Ca^{2+} influx, and insulin exocytosis. Additional mitochondrial signals may be generated that affect insulin secretion (15,16). A summary of potential regulatory mechanisms with respect to amino acid–stimulated insulin secretion is illustrated in Fig. 1.

NUTRIENT-INDUCED INSULIN SECRETION

In vivo, the β-cell is constantly monitoring nutrient availability and metabolic status and can generate appropriate secondary stimulus-coupling signals in response to the most minor changes in the concentration of specific metabolites. This is coupled with regulatory input from other signaling pathways, including the gut-derived incretins, vagal signals, and neuropeptides. The β-cell is metabolically distinct from almost all other mammalian cell types in several respects: *1*) it can use glucose in the

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Received for publication 21 March 2006 and accepted in revised form 11 May 2006.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

GABA, γ-amino butyric acid; GDH, glutamate dehydrogenase; K_{ATP} channel, ATP-sensitive K^+ channel; mTOR, mammalian target of rapamycin; TCA, tricarboxylic acid.

DOI: 10.2337/db06-S006

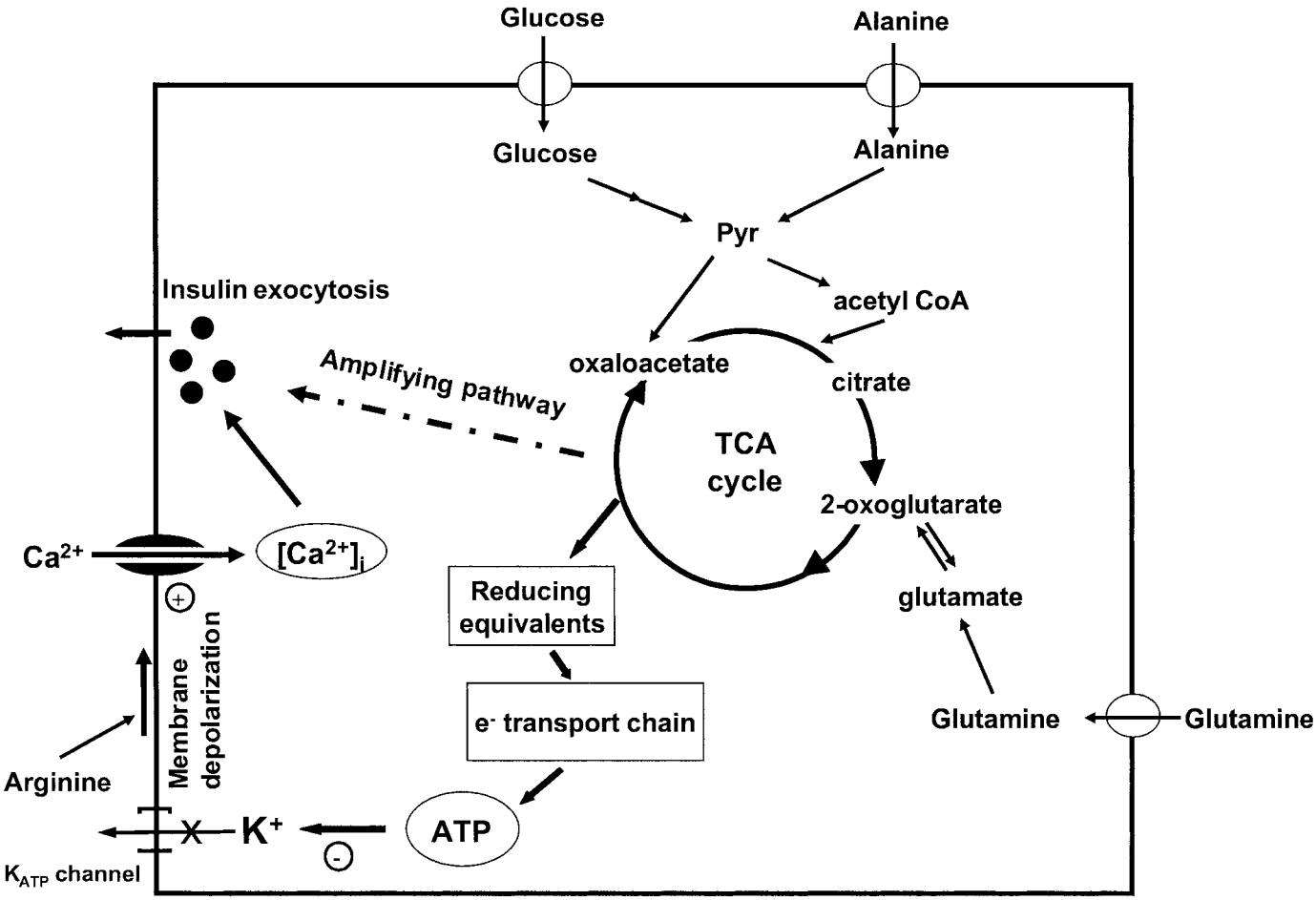

FIG. 1. Model for the regulation of insulin secretion in the β-cell stimulated by glucose and amino acids. Glucose equilibrates across the plasma membrane and is metabolized via glycolysis yielding pyruvate (Pyr), which is then metabolized further by the TCA cycle. Mitochondrial metabolism is also activated by amino acids such as alanine, glutamine, or glutamate. The TCA cycle generates reducing equivalents, which are transferred to the electron transport chain, leading to generation of ATP. The rise in ATP levels leads to closure of K_{ATP} channels and depolarizes the cell membrane. This opens voltage-dependent Ca^{2+} channels, increasing intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which triggers insulin exocytosis. Additional metabolic signals may be generated from TCA cycle intermediates, which "amplify" the initial Ca^{2+}-dependent stimulation for the exocytosis of insulin.

