Mitochondrial function in normal and diabetic beta-cells

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Abstract

The aetiology of type 2, or non-insulin-dependent, diabetes mellitus has been characterized in only a limited number of cases. Among these, mitochondrial diabetes, a rare subform of the disease, is the consequence of pancreatic beta-cell dysfunction caused by mutations in mitochondrial DNA, which is distinct from the nuclear genome. The impact of such mutations on beta-cell function reflects the importance of mitochondria in the control of insulin secretion. The beta-cell mitochondria serve as fuel sensors, generating factors that couple nutrient metabolism to the exocytosis of insulin-containing vesicles. The latter process requires an increase in cytosolic Ca2+, which depends on ATP synthesized by the mitochondria. This organelle also generates other factors, of which glutamate has been proposed as a potential intracellular messenger.

Reference


DOI : 10.1038/414807a
PMID : 11742413
Mitochondria are present in most eukaryotic cells, varying in number from hundreds to thousands, and have also been visualized as a continuous network. Their origin is generally thought to lie in the endosymbiotic association of oxidative bacteria and glycolytic proto-eukaryotic cells. The phenotype resulting from the absence of mitochondria is illustrated by mammalian peripheral red blood cells, which depend entirely on glycolysis for their energy supply. The endosymbiotic hypothesis of mitochondrial origin is supported, for example, by the double membrane surrounding the organelle and a unique genome in the form of circular mitochondrial DNA (mtDNA) with bacterial characteristics. Human mtDNA comprises only 37 genes (16,569 base pairs), the most notable of which are those encoding 13 polypeptides that are part of the multisubunit enzyme complexes of the respiratory chain.

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**Figure 1** A map of human mitochondrial DNA indicating diabetes-associated mutations. The mitochondrial genome encodes 37 genes (16,569 bp): 13 polypeptides, 22 tRNAs and 2 ribosomal RNAs. The polypeptides are constituents of the respiratory-chain complexes: 7 complex I subunits (NADH dehydrogenase), 1 subunit of complex III (ubiquinol: cytochrome c oxidoreductase), 3 subunits of complex IV (cytochrome c oxidase) and 2 subunits of complex V (ATP synthase). The genes for tRNAs are presented as one-letter symbols. Mutations in four of these tRNA genes are associated with diabetes: those for leucine (L), serine (S), lysine (K) and glutamic acid (E) tRNAs.
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The principal mitochondrial substrate is pyruvate, formed essentially from amino acids, fatty acids and carbohydrates, the latter of which are derived from the dietary intake of starch and glycogen. In the cytosol, pyruvate is oxidized with the formation of CO$_2$ and the reduction of NAD$^+$ and FAD to NADH and FADH$_2$, respectively. These provide electrons to the respiratory chain upon their reoxidation (Fig. 2).

The electron flow along the respiratory chain drives the extrusion of protons from the mitochondrial matrix, which establishes a steep electrochemical gradient across the inner mitochondrial membrane. The mitochondrial membrane potential is created by complexes I, III and IV of the respiratory chain and is negative inside. ATP synthase (complex V) in the mitochondrial membrane catalyzes the condensation of ADP with inorganic phosphate to yield ATP. The generation of this "high-energy bond" is powered by the diffusion of protons back into the matrix through ATP synthase. Finally, ATP is transferred to the cytosol in exchange for ADP by the adenine nucleotide translocator (ANT). Electrons can enter the respiratory chain at both complexes I (NADH) and II (FADH$_2$). The latter complex, succinate dehydrogenase, is also an integral part of the TCA cycle. The entire bioenergetic process is regulated not only by substrate flux but also by Ca$^{2+}$, which increases the activity of several mitochondrial dehydrogenases. An increase in free cytosolic Ca$^{2+}$ that occurs at cell activation is relayed to the mitochondrial matrix by way of a Ca$^{2+}$ uniporter, thus ensuring that the energy requirements of the cell are covered.$^{13}$ Ca$^{2+}$ entry is favored by activation of the respiratory chain, for instance by glucose in the β-cell.

