Minireview: Implication of Mitochondria in Insulin Secretion and Action

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Mitochondria are essential for intermediary metabolism as well as energy production in the cell. Their aerobic metabolism permits oxidation of glucose and fatty acids for the generation of ATP and other intermediates that are exchanged with the cytoplasm for various biosynthetic and secretory processes. In the pancreatic β-cell, glucose carbons are quantitatively funneled to the mitochondria, where signals for the initiation and potentiation of insulin secretion are generated. After mitochondrial activation, the plasma membrane is depolarized with ensuing cytosolic calcium transients and exocytosis of insulin. Calcium also acts in a feed-forward manner on mitochondrial metabolism, which contributes to sustained second phase insulin secretion. Patients with mitochondrial diabetes and a corresponding mouse model display defective glucose-stimulated insulin secretion and reduced β-cell mass, leading to overt diabetes. Normal mitochondrial activity appears to be equally important in the action of insulin on its target tissues. The development of insulin resistance may involve impairment of glucose oxidation after short exposure to increased levels of circulating free fatty acids. Insulin resistance in the elderly and in relatives of type 2 diabetic patients has also been associated with mitochondrial dysfunction. Both prevention and treatment of type 2 diabetes should focus on mitochondrial targets for the improvement of nutrient-stimulated insulin secretion and their utilization in peripheral tissues. (Endocrinology 147: 2643–2649, 2006)
TCA cycle and cross the inner mitochondrial membrane (12). This process depends on the mitochondrial membrane potential. Thus, these metabolites can serve as precursors for many essential molecules in the mitochondrial matrix as well as in the cytoplasm. Importantly, glutamate aspartate transport is rate limiting to mitochondrial metabolism, because overexpression of aralar1 enhances glucose-stimulated insulin secretion (GSIS) (13). Anaplerotic metabolism in the β-cell may be used to generate coupling factors that contribute to GSIS (see below). Due to the very low lactate dehydrogenase expression in β-cells, NADH formed during glycolysis would accumulate and reduce glycolytic flux unless NADH is reoxidized. Therefore, the hydrogen shuttles (malate/aspartate and glycerophosphate shuttles) that transport reducing equivalents from the cytoplasm to the mitochondrial matrix are particularly active in the β-cell (8, 14).

Mitochondrially Derived ATP in Metabolism Secretion Coupling

Mitochondrial ATP production is essential for GSIS, which is abolished by inhibitors of mitochondrial ATP synthesis. Such inhibitors also attenuate β-cell glycolysis as cytosolic NADH accumulates (15). This is also shown directly in mitochondrial DNA (mtDNA)-deficient Rho0 cells (14), which lack mitochondrial encoded subunits of the respiratory chain complexes I, III, IV, and V. Based on these defects, mitochondrial ATP production is absent in Rho0 cells. GSIS is abolished in such cells, which, however, still generate enough nonmitochondrial ATP for insulin synthesis, storage, and the triggering of secretion by Ca2+-elevating agents (14, 16). Furthermore, stimulation of β-cells with substrates that activate mitochondrial metabolism directly, such as membrane-permeant methyl-succinate, is sufficient to effectively induce insulin secretion (17). Pyruvate itself evokes insulin secretion in islets transduced with the plasma membrane monocarboxylate transporter, which is normally not expressed in β-cells (17).

Optimal stimulatory concentrations of the different carbon sources stimulate insulin secretion to a similar extent, suggesting that mitochondrial metabolism becomes rate limiting and sets an upper limit on insulin secretion in the presence of excess substrate (18).

Thus, compelling evidence demonstrates that ATP is a key factor coupling mitochondrial metabolism to insulin secretion. ATP production is accelerated after glucose stimulation to provide the energy for insulin exocytosis, replenishing of insulin granules, and establishment of a new steady state of ion gradients. Despite increased ATP consumption, cytosolic ATP levels in β-cells are increased by about 40% after a shift from basal to high glucose (17). ATP synthesized in the mitochondrial matrix is transported to the cytosol in exchange for cytosolic ADP via adenine nucleotide translocators. The resulting increase in the cytosolic ATP:ADP ratio reduces the opening probability of the plasma membrane KATP channel. Among the molecular components regulating insulin secretion, the KATP channel has proven to be a very useful target, because its opening is blocked by sulfonylurea compounds (5). These are widely used in the treatment of type 2 diabetes. Functional KATP channels are essential for GSIS, and a common polymorphism in the KATP channel has been linked to type 2 diabetes (19). Closure of KATP channels depolarizes the plasma membrane, triggering the opening of voltage-dependent Ca2+-channels (Fig. 1). As in other exocytotic fusion events, Ca2+ is an essential trigger of insulin granule exocytosis (20). The Ca2+ signal resulting from mitochondrial activation completes the chain of events between glucose uptake into the β-cells and insulin exocytosis.