physiologically relevant range (2–20 mmol/l) as it expresses a combination of GLUT2 (high K_m glucose transporter) and glucokinase, *2*) low lactate dehydrogenase and plasma membrane mono-carboxylate pyruvate/lactate transporter activity and correspondingly high activity in the mitochondrial malate-aspartate shuttle so ensuring mitochondrial oxidation of NADH, and *3*) a high activity of both pyruvate dehydrogenase and pyruvate carboxylase, ensuring both anaplerotic and oxidative metabolism of glucose/pyruvate can coexist. All these specific metabolic adaptations are geared to enhancing mitochondrial tricarboxylic acid (TCA) cycle activity, oxidative phosphorylation, and efficient ATP production. An enhancement of the ATP-to-ADP ratio results in closure of the K_{ATP} channel, depolarization of the plasma membrane, opening of voltage-activated Ca^{2+} channels, influx of Ca^{2+}, and finally fusion of insulin-containing granules with the plasma membrane (17).

Lipid metabolism, via long-chain acyl-CoA formation, may also affect insulin secretion (18). Indeed, it is now recognized that citrate exported from the mitochondria to the cytosol is cleaved by ATP citrate lyase to generate oxaloacetate and acetyl-CoA, which subsequently forms malonyl-CoA in a reaction catalyzed by acetyl-CoA carboxylase, promoting fatty acid synthesis and accumulation of long-chain acyl-CoAs (19), thereby enhancing Ca^{2+}-evoked insulin exocytosis (18). Amino acids also play a role as modulators of lipid metabolism. Acetyl-CoA carboxylase, responsible for malonyl-CoA synthesis, is activated by glutamate-sensitive protein phosphatase type 2A (20), an effect demonstrated in islet β-cells (21). Acetyl-CoA carboxylase is also regulated by phosphorylation via AMP kinase, an enzyme sensitive to amino acid concentration (22). Recent work in our laboratory (see later gene expression section for details) has demonstrated that addition of 10 mmol/l L-alanine to the BRIN-BD11 β-cell line increased expression of ATP-citrate lyase by 2.0-fold. ATP citrate-lyase will convert citrate to acetyl-CoA in the cytosol, thus providing the key step in fatty acid synthesis, acetyl-CoA carboxylase, with substrate. In addition, we have also found that that addition of 10 mmol/l L-glutamine to BRIN-BD11 cells upregulated acetyl-CoA carboxylase expression at the mRNA and protein level (M. Corless, A. Kiely, N.H. McClenaghan, P.R. Flatt, P.N., unpublished data), thus stimulating fatty acid synthesis.

In the mitochondrial matrix, Ca^{2+} increases the activity of several dehydrogenases. In this manner, increased cytosolic Ca^{2+} occurring during cell activation is relayed to the mitochondria via a Ca^{2+} uniporter (23). Such Ca^{2+} entry is favored by activation of the respiratory chain, for

instance, by glucose in the β-cell. Therefore, hyperpolarization of the mitochondrial membrane permits the rise in mitochondrial Ca^{2+}, further activating NADH-generating dehydrogenases (24). The primary actions of glucose are mediated by potentiation of ATP concentration by enhanced TCA cycle substrate (oxidative and anaplerotic) supply. Generation of other additive factors derived from glucose metabolism might also be promoted by mitochondrial Ca^{2+} elevation (25).

Amino acids may acutely influence insulin secretion via a number of possible mechanisms, including generation of metabolic coupling factors, depolarization of the plasma membrane, or enhancement of mitochondrial function. These mechanisms are discussed in detail later in this review, but in the first instance, essential aspects of amino acid–dependent effects on signaling, gene expression, and metabolism will be covered.

SIGNALING ROLE OF AMINO ACIDS

Certain amino acids are now known to play important nutrient-sensing roles involving the mammalian target of rapamycin (mTOR)-mediated signaling pathway (26). mTOR is a component of a signaling pathway that couples insulin receptor stimulation and nutrient availability with protein synthesis via activation and phosphorylation of the ribosomal protein S6. Indeed, the mTOR pathway is an important regulator of cell size that coordinates the activity of the cell growth machinery with the levels of energy and nutrients. This is accomplished via activation of several downstream effectors including the 4E-BP1 (eukaryotic initiation factor 4E-binding protein) family of translational repressors and the protein kinases S6K1 and S6K2 (S6 kinases 1 and 2), which are sensitive to both mTOR and insulin signaling pathways. Leucine is the most effective amino acid in this regard. The activation of the mTOR pathway is likely to be important in the β-cell, where mTOR and growth factor/insulin signaling are likely to synergize so stimulating mitochondrial function, insulin secretion, and protein synthesis (27). Nutrients and cellular metabolism regulate mTOR effectors such as S6K1 through the interaction with the mTOR complex. In cells growing in nutrient-rich conditions, the mTOR kinase activity is high. In cells growing in nutrient-poor conditions, the mTOR kinase activity is low. It is not known how amino acids activate the mTOR complex, but it is probable that stimulation of a kinase or inhibition of a phosphatase that act upon mTOR as a substrate is involved (26–28).