**Stimulus-secretion coupling of insulin release**

Blood glucose level is tightly controlled by insulin secretion from pancreatic β-cells and insulin action on liver, muscle and other target tissues. The β-cell is poised to adapt insulin secretion to the fluctuations in blood glucose concentration (Fig. 3). Glucose equilibrates across the plasma membrane and its phosphorylation by glucokinase to glucose-6-phosphate determines the rate of glycolysis and the rate of pyruvate generation.$^{14,15}$ Thus, when blood glucose is high, the rate of glycolysis in the β-cell will increase. In the β-cell, pyruvate is the main product of glycolysis, as little lactate is produced.$^{16}$ The supply of cytosolic NAD$^+$, necessary to maintain high rates of glycolysis, is therefore ensured by mitochondrial shuttles.$^{17}$ Compared to other cell types, an unusually high proportion of glucose-derived carbon enters the mitochondria in the form of pyruvate and enters the TCA cycle.

Subsequent oxidative metabolism provides the link between the glucose stimulus and insulin secretion.$^{12,13}$ In the mitochondria, pyruvate is a substrate for both pyruvate dehydrogenase and pyruvate carboxylase, thereby ensuring anaplerosis (provision of carbons) to the TCA cycle. Electron transfer from the TCA cycle to the respiratory chain by NADH and FADH$_2$, promotes the generation of ATP, which is exported to the cytosol. The increase in the ATP:ADP ratio in the cytosol causes depolarization of the plasma membrane by way of ATP-sensitive K$^+$ channels (K$_{ATP}$)$.^{18}$ This allows for the opening of voltage-sensitive Ca$^{2+}$ channels.$^{19,20}$ Similar to those expressed in other excitable cells, this is the key step by which glucose stimulates insulin secretion, as the increase in cytosolic Ca$^{2+}$ is the main trigger for exocytosis, the process by which the insulin-containing secretory granules fuse with the plasma membrane.$^{21,22}$

The importance of membrane-potential control is illustrated by the syndrome of persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI). It is most frequently caused by mutations in one of the two subunits (the sulphonylurea receptor and KIR6.2) of the K$_{ATP}$ channel, resulting in uncontrolled Ca$^{2+}$-mediated hypersecretion of insulin.$^{23}$ However, PHHI patients often retain some glucose-stimulated insulin secretion above the constitutively increased basal rate.$^{24}$ This supports in vitro observations that suggest the existence of a K$_{ATP}$ channel-independent effect of glucose.$^{25}$

**Signals and messengers for insulin exocytosis**

Ultrastructural examination of the β-cell has suggested that the mitochondria are often in close proximity to the secretory insulin
granules (Fig. 4). This may facilitate metabolism-secretion coupling, as ATP is a major permissive factor for movement of insulin granules and for priming of exocytosis.22,30 This action of ATP is distinct and complementary to its action on the respiratory chain, leading to hyperpolarization of the mitochondrial membrane \( (\Delta \psi_m) \) and generation of ATP. Subsequently, closure of \( K_{ATP} \) channels depolarizes the cell membrane \( (\Delta \psi_m) \). This opens voltage-gated \( Ca^{2+} \) channels, raising the cytosolic \( Ca^{2+} \) concentration \( ([Ca^{2+}]_c) \), which triggers insulin exocytosis. Several putative messengers, or additive signals, proposed to participate in the metabolism-secretion coupling are indicated.

