β-Cell Mitochondria and Insulin Secretion

Messenger Role of Nucleotides and Metabolites

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The β-cell mitochondria are known to generate metabolic coupling factors, or messengers, that mediate plasma membrane depolarization and the increase in cytosolic Ca\(^{2+}\), the triggering event in glucose-stimulated insulin secretion. Accordingly, ATP closes nucleotide-sensitive K\(^{+}\) channels necessary for the opening of voltage-gated Ca\(^{2+}\) channels. ATP also exerts a permissive action on insulin exocytosis. In contrast, GTP directly stimulates the exocytotic process. cAMP is considered to have a dual function: on the one hand, it renders the β-cell more responsive to glucose; on the other, it mediates the effect of glucagon and other hormones that potentiate insulin secretion. Mitochondrial shuttles contribute to the formation of pyridine nucleotides, which may also participate in insulin exocytosis. Among the metabolic factors generated by glucose, citrate-derived malonyl-CoA has been endorsed, but recent results have questioned its role. We have proposed that glutamate, which is also formed by mitochondrial metabolism, stimulates insulin exocytosis in conditions of permissive, clamped cytosolic Ca\(^{2+}\) concentrations. The evidence for the implication of these and other putative messengers in metabolism-secretion coupling is discussed in this review. Diabetes 51 (Suppl. 1):S37–S42, 2002

Blood glucose control depends on the normal regulation of insulin secretion from the pancreatic β-cells and on insulin action on its target tissues. Most forms of type 2 diabetes display dysregulation of insulin secretion combined with insulin resistance. The central role of β-cell dysfunction in the development of type 2 diabetes is illustrated, on the one hand, by the various forms of maturity-onset diabetes of the young (MODY) (1), and, on the other, by a recently developed transgenic mouse model. In these mice, the β-cell–targeted overexpression of the flux-determining enzyme of the hexosamine pathway results in hypersecretion of insulin, followed by insulin resistance and diabetes (2).

These observations should encourage further dissection of the mechanism of metabolism–secretion coupling in the β-cell (3).

**Glucose recognition by the β-cell.** The rate of insulin secretion is adapted to the changes in blood glucose concentration by the β-cells. Glucose equilibrates across the plasma membrane and is phosphorylated by glucokinase, which determines the rate of glycolysis and the generation of pyruvate (4,5). High rates of glycolysis are maintained through the activity of mitochondrial shuttles, mainly the glycerophosphate and malate/aspartate shuttles (6,7), which allow the reoxidation of cytosolic NADH. The importance of additional shuttles generating cytosolic NADPH has also been emphasized (8,9). A particular feature of the β-cell is not only the tight link between glycolysis and mitochondrial oxidative metabolism, but also the extremely high proportion of glucose-derived carbons oxidized in the mitochondria (10). The very low expression of monocarboxylate transporters in the plasma membrane and the low activity of lactate dehydrogenase act conjointly to drive pyruvate into the mitochondria (11–14). In the mitochondria, pyruvate is a substrate for both pyruvate dehydrogenase and pyruvate carboxylase. The latter enzyme forms oxaloacetate, providing anaplerotic input to the tricarboxylic acid (TCA) cycle (9,10). Reducing equivalents generated by the TCA cycle activate the electron transport chain resulting in hyperpolarization of the mitochondrial membrane (ΔΨ\(_{m}\)) and formation of ATP (Fig. 1). Pyruvate dehydrogenase and two TCA cycle dehydrogenases are activated by Ca\(^{2+}\) (reviewed in reference 15), which may reinforce the production of metabolic coupling factors during glucose-stimulated insulin secretion (16). Mitochondrial metabolism increases the ATP/ADP ratio (17). The increase in cytosolic ATP (18,19) and/or the decrease in free ADP (5,20) promotes the closure of ATP-sensitive K\(^{+}\) channels (K\(_{ATP}\) Channels) and depolarization of the plasma membrane (21). As a consequence, cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)\(_{c}\)]\(_{i}\)) is raised by the opening of voltage-sensitive Ca\(^{2+}\) channels (21,22). The increase in [Ca\(^{2+}\)\(_{c}\)]\(_{i}\) is the main trigger for exocytosis, the process by which the insulin-containing secretory granules fuse with the plasma membrane (22–24). When measured simultaneously, glucose causes biphasic increases in both [Ca\(^{2+}\)\(_{c}\)]\(_{i}\) and insulin secretion (25,26). Insulin secretion is stimulated not only by glucose, but also by leucine and other amino acids (3). Nutrient-induced secretion is potentiated by the neurotransmitters acetylcholine and putative adenylate cyclase–activating polypeptide (PACAP), as well as by the gastrointestinal hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) (27,28).