Recent data has highlighted the importance of AMPK activity in the regulation of insulin secretion in pancreatic β-cells (29). Amino acids have been shown to be important regulators of AMPK activity, e.g., there was a marked reduction in AMPK activity on addition of the amino acids leucine, glutamine, and arginine (30). Indeed, AMPK activity and insulin secretion were inversely correlated for the amino acids investigated. It was proposed that metabolizable amino acids regulate AMPK via changes in the cytosolic ATP-to-AMP ratio and phosphorylation of LBKI kinase, a regulator of AMPK activity (30).

AMINO ACID–DEPENDENT GENE EXPRESSION IN THE β-CELL

In mammals, the impact of nutrients, especially amino acids and fatty acids, on gene expression has become an important area of research. Control of gene expression by nutrient availability has been well documented in prokaryotes and lower eukaryotes, which are able to adjust their metabolic activity to variations in the nutrient supply by altering their pattern of gene expression. However, the mechanisms responsible for amino acid control of mammalian cell gene expression have only recently been investigated. Amino acids may exert influence via mTOR-dependent stimulation of protein synthesis and indirectly, gene expression. Amino acid starvation can lead to tRNA accumulation, transcriptional factor activation, and upregulation of several genes that are involved in amino acid synthesis (31). Interestingly, supra-physiological concentrations of amino acids have been shown to regulate gene expression in hepatocytes via cell swelling–dependent events (32).

Expression of genes related to β-cell signal transduction, metabolism, and apoptosis are chronically regulated by L-alanine. Analysis performed using the Affymetrix rat genome RGU34A microarray revealed that a total of 66 genes were increased ≥1.8-fold after 24-h culture with L-alanine (33). These genes were grouped according to molecular function, and increased expression of some key metabolic genes, including ATP-citrate lyase and catalase, was confirmed by real-time PCR. L-alanine– and L-glutamine–dependent regulation of β-cell gene expression was recently reviewed (34).

It is known that L-glutamine specifically regulates proinflammatory cytokine gene expression in mononuclear cells of the immune system, as well as specific functional genes in the liver, kidney, muscle, lymphocytes, adipocytes, fibroblasts, and tumor cells (35). In context to the work described here, whereas L-glutamine only weakly stimulated insulin secretion from BRIN-BD11 cells at basal (1.1 mmol/l) glucose (14), the amino acid was actively metabolized by several different pathways (36). More recently, we have discovered that 10 mmol/l L-glutamine increased the chronic 24-h insulin secretion rate of this clonal β-cell line by 30% compared with 1 mmol/l glutamine, which was associated with upregulation of 148 genes at least 1.8-fold and downregulation of 18 genes (M. Corless, A. Kiely, N.H. McClenaghan, P.R. Flatt, P.N., unpublished data). Notably, BRIN-BD11 cells (in common with all transformed cell lines) required exposure to L-glutamine at a minimum concentration of 1 mmol/l, to avoid significant loss of viability during a chronic period of culture.

We additionally observed that 24-h exposure to L-glutamine strongly upregulated both the calcineurin catalytic and regulatory subunit mRNA expression in BRIN-BD11 cells. Calcineurin, or protein phosphatase 2B, is a calcium-binding protein that has been shown to contribute to the mechanism of somatostatin-induced inhibition of exocytosis in mouse pancreatic β-cells (37). In addition, it is now appreciated that the cAMP response element binding (CREB) protein transcription factor regulates specific pro-survival genes in the β-cell. CREB translocation to the nucleus is regulated by specific Ca^{2+}-dependent dephosphorylation of transducers of regulated CREB (TORC) by calcineurin (38). We also determined significant glutamine-dependent upregulation of PDX-1 and acetyl-CoA carboxylase at the mRNA level. Elevated PDX-1 transcriptional binding was confirmed by an electrophoretic mobility shift assay, and increased acetyl-CoA carboxylase protein expression was demonstrated by Western blotting (M. Corless, A. Kiely, N.H. McClenaghan, P.R. Flatt, P.N., unpublished data). Thus, glutamine may be required for the optimal in vivo and in vitro differentiation of pancreas-