Role of Ca2+ in Mitochondrial Activation

Cytoplasmic Ca2+ increases after the opening of voltage-gated Ca2+ channels. Ca2+ exerts multiple functions in β-cells in addition to its role in exocytosis. The cytoplasmic Ca2+ signal is relayed to the mitochondria (Fig. 1). This is
amplified by hyperpolarization of the inner mitochondrial membrane, increasing the driving force for Ca$^{2+}$ uptake (17). Inside the organelle, Ca$^{2+}$ activates several matrix dehydrogenases (pyruvate dehydrogenase, isocitrate dehydrogenase, and 2-oxoglutarate dehydrogenase). This is reflected by Ca$^{2+}$-driven oscillations of reduced nicotinamide adenine nucleotide phosphate, NAD(P)H, in glucose-stimulated $\beta$-cells (21). Ca$^{2+}$ has also been suggested to stimulate ATP synthase directly (22). These coordinated actions should reinforce GSIS, possibly during the second phase of insulin secretion. Both first and second phases of GSIS are attenuated in type 2 diabetic patients and first degree relatives (for review, see Ref. 23). Ca$^{2+}$ can also be sensed at the outer surface of the inner mitochondrial membrane, because the above-mentioned glutamate/aspartate transporter aralar1 is regulated by cytosolic Ca$^{2+}$ (24). Together, these mechanisms converge to ensure sustained ATP generation during cell activation.

Ca$^{2+}$ accumulated by mitochondria must be removed. Increases in matrix Ca$^{2+}$ are counteracted principally by the Na$^+$/Ca$^{2+}$ exchanger. Its activity is an important determinant of the extent and duration of mitochondrial Ca$^{2+}$ transients. Inhibition of the Na$^+$/Ca$^{2+}$ exchanger has been exploited as a potential target in diabetes research. An inhibitor of the exchanger (CGP37157) was shown to prolong mitochondrial Ca$^{2+}$ signals and increase insulin secretion (25). The same compound has been shown to prolong mitochondrial Ca$^{2+}$ signals and ATP production in fibroblasts from patients with complex I deficiency (26).

**Coupling Factors in the Amplifying Pathway of Insulin Exocytosis**

Glucose-induced insulin secretion depends on additional, as yet unidentified mitochondrial signals. When the $K_{ATP}$ channel opener diazoxide is added, Ca$^{2+}$ increases, and GSIS are completely blocked. Because Ca$^{2+}$ signals are essential for granule exocytosis, cytoplasmic Ca$^{2+}$ concentrations were elevated artificially to create a semipermissive condition for granule fusion. Using this experimental setup, it could be demonstrated that glucose metabolism can stimulate insulin secretion independent of $K_{ATP}$ channel closure (27). In α-toxin-permeabilized cells, permitting clamping of cytoplasmic Ca$^{2+}$ and ATP, succinate stimulates insulin secretion through mitochondrial activation (28, 29). Interestingly, glycerophosphate was unable to stimulate insulin secretion under the same conditions. Via the glycerol phosphate shuttle, glycerophosphate can provide reducing equivalents, but not carbons, to the mitochondria, demonstrating the need for anaplerotic input to the TCA cycle for the potentiation of insulin secretion. Thus, the synthesis of coupling factors supporting the action of ATP could be linked to the anaplerotic pathway of pyruvate metabolism. In support of this possibility, inhibition of pyruvate carboxylase impairs GSIS (11, 30). Several mitochondria-derived molecules have been proposed to serve such a role.