**Figure 3** Model for coupling of glucose metabolism to insulin secretion in the \( \beta \)-cell. Glucose is phosphorylated by glucokinase (GK) and converted to pyruvate \( (Pyr) \) by glycolysis. Pyruvate preferentially enters the mitochondria and fuels the TCA cycle, resulting in the transfer of reducing equivalents to the respiratory chain, leading to hyperpolarization of the mitochondrial membrane \( (\Delta \psi_m) \) and generation of ATP. Subsequently, closure of \( K_{ATP} \) channels depolarizes the cell membrane \( (\Delta \psi_m) \). This opens voltage-gated \( Ca^{2+} \) channels, raising the cytosolic \( Ca^{2+} \) concentration \( ([Ca^{2+}]_c) \), which triggers insulin exocytosis. Several putative messengers, or additive signals, proposed to participate in the metabolism-secretion coupling are indicated.

**Figure 4** Electron micrograph of part of a rat \( \beta \)-cell showing mitochondria (m) and insulin-containing secretory granules (sg). The scale bar represents 0.5 \( \mu \)m. Reprinted with permission from ref. 13.

Cyclic AMP (cAMP) — the universal second messenger — is generated at the plasma membrane from ATP and potentiates glucose-stimulated insulin secretion. Many neurotransmitters and hormones, including glucagon and the intestinal hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), increase \( Ca^{2+} \) levels in the \( \beta \)-cell by activating adenyl cyclase. Although glucose itself is inefficient in stimulating the production of cAMP, permissive levels of cAMP are necessary for normal responsiveness of secretion.27 The most important target of cAMP is the exocytotic machinery, where the messenger acts both as a protein kinase A-dependent and independent manner.22,23,28-30. Among other putative nucleotide messengers, NADH and NADPH are generated by the TCA cycle intermediates.56,57. &\& The rise in cytosolic \( Ca^{2+} \) (ref. 31) and their levels rise more rapidly in the cytosol than in the mitochondria.29,58,59. &\& It remains to be determined whether pyridine nucleotides participate in \( \beta \)-cell activation through effects on ion fluxes or on the process of exocytosis.

In glucose-stimulated \( \beta \)-cells, the TCA cycle intermediate citrate is exported from mitochondria. In the cytosol, citrate carbons are transferred to coenzyme A (CoA) to form malonyl-CoA, which is a lipid precursor. Malonyl-CoA inhibits transport of fatty acids into the mitochondria and their subsequent oxidation, thereby favouring the synthesis of long-chain acyl-CoAs in the cytosol. This metabolic switch led to the proposal that malonyl-CoA acts as a metabolic coupling factor in insulin secretion.55,56,58,59. &\& The long-chain acyl-CoA hypothesis was substantiated by the observation that palmitoyl-CoA enhances \( Ca^{2+} \)-evoked insulin exocytosis.58,59. Disruption of malonyl-CoA accumulation during glucose stimulation did not, however, attenuate the secretory response.59,60. Therefore, the role of long-chain acyl-CoA derivatives in metabolism-secretion coupling remains controversial.

Studies in a permeabilized \( \beta \)-cell model have shown a direct link between mitochondrial activation and insulin exocytosis.60. Under conditions of permissive \( Ca^{2+} \) concentrations, stimulation of mitochondrial metabolism55,56,57,58,59 indicated the generation of a coupling factor.60. The factor was subsequently identified as glutamate, which can be produced from the TCA cycle intermediate \( \alpha \)-ketoglutarate or
by transamination reactions. In non-permeabilized cell preparations, a membrane-permeant derivative of glutamate was also shown to enhance the action of glucose on insulin secretion. Further studies demonstrated a positive correlation between cellular glutamate concentrations and the secretory response to glucose. At present, the mechanism of glutamate action on exocytosis is unknown. In this scenario, the classical neurotransmitter glutamate is allocated a new role, that of an intracellular messenger or cofactor in insulin secretion.

Mitochondrial dysfunction in the β-cell

It was established several decades ago that blockade of the respiratory chain inhibits glucose-stimulated insulin secretion. This conclusion was based on the use of various mitochondrial poisons and lowering of the oxygen supply to the β-cell. More recently, the activity of the respiratory chain was impaired by suppressing the production of those enzyme subunits encoded by mtDNA, which creates so-called rho0 cells. In this way, ethidium bromide and other chemical agents were still present, as a result of defective respiratory-chain activation and the loss of mitochondrial ATP production. The defect is of mitochondrial origin, as specific mitochondrial substrates were equally inefficient, whereas the secretory response to Ca2+-raising agents was still present. Elegant experiments showed that the secretory response to glucose could be restored by replenishing rho0 β-cells with normal mitochondria.