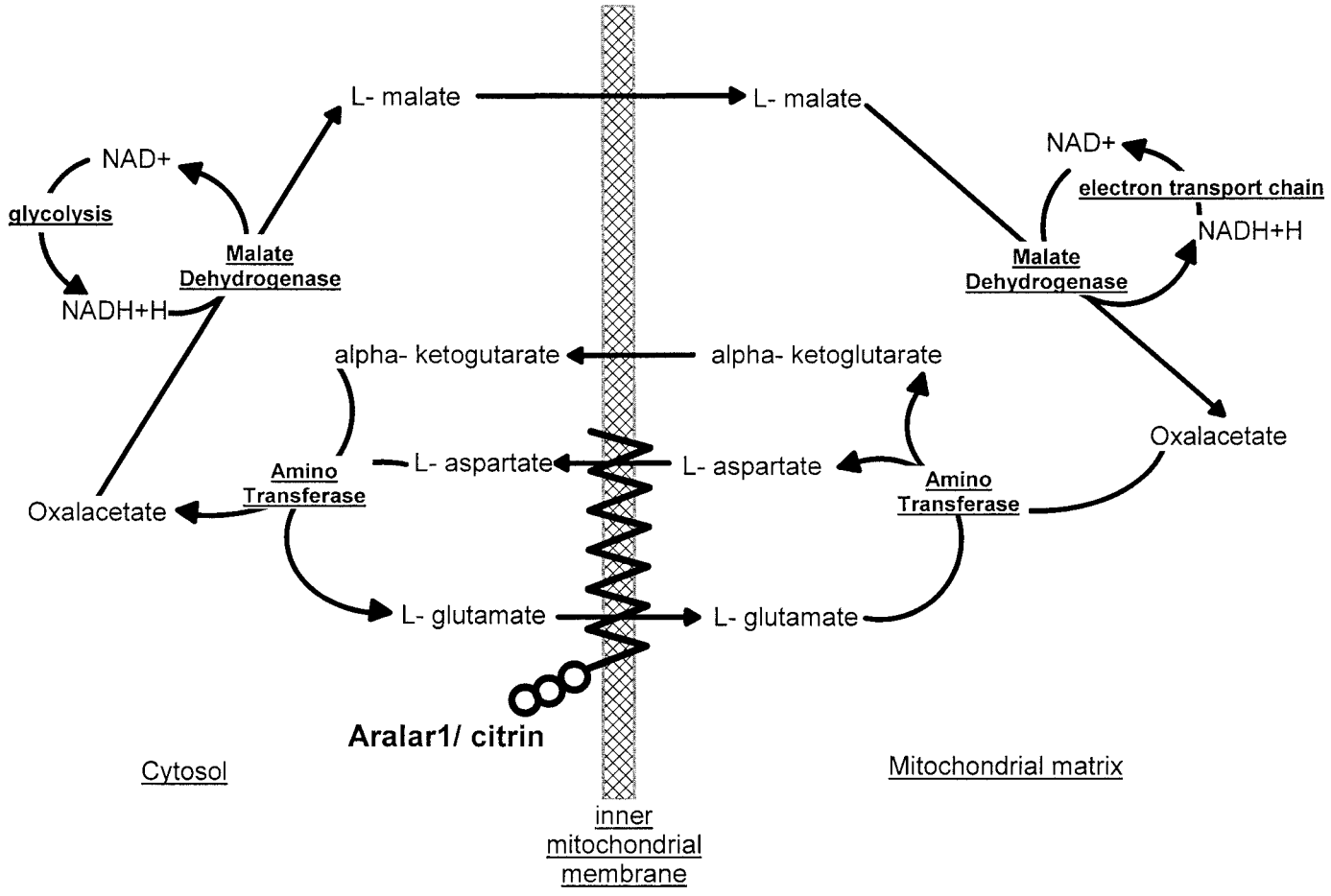

FIG. 2. The malate-aspartate shuttle. The malate-aspartate shuttle is the principal mechanism for the movement of reducing equivalents in the form of NADH from the cytoplasm to the mitochondrion. Cytoplasmic malate dehydrogenase reduces oxalacetate to malate while oxidizing NADH to NAD^+. Malate then enters the mitochondrion, where the reverse reaction is carried out by mitochondrial malate dehydrogenase. Movement of mitochondrial oxaloacetate to the cytoplasm to maintain this cycle is achieved by transamination to aspartate, with the amino group being donated by glutamate. The 2-oxoglutarate (α-ketoglutarate) generated leaves the mitochondrion for the cytoplasm.

derived stem cells toward the β-cell phenotype and optimal lipid synthesis.

In summary, whereas L-alanine and L-glutamine may acutely regulate insulin secretion (as described in detail below), they also play a role in regulating β-cell gene expression, which will affect the ability of the β-cell to chronically respond to nutrient availability, metabolism, hormonal stimuli of insulin secretion, and regulators of functional integrity.

ROLE OF AMINO ACIDS IN NADH MITOCHONDRIAL SHUTTLES AND STIMULATION OF ENERGY METABOLISM

In pancreatic β-cells, the activities of the NADH shuttles play an important role in glucose metabolism. This is as a consequence of low lactate dehydrogenase activity resulting in β-cell dependence on NADH shuttles to regenerate cytosolic NAD^+. The transport of glycolysis-derived reducing equivalents from the cytosol to the mitochondrial matrix also results in the coupling of glycolysis to mitochondrial energy metabolism. Amino acids such as aspartate and glutamate play a key role in such shuttles. After transport into the mitochondria, glycolysis-derived electrons are transferred to the electron transport chain, which creates the proton electrochemical gradient driving ATP synthesis. The formation of a robust proton gradient limits the production of mitochondrial coupling factors.