Citrate exported from mitochondria can act as an allosteric activator of acetyl-CoA synthase, which leads to a rapid increase in the malonyl-CoA concentration after glucose stimulation of the $\beta$-cell (31). This suggests that malonyl-CoA could have a direct impact on insulin secretion. Suppressing the increases in malonyl-CoA by the overexpression of malonyl-CoA carboxylase, however, did not impair the glucose responsiveness of $\beta$-cells. Although these results argue against a direct involvement of malonyl-CoA in GSIS, it is likely to facilitate fatty acid-mediated potentiation by diminishing fatty acid oxidation, favoring their actions on exocytosis (31, 32). Although short-term exposure of islets to palmitate and other free fatty acids acutely enhances GSIS, longer exposure of islets is inhibitory. It is of interest in this context that infusion of free fatty acids for 4 d into healthy members of families with a history of type 2 diabetes causes marked inhibition of GSIS, contrasting with slight stimulation in control subjects (33).

Another possible metabolic event linked to insulin secretion is pyruvate cycling. This process is initiated in response to glucose as pyruvate is carboxylated to form oxaloacetate. In pyruvate cycling, malate and/or citrate derived from oxaloacetate are transported out of the mitochondria, where they can be converted to pyruvate. The last step of these reactions is mediated by malic enzyme, which oxidatively decarboxylates malate, also generating NADPH (Fig. 1). Pyruvate completes the cycle to be used again as substrate for mitochondrial matrix enzymes. As measured by $^{13}$C nuclear magnetic resonance, pyruvate cycling is stimulated 2- to 3-fold after a shift of INS-1 cells to elevated glucose. Using different INS-1 clones that vary in their secretory response, it was also shown that pyruvate cycling, but not pyruvate oxidation, was proportional to GSIS (11). However the correlation between pyruvate cycling and insulin secretion is lost in the presence of free fatty acids (34). Both malonyl-CoA and pyruvate cycling in insulin secretion merit additional investigation.

Pyruvate cycling, as described above, generates reducing equivalents in the cytosol in the form of NADPH. A recent study suggests that NADPH can stimulate Ca$^{2+}$ induced insulin exocytosis, as monitored by capacitance changes (35). This is an attractive mechanism, because glucose increases the ratios of both NADH:NAD$^+$ and NADPH:NADP$^+$. Interestingly, the effect was specific for NADPH, because injection of NADH did not influence capacitance. However, glutathione as well as glutaredoxin could also stimulate exocytosis (35). In summary, the results suggest that the redox state of the cell could have a direct impact on the granule fusion machinery.

Glutamate is generated from the TCA cycle intermediate 2-oxoglutarate by glutamate dehydrogenase, from other transamination reactions, or from glutamine in the cytoplasm. In response to glucose, glutamate levels rise in islets (29, 36), as also shown by nuclear magnetic resonance in clonal $\beta$-cells (11, 37). The same technique was used to demonstrate anaplerotic input to the TCA cycle from glucose and cataplerotic generation of glutamate via glutamate dehydrogenase under conditions favoring GSIS (10). As argued above, glutamate, therefore, has the potential to act as an intermediate that stimulates insulin secretion. Because glutamate levels rise only 5–10 min after glucose addition, it is likely to impact mainly on second phase insulin secretion (29). Consistent with a role in insulin secretion, the cell membrane-permeable glutamate analog dimethylglutamate in-
creases glucose sensitivity, resulting in insulin secretion. Additional evidence for the implication of glutamate in GSIS is suggested by down-regulation of glutamate dehydrogenase, resulting in a 50% inhibition of insulin secretion in response to glucose (6). Glucose-derived glutamate is generated by mitochondria and acts downstream as a possible link between mitochondrial metabolism and the exocytotic machinery (Fig. 1). Glutamate directly enhances Ca^{2+}-stimulated exocytosis, as demonstrated in both permeabilized cells and capacitance recordings, under conditions of clamped ATP (29, 38). The underlying mechanism remains to be established. It is of interest that familial hyperinsulinism has been linked to gain of function mutations in glutamate dehydrogenase, which render the β-cell sensitive to glutamine (normally not insulinotropic) as a secretagogue (39).