Conversely, knockout of the GIP receptor in mice leads to

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BCH, 2-aminobicyclo (2,2,1)heptane-2-carboxylic acid; [Ca\(^{2+}\)\(_{i}\)], cytosolic Ca\(^{2+}\); GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; K\(_{ATP}\) channel, ATP-sensitive K\(^{+}\) channels; PACAP, pituitary adenylate cyclase–activating polypeptide; TCA, tricarboxylic acid.
glucose intolerance (29). Insulin secretion is also subjected to paracrine regulation by glucagon release from the islet \( \alpha \)-cells (30,31). In addition, insulin exocytosis is under the direct negative control of norepinephrine, somatostatin, and circulating epinephrine (23,27,32,33). The reader is referred to the cited reviews for the detailed descriptions of the actions of hormones and neurotransmitters.

**K\(_{\text{ATP}}\)**-independent effect of glucose. The \( \text{Ca}^{2+} \) signal in the cytosol is necessary, but not sufficient, for the full development of biphasic insulin secretion. It was first proposed in 1988 by using sulfonylureas that glucose evokes \( K_{\text{ATP}} \)-independent stimulation of insulin secretion (34). This \( K_{\text{ATP}} \)-independent pathway was further characterized in 1992, when it was demonstrated that glucose elicits secretion under conditions of clamped, elevated \( [\text{Ca}^{2+}]_c \) (35–37). More recently, knockout mouse models lacking either of the two functional subunits of the \( K_{\text{ATP}} \) channel show marked reduction (albeit not elimination) of glucose-stimulated insulin secretion (38,39). It is noteworthy that these \( \beta \)-cells show a partial secretory response to glucose without changes in \( [\text{Ca}^{2+}]_c \), which is already elevated at low glucose concentration. Taken together, all these studies suggest the existence of metabolic coupling factors generated by glucose.

Nucleotides as mediators of the glucose effect. It is well established that intracellular ATP is required for insulin exocytosis (40–42). A higher ATP/ADP ratio is needed for the closure of \( K_{\text{ATP}} \) channels compared with the requirement of the exocytotic process itself (41). However, at nonstimulatory \( \text{Ca}^{2+} \) concentrations, ATP does not cause insulin secretion in permeabilized cells (40). In the presence of stimulatory \( \text{Ca}^{2+} \), ATP enhances the process (40,42,43). Conversely, glucose-induced ATP elevation does not promote insulin release in the absence of extracellular \( \text{Ca}^{2+} \) (44). There was, however, a correlation between the generation of ATP and the \( K_{\text{ATP}} \)-independent insulin secretion evoked by glucose or by the combination of glutamine plus leucine. In the same report, glutamine alone also enhanced \( K_{\text{ATP}} \)-independent insulin release, but no measurements of ATP were shown (44). Therefore, ATP produced from nutrient metabolism could be involved in the \( K_{\text{ATP}} \)-independent secretion.

Glucose also increases the GTP/GDP ratio in islets both under control conditions and under conditions of the \( K_{\text{ATP}} \)-independent secretion (17 and references therein). In contrast to ATP, GTP is capable of initiating insulin exocytosis in a \( \text{Ca}^{2+} \)-independent manner both in native (45,46) and in clonal \( \beta \)-cells (24,40,45). Thus, GTP is generated by glucose and also modulates exocytosis di-
rectly, which qualifies it as a messenger molecule (47). Whether heterotrimeric G-proteins or the monomeric G-protein Rab3 is the target for GTP remains to be established (24,48).