In β-cells, NADH may be transported to the mitochondrial matrix by either the glycerol-phosphate or the malate-aspartate shuttle (39). Inhibition of the malate-aspartate shuttle by amino-oxycetate (which acts on transamination reactions and inhibits cytosolic NADH reoxidation) attenuated the secretory response to nutrients, thus demonstrating the dominance of this latter shuttle in the β-cell. One key constituent of the malate-aspartate NADH shuttle is the mitochondrial aspartate-glutamate transporter with its two Ca^{2+}-sensitive isoforms Citrin and Aralar1, which are expressed in excitatory tissues. However, Aralar1 is the only aspartate-glutamate transporter isoform expressed in β-cells. The function of this transporter in the malate-aspartate shuttle is illustrated in Fig. 2. Adenoviral-mediated overexpression of Aralar1 in INS-1E β-cells and rat pancreatic islets enhanced glucose-evoked NAD(P)H generation, electron transport chain activity, and mitochondrial ATP formation (40). Aralar1 was demonstrated to exert its effect on insulin secretion upstream of the TCA cycle (40). Indeed, the capacity of the aspartate-glutamate transporter appeared to limit NADH shuttle activity and subsequent mitochondrial metabolism. Our laboratory is now investi-

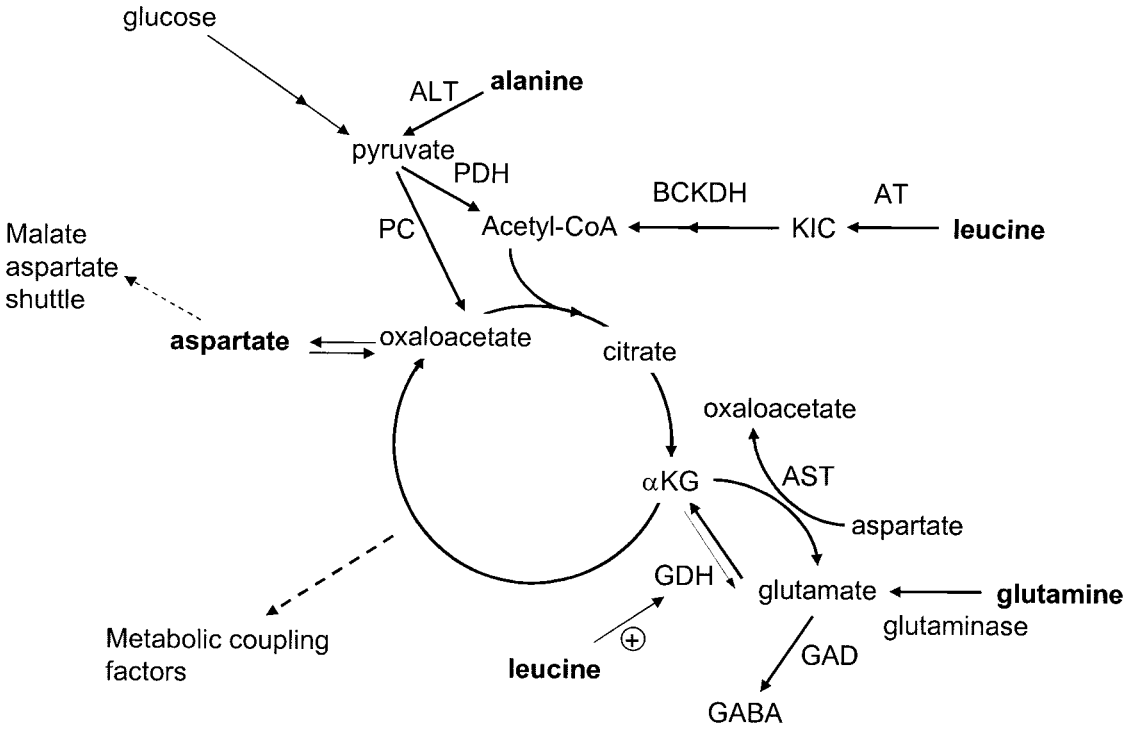

FIG. 3. Schematic describing metabolism of selected amino acids and the related production of metabolic stimulus-secretion coupling factors involved in insulin release. The pathway of glutamine metabolism via glutaminase, GDH, and entry into the TCA cycle (glutaminolysis) is illustrated along with essential points of amino acid interaction with glutamine and glucose metabolism. αKG, α-ketoglutarate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AT, aminotransferase; BCKDH, branched-chain α-keto-acid dehydrogenase; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; KIC, α-ketoisocaproic acid.

gating the role of the Aralar1 transporter in β-cell amino acid metabolism and insulin secretion. We have demonstrated that in Aralar1-overexpressing INS-1E β-cells, an L-alanine addition resulted in increased NAD(P)H production, electron transport chain activity, and insulin secretion (K.B., P. Maechler, P.N., unpublished data).

MECHANISMS OF AMINO ACID–DEPENDENT STIMULATION OF INSULIN SECRETION

L-Glutamine. Glutamine may be released by a number of cells and tissues (for example, skeletal muscle and liver), but glutamine synthesis and release should be considered in the context of glutamine consumption by other cells and tissues. Indeed, blood glutamine concentration is a reflection of the balance between synthesis and release and consumption by the relevant cell types. A sudden change in this balance (for example, rapid expansion and rapid consumption by cells of the immune system) must be balanced by enhanced synthesis and release by, for example, skeletal muscle.