In summary, a good case has been made for several mitochondrial-derived factors that could potentiate GSIS. Changes in mitochondrial metabolism after glucose stimulation of the β-cell is associated with changes in the concentration of many metabolites. Several of these metabolites could synergize to optimally stimulate insulin secretion; for example, NADPH and glutamate may affect different processes required for insulin exocytosis. Although malonyl-CoA would potentiate the effect of fatty acids, glutamate could enhance the second phase of GSIS.

Unfortunately, mechanistically we know rather little about the downstream mediators of the proposed coupling factors. The identification of components involved in insulin secretion that are directly regulated by coupling factors would certainly open up new possibilities for the treatment of type 2 diabetes.

**Fatigue of β-Cell Mitochondria in Type 2 Diabetes**

A critical role for mitochondria in normal β-cell function has also become evident from the study of mitochondrial diabetes (40, 41). This hereditary disorder accounts for only about 1% of all diabetes cases, but observations from the analysis of mitochondrial diabetes may well aid in understanding some of the β-cell changes in the more common forms of the disease. Patients with mitochondrial diabetes have mutations or deletions in mtDNA, which is maternally inherited. Each cell contains many mitochondria and many copies of mtDNA (100–1000 copies/cell). Patient mitochondria display heteroplasmy with regard to mtDNA, the degree of which determines the impact of the mutation. The mitochondrial genome only encodes 37 genes, including 13 subunits of the respiratory chain complexes, ribosomal RNAs, and tRNAs. Most mitochondrial proteins are encoded by nuclear DNA and are imported into the mitochondria posttranslationally. Defects in mitochondrial DNA consequently result in impaired oxidative phosphorylation (OXPHOS).

The impact of mitochondrial DNA mutations is most pronounced in tissues with a low mitotic rate, such as brain, muscle, and islet cells. Cells depending on high ATP production, such as β-cells, are particularly susceptible to a diminished fraction of functional mitochondria, which tend to decompensate with time. This leads to apoptosis and decreased β-cell mass, which, together with defective secretion coupling, cause impaired GSIS. These patients may even require insulin therapy (41, 42). Mitochondrial diabetes illustrates how the gradual loss of mitochondrial activity is deleterious for β-cell secretory function and survival.

In analogy, decreased mitochondrial activity in β-cells would predispose individuals to develop β-cell dysfunction in type 2 diabetes. In individuals with a family history of type 2 diabetes, mitochondrial respiratory function may be sufficient during a normal workload, but may deteriorate under conditions that put stress on the β-cells, such as insulin resistance or already slightly increased glycemia (43). These states require increased insulin output to maintain glucose homeostasis. This confirms that both genetic predisposition and environmental factors could precipitate β-cell dysfunction.

The prevalence of type 2 diabetes increases with age. Unfortunately, measurements of mitochondrial activity during aging are only available for insulin target tissues. One of the take-home messages is that mitochondrial function decreases with increasing age (see below).

A key determinant of aging is the production of reactive oxygen species (ROS) (44). Irreversible damage caused by ROS interferes with mitochondrial function. Interestingly, it is the mitochondria themselves that in large part are responsible for ROS production resulting in damage to their lipids, proteins, and mtDNA. ROS are produced in the mitochondria as a side reaction of respiration. Rather than reducing molecular oxygen to water at complex IV, some electrons escape to reduce oxygen directly to form superoxide. The superoxide radical can then react further to give rise to H2O2 and hydroxyl radicals. The mtDNA is vulnerable to the formed ROS. First, ROS are generated nearby in the matrix space, and second, mtDNA is not protected by histones. A role for mtDNA damage in aging has been convincingly demonstrated in a mouse model expressing a mitochondrial DNA polymerase lacking proof-reading activity (45). These mice accumulate more mtDNA mutations and show all the phenotypes of premature aging. mtDNA mutations have also been shown to accumulate in humans as a function of age (46). β-Cell-targeted disruption of the frataxin gene, encoding a mitochondrial iron-binding protein, causes diabetes in the mouse. This was linked to increased islet ROS production and decreased β-cell mass. Patients with mutations in the frataxin gene develop a neurodegenerative disease, associated with type 2 diabetes in 20% of cases (47). Whether these patients or patients with common forms of type 2 diabetes have reduced β-cell mass as a consequence of mitochondrial dysfunction remains to be firmly established.