It has been known for more than three decades that cAMP potentiates glucose-stimulated insulin secretion. GLP-1, GIP, PACAP, and glucagon increase cAMP levels in β-cells (27,28). Glucagon has been shown to render β-cells glucose-responsive through the generation of cAMP (30). This paracrine effect of glucagon was also recently demonstrated in human islets (31). cAMP exerts at least three actions that may render the β-cell glucose-competent and enhance insulin secretion: 1) the Ca\(^{2+}\) current through L-type Ca\(^{2+}\) channels is increased (49); 2) β-cells refractory to glucose depolarization become responsive, showing K\(_{ATP}\)-channel closure (50); and 3) the secretory machinery is sensitized to Ca\(^{2+}\) (40,49,51,52). All these actions are mediated by cAMP-dependent protein kinase A. A direct protein kinase A–dependent enhancement of insulin exocytosis involving the cAMP-GEFII protein (GEF, guanosine nucleotide exchange factor) has recently been described (53). Although glucose has been found to increase cAMP levels in a minority of studies, such an effect is not observed in purified β-cells (30). Therefore, the role of cAMP in glucose-stimulated insulin release is that of a potentiator rather than a mediator.

Among other putative nucleotide messengers, NADH and NADPH are generated by glucose metabolism (for a review, see reference 54). Single β-cell measurements of NAD(P)H fluorescence have demonstrated that the rise in pyridine nucleotides precedes the rise in [Ca\(^{2+}\)] (55) and that the elevation in the cytosol is reached more rapidly than in the mitochondria (56). Cytosolic NADPH is generated by glucose metabolism via the pentose phosphate shunt (57) and by mitochondrial shuttles (9). An action of NADPH on insulin secretory granules has been proposed from experiments on toadfish islets (58). Another nucleotide, diadenosine polyphosphate, which is produced during glucose stimulation, closes K\(_{ATP}\) channels and may play a role in stimulus-secretion coupling (59).

This enumeration of possible nucleotide messengers is not exhaustive and, for all of them, their role in insulin exocytosis needs to be substantiated.

**Metabolites as putative messengers.** Malonyl-CoA has been proposed as a metabolic coupling factor in insulin secretion (60) and as a messenger derived from citrate generated in the mitochondria (9). However, disruption of malonyl-CoA accumulation during glucose stimulation did not affect the secretory response (61), even under conditions in which only the K\(_{ATP}\)-independent pathway is operative (62). In view of the inhibition of metabolism-secretion coupling in lipid-depleted β-cells (63,64), a permissive role of long-chain acyl-CoAs in insulin release cannot be excluded.

To study the link between mitochondrial activation and insulin exocytosis, we have established a *Staphylococcus aureus* α-toxin permeabilized β-cell model permitting the clamping of [Ca\(^{2+}\)] and nucleotides such as ATP. As shown in Fig. 2, the TCA cycle intermediate succinate (1 mmol/l) enhances insulin secretion at the permissive concentration of 500 mmol/l Ca\(^{2+}\) and at 10 mmol/l ATP in such permeabilized INS-1 cells (65). The magnitude of the response is similar to a rise in free Ca\(^{2+}\) from 500 mmol/l to 1.3 mmol/l. Other TCA cycle intermediates, α-ketoglutarate, malate (Fig. 2), or citrate (65) are inefficient. The provision of carbons to the TCA cycle is not sufficient for insulin exocytosis. Indeed, the effect of succinate also requires an increase in intramitochondrial Ca\(^{2+}\) occurring at permissive cytosolic Ca\(^{2+}\) secondary to hyperpolarization of the mitochondrial membrane (65,66). Exposure of isolated INS-1 cell mitochondria to succinate results in a pronounced production of glutamate (67). Glutamate can be generated through several biochemical pathways including transamination reactions (68). In mitochondria, glutamate dehydrogenase forms glutamate from the TCA cycle intermediate α-ketoglutarate (69). In permeabilized INS-1 cells, glutamate (1 mmol/l) stimulates insulin secretion, reproducing the effect of succinate, both at 10 mmol/l ATP (Fig. 2) and at 1 mmol/l ATP (66). In contrast to succinate, the secretory response to glutamate does not require activation of mitochondrial metabolism (66). As the effect of glutamate is similar at low and high ATP concentrations, it is most unlikely that ATP mediates the glutamate-evoked exocytosis. The precise site of glutamate action downstream of mitochondria remains to be defined.