Intracellular glutamine concentration varies between 2 and 20 mmol/l (depending on cell type), whereas its extracellular concentration averages 0.7 mmol/l (35). Glutamine plays an essential role, promoting and maintaining function of various organs and cells, such as kidney, intestine, liver, heart, neurons, lymphocytes, macrophages, neutrophils, pancreatic β-cells, and white adipocytes (35). At the most basic level, glutamine serves as an important fuel in these cells and tissues. A high rate of glutamine uptake is characteristic of rapidly dividing cells, such as enterocytes, fibroblasts, and lymphocytes (35), where glutamine is an important precursor of peptides and proteins, as well as of amino sugars, purines, and pyrimidines, thus participating in the synthesis of nucleotides and nucleic acids (35). Glutamine metabolism additionally provides precursors for the synthesis of key molecules, such as glutathione (35). In a recent study, we reported that both rat islets and BRIN-BD11 cells consumed L-glutamine at high rates (4). Islets may have a high rate of protein turnover even under basal conditions, which would require L-glutamine for purine and pyrimidine synthesis, subsequent mRNA production, and, in addition, protein synthesis. Despite the fact that L-glutamine is rapidly taken up and metabolized by islets, it alone does not stimulate insulin secretion or potentiate glucose-induced insulin secretion (14). However, it enhanced L-leucine–induced insulin secretion (5). This action has been attributed to activation of glutamate dehydrogenase (GDH) by L-leucine, which leads to an increased entry of L-glutamine–derived carbon into the TCA cycle and subsequent oxidation. It is known that glucose inhibits glutaminolysis in β-cells (41), presumably via GTP-dependent allosteric inhibition of GDH, resulting in accumulation of L-glutamate and thus product-dependent inhibition of glutaminase, and as a result blocks leucine-stimulated insulin secretion (see Fig. 3 for details).

It has been reported that L-glutamine is converted to γ-amino butyric acid (GABA) in islets (42). A recent study has suggested the conversion of glutamine to GABA as a means to explain the paradox that L-glutamine alone does not stimulate insulin release (43). The latter study reported that L-glutamine was metabolized preferentially to GABA and L-aspartate in islets and that, in the presence of L-leucine, there was increased metabolism of both L-glutamate and GABA via the GABA shunt (glutamate decarboxylase, GABA aminotransferase, and succinate-semialdehyde dehydrogenase). The production of $^{14}CO_2$ from L-[U-^{14}C]glutamine was attributed mainly to the for-

mation of GABA (via glutamate decarboxylase), and the accumulation of L-aspartate was suggested to be as a result of oxaloacetate transamination, where the oxaloacetate was produced by a combination of the GABA shunt pathway and the TCA cycle. Under this scheme, there is no oxidation of L-glutamine via the TCA cycle, and the authors suggest that this may explain the poor ability to induce insulin secretion.

Using ^{13}C-labeled glutamine and nuclear magnetic resonance spectroscopy, we showed that the major products of L-[1,2^{13}C]glutamine metabolism are L-[1,2^{13}C]glutamate and L-aspartate labeled at positions C1 and C4 in BRIN-BD11 β-cells (39). L-Aspartate is formed after entry of L-glutamate into the TCA cycle. Additionally, the L-glutamate produced from glutamine entered the γ-glutamyl cycle and resulted in an increased production of glutathione (36). There was no nuclear magnetic resonance–based evidence for the production of GABA in our experimental conditions. Addition of glucose caused an increase in glutamate concentration but no increase in glutamine consumption, agreeing with previous reports that glucose can inhibit glutaminolysis (36). Using ^{13}C-isotopomer analysis of anaplerotic flux in INS-1 cells, Cline et al. (44) demonstrated that glutamine addition increased flux through glutamate dehydrogenase, but this was not correlated with insulin secretion.

Recently, a signaling role for glutamine in insulin secretion was proposed (45). Using normal mouse islets, the authors used a potential glutamine synthetase inhibitor, methionine sulfoximine, to investigate the role of intracellularly generated glutamine in insulin secretion. They reported that, in the presence of this inhibitor, the insulin released in response to a glucose ramp was abolished and that this inhibition could be reversed by addition of L-glutamine or its nonmetabolizable analog, 6-diazo-5-oxo-L-norleucine. However, caution should be exercised when interpreting the results from this study because methionine sulfoximine is not a specific glutamine synthetase inhibitor (46) and indeed inhibits a number of glutamate-utilizing enzymes, including γ-glutamylcysteine synthetase, thus blocking potential metabolism of glucose-derived glutamate via the γ-glutamyl cycle. Although this issue was partially addressed by use of buthionine sulfoximine (BSO), an inhibitor of γ-glutamylcysteine synthetase, the mechanisms involved in glutamine-based "signaling" have yet to be identified.