In the β-cell, glucose has been reported to promote ROS production through its hyperpolarizing effect on mitochondrial membrane potential (48, 49). When a similar methodology was combined with measurements of reduced pyrimidine and flavin nucleotide fluorescence in β-cells, surprisingly, physiological as well as high glucose concentrations lowered superoxide production. This was related to the initial metabolic state of the cells, i.e. suppression was most pronounced in cells with the highest initial NADPH levels (50). In view of these conflicting results, ROS generation as well as their putative detrimental actions in the β-cell need additional study.

Despite the reported low expression of superoxide dismutase and catalase in β-cells under normal conditions, an-
tioxidant systems are sufficient to prevent oxidative damage (51). Exogenous antioxidants have been investigated for the treatment of diabetes (52). For detailed discussion of β-cell apoptosis in general and implication of mitochondria in particular, the reader is referred to the recent literature (53, 54).

How can we boost the fatigued β-cell? Uncoupling protein 2 (UCP2) is an inner mitochondrial membrane protein receiving considerable attention as target for improved β-cell function (3). UCP2 is homologous to UCP1, which is expressed in brown adipose tissue. UCP1 has been shown to uncouple mitochondria to generate heat by transporting protons down their electrochemical gradient. By doing so, UCP1 dissipates the energy, disconnecting respiration from ATP synthesis. Whether UCP2 in β-cells and UCP3 in skeletal muscle carry out an identical molecular function is not yet known. It has been clearly demonstrated that increased expression of UCP2 has a negative impact on GSIS both in vitro and in vivo (3). A common human polymorphism in the promoter of UCP2 that leads to its overexpression attenuates GSIS both in vivo and in incubated islets (55). Most interestingly, in UCP2−/− mice, GSIS is stimulated (56). Introduction of the UCP2−/− mutation in the ob/ob mouse background improved glycemic control as well as GSIS in vivo. It was therefore suggested that inhibition of UCP2 function increases the coupling efficiency of respiration and ATP production. The more pronounced increase in mitochondrial membrane potential also augmented ROS production (49). Superoxide can activate UCP2. This may be a trick of nature to prevent excessive mitochondrial damage in cells that tend to hyperpolarize their inner mitochondrial membrane. Indeed, overexpression of UCP2 reduces cytokine induced ROS production and apoptosis (57). Treatment of type 2 diabetes by inhibition of UCP2 may therefore be fraught with danger because of the long-term deleterious effects on β-cells.

Among the possible treatments for type 2 diabetes is increased biogenesis of active mitochondria to replace those harboring mtDNA defects. The nuclear-encoded mitochondrial transcription factor A (TFAM) is essential for control of mtDNA copy number and transcription (58). β-Cell-specific disruption of TFAM in the mouse results in a phenotype resembling human mitochondrial diabetes with early impairment of GSIS and late loss of β-cell mass (59). Dominant negative suppression of the maturity-onset diabetes of the young 4 gene, Pdx1, in islets reduced the expression of TFAM and consequently mitochondrially encoded genes. Such islets are refractory to glucose-stimulated ATP generation and insulin secretion (60). Moreover, Pdx1−/− mice show age-dependent β-cell apoptosis (61). Conversely, increasing TFAM expression should, in theory, increase mitochondrial function. Unfortunately, TFAM-transgenic mice with up-regulated mtDNA copy number do not display increased number and function of mitochondria (58). It should be tested whether other nuclear factors are more efficient in promoting mitochondrial biogenesis.

**Fatigue of Target Tissue Mitochondria in Type 2 Diabetes**

Blood glucose is lowered by the action of insulin on its receptor on target cells. The responsiveness of insulin-sensitive tissues determines the quantity of secreted insulin required to properly regulate blood glucose concentrations. Diabetes is generally associated with insulin resistance in the main target tissues, including liver, muscle, fat, and hypothalamus (for review, see Ref. 62). Environmental factors strongly influence the development of insulin resistance. The two most important are overnutrition and lack of exercise. The resulting overweight is always associated with insulin resistance. In this article, we restrict ourselves to a brief discussion of the role of mitochondria in the pathophysiology of insulin action. Recent evidence suggests that fat tissue-derived factors play important roles in the modulation of insulin responsiveness. More classically, intracellular fatty acids, including acyl-CoA, diacylglycerol, and other metabolites, have been found to act as messengers of the well-fed state that lead to insulin resistance.