Glucose increased cellular glutamate content in INS-1 cells and human islets (66). In rat islets, glutamate was the only amino acid out of 12 whose content increased during glucose stimulation, whereas the content of aspartate, a possible NH\(_2\) donor, decreased (70). In other reports, glucose did not change glutamate levels in islets isolated from rats (71) or mice (72). However, measurements of total glutamate contents do not reflect fluctuations in the cytosolic compartment of the putative cofactor of insulin exocytosis (16).

Exposure of islets to extracellular glutamine causes a marked increase in their glutamate levels without any increase in insulin secretion (73,74). Glutamine enhances the ion NH\(_4\)\(^{+}\) in cells, which has been shown to inhibit insulin release in both mouse and rat islets secondary to intracellular alkalinization (44,75). Under certain condi-
tions, e.g., in the presence of leucine or by counteracidification, glutamate is capable of eliciting secretion (44,74,76). Therefore, glutamine exhibits dual effects of enhancing and inhibiting insulin release. The insulinotropic action of the membrane-permeant dimethyl ester of glutamate is also restricted to permissive conditions, e.g., at intermediate glucose levels or in the presence of a sulfonfonylurea (77). Taken together, these results demonstrate that intracellular glutamate itself is not sufficient to elicit insulin secretion. This is in agreement with our observation that glutamate stimulates insulin exocytosis at permissive but not at basal Ca\(^{2+}\) in permeabilized cells (66). Mitochondrial metabolism of glutamine/glutamate and ATP production is only weak in nonstimulated islets (73,77). This is explained by the sluggish conversion of glutamate to \(\alpha\)-ketoglutarate by glutamate dehydrogenase (73). Activation of the enzyme by leucine or its nonmetabolizable analog 2-aminobicyclo (2.2.1)heptane-2-carboxylic acid (BCH) increases glutamine oxidation and insulin secretion (78,79). Conversely, in the presence of glucose, glutamine oxidation stimulated by the presence of BCH is inhibited (79). This preferred directional flux has also been observed in mouse islets in which the incorporation of glucose carbons into glutamate was augmented by glucose stimulation, even without changing cellular glutamate content (80). As glutamate does not generate ATP, it is capable of neither depolarizing the plasma membrane nor raising cytosolic Ca\(^{2+}\) in native \(\beta\)-cells. In rat islet cell lines RINm5F cells, in which nutrient metabolism predominantly generates acidic intermediates (11), glutamate elevates cytosolic Ca\(^{2+}\) and consequently stimulates insulin secretion (81). In mouse islets, glutamine moderately enhances insulin release in \(K_{\text{ATP}}\)-pathway-independent conditions, i.e., when cytosolic Ca\(^{2+}\) is maintained at permissive levels by diazoxide and high K\(^{+}\), although this stimulatory effect might be blunted because of the inhibitory action of the generated NH\(_{4}\)\(^{+}\) (44). Unlike glutamine, glucose—the main nutrient secretagogue—increases not only ATP and cytosolic Ca\(^{2+}\), but also glutamate to promote optimal signaling for insulin exocytosis.

CONCLUSIONS
Numerous nucleotides and metabolites have been proposed to couple glucose metabolism to insulin secretion. Of these, GTP can be considered as a bona fide messenger molecule, whereas the role of ATP requires further investigation. According to current knowledge, glutamate could well be a cofactor acting in concert with Ca\(^{2+}\) and other putative molecules. The principal role for glutamate could be the augmentation of the sustained, second phase of glucose-stimulated insulin secretion (82).

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