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


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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.

The mtDNA is transcribed and translated within the mitochondrion. The nuclear genome specifies the remainder (the majority) of the enzyme subunits and the other factors of which glutamate has been proposed as a potential intracellular messenger.

**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.

**Genes:**
- Complex I (NADH dehydrogenase)
- Complex III (Ubiquinol-Cyt c oxidoreductase)
- Complex IV (Cyt c oxidase)
- Complex V (ATP synthase)

**Transfer RNAs**
- Ribosomal RNAs
- Mutations associated with diabetes
The electron flow along the respiratory chain drives the extraction 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 catalyses 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 (FADH₂). 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 Ca²⁺, which increases the activity of several mitochondrial dehydrogenases. An increase in free cytosolic Ca²⁺ that occurs at cell activation is relayed to the mitochondrial matrix by way of a Ca²⁺ uniporter, thus ensuring that the energy requirements of the cell are covered⁷. Ca²⁺ entry is favoured 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 generation¹⁸. 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 produced¹⁴. The supply of cytosolic NAD⁺, necessary to maintain high rates of glycolysis, is therefore ensured by mitochondrial shuttles. 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 secretion¹²,¹³. 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 FADH₂ 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 the closure of ATP-sensitive K⁺ channels (K_{ATP})¹⁴. This allows the opening of voltage-sensitive Ca²⁺ channels similar to those expressed in excitatory cells. This is the key step by which glucose stimulates insulin secretion, as the increase in cytosolic Ca²⁺ is the main trigger for exocytosis, the process by which the insulin-containing secretory granules fuse with the plasma membrane⁵,¹⁶.

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 Ca²⁺-mediated hypersecretion of insulin¹⁷. However, PHHI patients often retain some glucose-stimulated insulin secretion above the constitutively increased basal rate. This supports in vitro observations that suggest the existence of a K_{ATP} channel-independent effect of glucose. Glucose is thus capable of eliciting a partial secretory response under conditions of clamped, elevated cytosolic Ca²⁺ concentration without affecting the plasma membrane potential. It can be concluded that ATP generated in the mitochondria is the main coupling messenger in insulin secretion, but that other metabolic factors are necessary for the full development of the secretory response.

### 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 exocytosis24,25. This action of ATP is distinct and complementary to its action on the KATP channel. Thus, channel activity and exocytosis are both determined by the cytosolic ATP:ADP ratio. Glucose also generates GTP, which could initiate insulin exocytosis through GTPases21,22. GTP is formed in mitochondria by the TCA cycle, but is trapped in the organelle. In the cytosol, GTP is formed mainly through the action of nucleoside diphosphate kinase. In contrast to ATP, GTP is capable of initiating insulin exocytosis in a Ca2+-independent fashion, which qualifies it as a messenger molecule22–24. It is not known whether GTP acts by way of a monomeric or heterotrimeric G protein that directly controls exocytosis22,25.

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 cAMP levels in the β-cell by activating adenylyl cyclase26. Although glucose itself is inefficient in stimulating the production of cAMP, permissive levels of cAMP are necessary for normal responsiveness of secretion27. The most important target of cAMP is the exocytotic machinery, where the messenger acts both in a protein kinase A-dependent and independent manner22,23,28–30. Among other putative nucleotide messengers, NADH and NADPH are generated by glucose metabolism9. The rise in pyridine nucleotides precedes the increase in cytosolic Ca2+ (ref. 31) and their levels rise more rapidly in the cytosol than in the mitochondria32. It remains to be determined whether pyridine nucleotides participate in β-cell activation through effects on ion fluxes or on the process of exocytosis.

In glucose-stimulated β-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 favoring 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 secretion39. The long-chain acyl-CoA hypothesis was substantiated by the observation that palmitoyl-CoA enhances Ca2+-evoked insulin exocytosis40. Disruption of malonyl-CoA accumulation during glucose stimulation did not, however, attenuate the secretory response40. Therefore, the role of long-chain acyl-CoA derivatives in metabolism–secretion coupling remains controversial.

Studies in a permeabilized β-cell model have shown a direct link between mitochondrial activation and insulin exocytosis40. Under conditions of permissive Ca2+ concentrations, stimulation of mitochondrial metabolism39 indicated the generation of a coupling factor40. The factor was subsequently identified as glutamate39, which can be produced from the TCA cycle intermediate α-ketoglutarate or
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 rh0 cells. In this way, ethidium bromide and other chemical agents were still present. Elegant experiments showed that the secretory response to glucose could be restored by replenishing rh0 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 is lethal. 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 T-fam gene caused a diabetic phenotype. The islets of these mice showed attenuated respiratory-chain activation and diminished secretory 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 pathological 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 called diabetes mellitus, 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) and as defective insulin secretion. Although the contribution of variations in mtDNA to the development of type 2 diabetes is unknown, a 15% 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 in 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 defenses (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 differently on cell viability according to tissue sensitivity to apoptosis. The A3,243G mutation has been associated with reduction of islet mass involving both β-cells and the neighbouring glucagon-producing α-cells. In post-mortem studies, diabetic patients with the A3,243G mutation showed a degree of heteroplasmly ranging from 32% to 63% (refs 55, 56). It is possible that the extent of heteroplasmly may participate in the lowering of the cellular energy capacity below the bioenergetic threshold. The consequence of this would be that the organelle could no longer fulfill the energetic and signalling requirements for glucose-stimulated insulin secretion, a concept also supported by clinical observations. In contrast, secretion induced by the receptor agonist glucagon, acting by way of cAMP effects on exocytosis, was preserved in A3,243G patients. This form of diabetes may or may not require insulin injections, and is usually not associated with resistance of muscle and other tissue to the action of the hormone.

The molecular diagnosis of mitochondrial diabetes is complicated by an invariably low degree of heteroplasmly in the peripheral white blood cells usually used for genetic analysis. Ex vivo mitochondrial dysfunction associated with the A3,243G mutation was demonstrated in skin fibroblasts or in cells enriched for patient mutant mitochondria. A similar conclusion was drawn from studies on another mtDNA mutation associated with a neurodegenerative disease. The mitochondrial diabetes phenotype illustrates the importance of normal respiratory-chain function in the β-cell for glucose homeostasis.

Other pathophysiological considerations

In contrast to the rare monogenic mitochondrialdiabetes, 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 15% 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 in such patients even before the onset of the disease. The corresponding information for β-cells is lacking.

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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 β-cell ATP generation and insulin secretion during glucosestimulation. In obese, diabetic hyperlipidaemic rodent models, UCP2 levels in islets were reported to be either lower or higher than lean controls. Thus, there is no simple relationship in vivo between circulating lipids and UCP2 function. Type 2 diabetic patients 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 sulphonylureas. 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. Dimethylsuccinate, 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 resulted in improved insulin secretion but, disappointingly, did not affect diabetic complications (nephropathy, retinopathy and neuropathy). More efficient treatments should be envisaged for such patients. The ultimate goal is the replacement of mutated DNA with normal mtDNA by either gene or cell therapy. Despite much research, these techniques are not yet available. It was, however, shown recently that complementation of normal mtDNA in mice carrying mutated mtDNA restores mitochondrial function. This may open new perspectives for gene therapy of mitochondrial diseases.

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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