Expression of mtDNA is controlled by a nucleus-encoded transcription factor, TFAM, and disruption of this gene in the mouse leads to impaired mitochondrial function. The phenotype of the heterozygous knockout mouse revealed that the heart is highly sensitive to respiratory-chain deficiency. The β-cell-specific deletion of the Tifam gene caused a diabetic phenotype. The islets of these mice showed attenuated respiratory-chain activation and diminished secretion response to glucose. These transgenic animals represent the first model of human mitochondrial diabetes, which, it should be noted, has been linked mostly to mutations in tRNA genes (Fig. 1). Taken together, the in vitro and in vivo studies highlight the pivotal role of mitochondria in stimulus–secretion coupling in the β-cell.

mtDNA mutations and diabetes

Point mutations or deletions in mtDNA have been associated with a large spectrum of diseases, with symptoms such as muscle weakness, cardiomyopathy, optic nerve atrophy, retinal dystrophy, impaired hearing and hyperglycaemia (diabetes mellitus). Point mutations in the mitochondrial tRNA genes are the primary cause of these pathologic manifestations (Fig. 1). A specific, maternally inherited form of diabetes mellitus was first linked to mutations in the mtDNA in 1992 (refs 50, 51). Often associated with neurosensory deafness, it is also called maternally inherited mitochondrial diabetes and deafness (MIDD). The most frequent mutation encountered is the A3243G mutation in the gene for tRNALeu (bearing the anticodon UUR) (refs 51, 52). Altogether, mitochondrial diabetes accounts for approximately 1% of all cases of diabetes. The mutation reduces the stability of the oxygen supply to the mitochondria, while concurrently the defence mechanisms against these free radicals are diminishing. The mitochondria are the principal source of ROS resulting from imperfect electron transport. Normally, only 0.1% of total oxygen consumption leaks to ROS generation, but the percentage becomes greater in the ageing tissue. This deleterious process is amplified by the diminishing natural enzymatic defences (for example, catalase and superoxide dismutase). The low expression of these protective enzymes makes the β-cell particularly susceptible to ROS action. In addition to their acute effects, ROS may also lead to increased mutation in mtDNA, exacerbated by the limited repair capacity. These considerations have prompted the suggestion that ROS may participate in the impairment of glucose-induced insulin secretion seen both in ageing and in type 2 diabetes.

Further clues on β-cell function come from other forms of monogenic diabetes. Different subclasses of maturity-onset diabetes of the young (MODY) represent such monogenic forms of diabetes with autosomal dominant transmission. They are characterized by β-cell dysfunction as a result of mutations in nuclear genes. MODY1 and MODY3 have been linked to mutations in the transcription factors hepatocyte nuclear factor HNF-4α and HNF-1α, respectively. MODY3 is the most common form of this inherited disease and explains 2–5% of diabetic cases. Suppression of the HNF-1α gene in mice results in diabetes and impairment of glucose-induced insulin secretion assessed in vitro. In cellular model systems, the molecular impact of these transcription factors on β-cell function is being characterized.

Other pathophysiological considerations

In contrast to the rare monogenic mitochondrial diabetes, type 2 or non-insulin-dependent diabetes mellitus is common and polygenic in nature. Patients usually display both resistance to insulin at its target tissues (mainly skeletal muscle) as well as defective insulin secretion. Although the contribution of variations in mtDNA to the development of type 2 diabetes is unknown, a 50% decrease in mtDNA copy number in skeletal muscle of type 2 diabetics was observed. A reduced mtDNA content was also reported in peripheral blood cells from such patients even before the onset of the disease. The corresponding information for β-cells is lacking.