L-Glutamate. L-glutamate is the most highly debated amino acid with respect to stimulation of insulin secretion and the possible molecular mechanisms of its action on promotion of secretion. Intracellular generation of L-glutamate has been proposed to participate in nutrient-induced stimulus-secretion coupling, as an additive factor in the amplifying pathway of glucose-stimulated insulin secretion (47). During glucose stimulation, total cellular glutamate levels have been reported to increase in human, mouse, and rat islets as well as in clonal β-cells (3,47–50), whereas in other studies, no change was detected (51,52). The finding that mitochondrial activation in permeabilized β-cells directly stimulates insulin exocytosis (25) pioneered the identification of glutamate as a putative intracellular messenger (47,53). It has been suggested that glutamate could be transported into secretory granules, thereby promoting Ca^{2+}-dependent exocytosis (47,53). Such a model has been substantiated by demonstration that clonal β-cells express vesicular glutamate transporters and that glutamate transport characteristics are similar to neuronal transporters (54). Other evidence in support of the L-glutamate hypothesis comes from work with β-cells overexpressing L-glutamate decarboxylase (GAD): overexpression of GAD reduced L-glutamate content in INS-1E and islet β-cells and reduced secretory responses to high glucose (48).

In recent years, the role of L-glutamate in insulin secretion has been robustly challenged (49,52). An increase in intracellular L-glutamate concentration on addition of glucose (16.7 mmol/l) in rat islets was not observed in a key study (52). Incubation with L-glutamine (10 mmol/l) increased the L-glutamate concentration 10-fold but did not stimulate insulin release, leading the authors to cast doubt on the proposed role of L-glutamate. In a separate study, it was demonstrated that, on incubation with glucose, a significant increase in L-glutamate concentration occurred in depolarized mouse and rat islets (49). However, the latter authors argued against the glutamate hypothesis on the basis of experiments with L-glutamine: L-glutamine caused an increase in L-glutamate content with no effect on insulin secretion. Additionally, in this study, activation of GDH by BCH lowered L-glutamate levels but increased insulin secretion. However, addition of L-glutamine as a precursor for L-glutamate may lead to saturating concentrations of L-glutamate without activation of the K_{ATP}-dependent pathway and thus may not result in an increase in insulin secretion (50).

L-Alanine. L-Alanine is consumed at high rates in both BRIN-BD11 cells and rat islets (~2 and 8 μmol/mg protein/20 min for BD11 cells and islets, respectively [4]). Addition of 10 mmol/l L-alanine to 16.7 mmol/l glucose significantly increased glucose consumption in BRIN-BD11 cells (3), suggesting a critical role for L-alanine in β-cell function.

Early studies have shown that L-alanine is taken up and oxidized by *ob/ob* mouse islets (55). Recently, L-alanine has been shown to have insulinotropic effects both in β-cell lines and in rat islets (4,14). Addition of 10 mmol/l L-alanine to an incubation medium containing 1.1 mmol/l D-glucose increased insulin secretion 3- and 1.6-fold for BRIN-BD11 cells and islets, respectively (4). It was suggested that, in RINm5F cells, the insulinotropic action of L-alanine was due to co-transport with Na^+, which resulted in membrane depolarization that led to the generation of Ca^{2+} spike potentials and an increase in intracellular Ca^{2+} (56). Other studies using the pancreatic β-cell line BRIN-BD11 demonstrated that L-alanine influenced glucose-induced insulin secretion by electrogenic Na^+ transport (57). More recently, using ^{13}C nuclear magnetic resonance, L-alanine was shown to undergo substantial metabolism in BRIN-BD11 cells (3), resulting in glutamate, aspartate, and lactate production. Additionally, by use of the respiratory poison oligomycin, the metabolism and oxidation of alanine was shown to be important for its ability to stimulate insulin secretion (58).

In contrast to our own work, others have reported that addition of L-alanine did not stimulate insulin secretion from rat islet cells. However, in the presence of L-leucine or 2-ketoisocaproate, alanine promoted insulin secretion (59). Additionally, L-alanine induced an increase in Ca^{2+} uptake and was oxidized by the β-cell. It was concluded that L-alanine could stimulate insulin secretion under specific conditions of nutrient availability and that the mode of induction of insulin secretion may be a combination of increased ATP production and Na^+ co-transport (59).

L-Leucine. The proposed mechanisms by which L-leucine stimulates insulin release from pancreatic β-cells include *1*) increased mitochondrial metabolism by activation of GDH and *2*) increased ATP production (and subsequent K_{ATP} channel–dependent membrane depolarization) by transamination of leucine to α-ketoisocaproate and subsequent entry into the TCA cycle via acetyl-CoA (5,60,61; Fig. 3). In the presence of high glucose, leucine-induced insulin secretion is inhibited (62), since high glucose inhibits flux through glutaminase and GDH. Recently, there has been renewed interest in L-leucine metabolism as a result of the observation of hyperinsulinism in patients with mutations in the regulatory site of GDH (63,64). Affected patients have increased β-cell responsiveness to leucine and develop hypoglycemia after a protein meal. Key mutations in the inhibitory allosteric site in GDH (GTP binding) result in the loss of negative allosteric regulation. Although one of the proposed mechanisms by which leucine induces insulin secretion is the conversion of leucine to α-ketoisocaproate, a recent report showed that leucine and α-ketoisocaproate stimulated insulin release via distinct mechanisms (65). α-Ketoisocaproate was proposed to stimulate insulin release by a combination of mechanisms including its own catabolism and transamination to leucine with production of 2-oxoglutarate (α-ketoglutarate). However, others have demonstrated that α-keto acids can directly inhibit K_{ATP} channel activity and therefore stimulate insulin secretion (66).