Fatty acid oxidation takes place inside mitochondria. In a well-balanced system, the mitochondria, for example of skeletal muscle, will oxidize fatty acids efficiently to prevent the intramyocellular accumulation of triglycerides. Flooding the system with free fatty acids may overwhelm the mitochondria, leading to the accumulation of intracellular lipids and the development of insulin resistance (63). Elevation of free fatty acids during a hyperinsulinemic euglycemic clamp can induce insulin resistance over a time frame of only a few hours (64). More modest elevation of fatty acids over longer periods of time in combination with a diminished oxidative capacity of mitochondria may also lead to the accumulation of triglycerides inside liver muscle or β-cells.

With increasing age, mitochondrial activity decreases, with a consequent reduction in fatty acid oxidation. This has been demonstrated, for instance, by comparing lean subjects in different age groups. The elderly (61–84 yr) in this study had developed insulin resistance, showed increased levels of intramyocellular and intrahepatic lipid accumulation, and, most importantly, had a 46% decrease in the mitochondrial ATP synthesis rate (65). Similarly, in young lean offspring of patients with type 2 diabetes, intramyocellular triglycerides were elevated, and muscle ATP synthesis was reduced (66). The reduced ATP synthesis in these experiments may either be the cause or the consequence of impaired insulin action. Reduced insulin signaling or the accumulated triglycerides may affect mitochondrial biogenesis or function (67). Alternatively, mitochondrial dysfunction could cause insulin resistance. Most likely, these two mechanisms act together in a vicious cycle of insulin resistance. Glucose tolerance remains normal, provided the β-cells can compensate by maintaining elevated circulating insulin. The development of type 2 diabetes is mainly determined by genetic factors controlling β-cell function and occurs in approximately 20% of overweight, insulin-resistant subjects. Compensation occurs by both hypersecretion of insulin and increased β-cell mass (66) (53). In summary, partial loss of mitochondrial activity goes hand in hand with the development of insulin resistance.

AMP-dependent kinase (AMPK) has been proposed as a target for prevention of the metabolic syndrome, characterized by dislipidemia, obesity, hypertension, and insulin resistance, eventually leading to type 2 diabetes. Malonyl-CoA is the gatekeeper of fatty acid oxidation by inhibition of mitochondrial carnithine-palmitoyl transferase I. The activation of AMPK by exercise, leptin, or the antidiabetic drug
metformin lowers the concentration of malonyl-CoA in the muscle through several mechanisms, including inhibition of acetyl-CoA carboxylase by phosphorylation (reviewed in Ref. 68). In contrast, metformin-induced activation of AMPK is associated with inhibition of GSIS in human islets (69). This is one of several examples of differential responses in β-cells and insulin target cells.

AMPK also signals to increase the expression of OXPHOS genes. This is mediated by the peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α). This transcription factor coordinately regulates the expression of a large set of OXPHOS genes, enhancing mitochondrial activity (70).

Interestingly, an impressive 89% (94 of 106) genes classified of OXPHOS genes, showing lower expression in muscle of type 2 diabetic patients. Two thirds of the identified OXPHOS genes are copregulated by PGC1α. The results clearly demonstrate that transcriptional regulation contributes to mitochondrial defects in the target tissues of diabetic patients (67, 70). This role of PGC1α is particularly interesting, because it was also found to be a positive regulator of mitochondrial biogenesis. However, the effects of PGC1α are tissue specific. For instance, overexpression of PGC1α in β-cells decreased the levels of several glycolytic enzymes, causing glucose intolerance after islet transplantation in vivo and impaired GSIS in vitro (71).

Nonetheless, improving mitochondrial respiration and fatty acid oxidation in insulin target tissues are attractive drug targets in type 2 diabetes. In contrast, increased levels of malonyl-CoA in the hypothalamus result in reduced food intake and weight loss in obese animals (72). This and the above-mentioned examples illustrate the difficulty of finding novel drugs for the prevention and improved treatment of type 2 diabetes. One of the difficulties lies in the multigain regulation of normal blood glucose homeostasis.

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