The diabetic state is generally characterized by accelerated tissue ageing, perhaps related to mitochondrial dysfunction. Accumulation of point mutations in mtDNA has been reported to occur in an age-dependent manner in humans. There is an age-related increase in the production of reactive oxygen species (ROS), while concurrently the defence mechanisms against these free radicals are diminishing. The mitochondria are the principal source of ROS resulting from imperfect electron transport. Normally, only 0.1% of total oxygen consumption leaks to ROS generation, but the percentage becomes greater in the ageing tissue. This deleterious process is amplified by the diminishing natural enzymatic defences (for example, catalase and superoxide dismutase). The low expression of these protective enzymes makes the β-cell particularly susceptible to ROS action. In addition to their acute effects, ROS may also lead to increased mutation in mtDNA, exacerbated by the limited repair capacity. These considerations have prompted the suggestion that ROS may participate in the impairment of glucose-induced insulin secretion seen both in ageing and in type 2 diabetes.

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basis of the defect has been attributed to deranged mitochondrial metabolism. In particular, the defective respiratory-chain activation correlated with downregulation of the TCA cycle enzyme α-ketoglutarate dehydrogenase, accompanied by an upregulation of uncoupling protein 2 (UCP2).

UCP2 is an inner mitochondrial membrane protein that tends to diminish the proton gradient generated by the respiratory chain. Its overexpression in β-cells attenuates ATP generation and insulin secretion in response to glucose. It is of interest that deletion of the UCP2 gene in mice enhances islet ATP generation and insulin secretion during glucose stimulation. In obese, diabetic hyperlipidaemic rodent models, UCP2 levels in islets were reported to be lower than or higher than lean controls. Thus, there is a simpler relationship in vivo between circulating lipids and UCP2 function. Type 2 diabetes usually have both hyperglycaemia and hyperlipidaemia. This is thought to induce the phenomenon of ‘glucolipotoxicity’ in the β-cell, leading to lipid accumulation, impaired glucose metabolism and alterations in mitochondria. Chronic exposure of β-cells to fatty acids induces changes in the expression of numerous genes. Among them, UCP2 is induced, which correlates with reduced glucose-evoked insulin secretion. This may be part of an adaptive mechanism protecting the β-cell against oxidants, as indicated by in vitro experiments. Elucidation of the adaptation of the mitochondrial machinery is complicated by the multiple influences on the β-cell in the course of the development of the diabetic state.

Possible therapeutic interventions and perspectives

Patients with mitochondrial diabetes or with one of the MODY subforms are treated like any other case of type 2 diabetes. Treatment begins with diet, but usually needs to be supplemented with oral hypoglycaemic agents. In particular, the sulphfonylureas. Eventually, blood glucose control may require insulin injections.

Mitochondrially targeted therapy of the insulin secretory defect in a rat model of type 2 diabetes has been proposed. Dimethylsucinate, a precursor of the TCA cycle intermediate succinate, was found to improve the secretory response. Diabetic patients with a mitochondrial DNA mutation have been given long-term treatment with coenzyme Q10, a component of the respiratory chain. This may be part of an adaptive mechanism protecting the β-cell against oxidants, as indicated by in vitro experiments. Elucidation of the adaptation of the mitochondrial machinery is complicated by the multiple influences on the β-cell in the course of the development of the diabetic state.

In view of the requirement for optimally functioning mitochondria, measures directed to protecting these organelles should be envisaged in diabetes prevention. Even after the disease is manifest, such therapy could preserve partial β-cell sensitivity to glucose. Further definition of the molecular mechanisms underlying the role of mitochondria in cell activation will help to target interventions in diabetes and other metabolic diseases.
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Acknowledgements
We apologize to colleagues whose papers were not cited owing to space limitations. We are grateful to L. Orci for kindly providing the Fig. 4, to T. Pozzan and P. Antinoozi for most helpful discussions and to the Swiss National Science Foundation for continued support of our research.