Prolonged culture with leucine resulted in increased ATP, cytosolic Ca^{2+}, and glucose-induced insulin secretion in rat islets (67). Additionally, chronic periods of culture with leucine upregulated ATP synthase and glucokinase leading to the proposal that this combined upregulation sensitizes the β-cell to glucose-induced insulin secretion (67).

Leucine along with other members of the branched-chain amino acids activate the mTOR signaling pathway in β-cells as previously described. Mitochondrial signals generated by metabolism of leucine have been suggested to be important for activation of the mTOR mitogenic signaling pathway in insulin-sensitive tissues (27).

L-Arginine. The stimulation of insulin release by L-arginine has been proposed to involve the transport of the cationic amino acid into the β-cell, which leads to membrane depolarization (1,68,69). A recent detailed study agreed with this argument (70). L-Arginine was shown to cause an elevation in intracellular Ca^{2+} concentration as a result of its electrogenic transport into the β-cell via the amino acid transporter mCAT2A. Depolarization of the plasma membrane will then result in activation of voltage-dependent calcium channels, an increase in cytosolic Ca^{2+}, and subsequent stimulation of insulin secretion. Clinical assessment of administered L-arginine has revealed only limited beneficial effects, possibly due to rapid removal of the amino acid in the epithelial cells of the intestine, where it can be rapidly converted to ornithine and citrulline, then exported to the kidney (71) or the liver, where it can be converted to proline for export (71).

Alternatively, L-arginine metabolism in the β-cell can give rise to urea production via arginase activity, or nitric oxide production via nitric oxide synthase. Inducible nitric oxide synthase may be upregulated in the presence of proinflammatory cytokines (72) or indeed specific fatty acids (73), and under these conditions, L-arginine consumption and metabolism may have a negative impact on β-cell function. Chronically elevated nitric oxide levels will reduce insulin secretion, possibly by interfering in mitochondrial function and generation of key stimulus-secretion coupling factors. The impact of arginase activity and urea production are currently unknown. L-Arginine may alternatively be converted to L-glutamate and thus can influence insulin secretion as described above (50). However, no studies have yet explored L-arginine metabolism in detail in the β-cell; thus, the potential for L-glutamate generation remains to be determined.

Homocysteine. Elevated plasma homocysteine levels have been reported in hyperinsulinemic obese subjects and in subjects with type 2 diabetes with preexisting coronary vascular disease (74). In contrast to all the amino acids discussed above, homocysteine has a negative impact on insulin secretion in pancreatic β-cells. A recent study has revealed that homocysteine inhibits insulin section from the BRIN-BD11 cell line (75). In particular, homocysteine caused a significant and dose-dependent inhibition of insulin secretion with initial effects at 50 μmol/l. Nuclear magnetic resonance spectroscopy studies revealed that homocysteine caused a significant reduction in the labeling of glucose-derived TCA cycle–dependent end products, which may subsequently affect the triggering and potentiation of insulin secretion. The effects of homocysteine were not limited to glucose but also impaired amino acid–stimulated insulin secretion: acute incubation with homocysteine resulted in concentration-dependent inhibition of alanine-, arginine-, and α-ketoisocaproic acid–induced insulin secretion. Further studies into the mechanism of homocysteine-mediated reduction in insulin secretion should shed some light on the possible role of hyperhomocysteinemia in the development of type 2 diabetes.

CONCLUSION

Amino acid metabolism is essential to normal pancreatic β-cell function, as highlighted in this review. Acutely, key amino acids such as alanine and glutamine can regulate β-cell function and insulin secretion. The mechanisms by which these amino acids confer their regulatory effects are complex and involve mitochondrial metabolism. L-Glutamine metabolism is important for L-glutamate production, which may act as an important stimulus-secretion coupling factor in the presence of glucose. D-Glucose conversion to L-glutamate can occur in specific conditions of glutamine limitation, via 2-oxoglutarate in a transamination reaction. The rate of L-glutamine metabolism appears to be especially high in the β-cell, due to optimization of mitochondrial function. Thus, L-glutamine is used by the β-cell, not only for oxidation, but also for glutathione production. We speculate that in conditions where activation or overexpression of GDH activity is promoted, the metabolism of L-glutamine will contribute to a rise in the ATP/ADP ratio, thus inactivating the K_{ATP} channel and depolarizing the plasma membrane, resulting in intracellular Ca^{2+} elevation and insulin secretion. Glutamate may play a key role in stimulation of insulin secretion either directly or via metabolism or via its role in enhancing malate-aspartate shuttle activity. Other amino acids such as leucine or arginine may play a role in enhancing insulin secretion by allosteric activation of metabolism or membrane depolarization, or a combination of these two possibilities. Chronic effects of changes in amino acid concentration in vivo and in vitro on β-cell function and integrity have not yet been investigated in

detail, but initial experiments indicate an important role for alanine and glutamine in the regulation of β-cell lipid metabolism and signal transduction. Understanding the mechanisms by which amino acids regulate insulin secretion in vivo may reveal novel sites for targeting drugs for the therapy of type 2 diabetes in the future.

ACKNOWLEDGMENTS

We thank the Health Research Board of Ireland, Enterprise Ireland, and the Wellcome Trust for funding our work. We thank Professor Pierre Maechler of the University of Geneva, Switzerland, for provision of Aralar1, technical advice, and training.

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