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Reference


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Role of mitochondria in metabolism-secretion coupling of insulin release in the pancreatic β-cell

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Abstract. The control of insulin secretion from the pancreatic β-cell involves a complex cascade of events in which the mitochondria play a central role. In the consensus model this role is essentially restricted to the production of ATP promoting membrane depolarisation and a rise in cytosolic [Ca\(^{2+}\)]. Evidence for the generation of an additional mitochondrial factor implicated in metabolism-secretion coupling is provided in this review. Although not yet identified, the formation of this putative factor requires an increase of [Ca\(^{2+}\)] in the mitochondrial matrix together with a supply of carbons to the tricarboxylic acid (TCA) cycle. In this model, calcium activates matrix dehydrogenases, in particular those of the TCA cycle. This enables the synthesis of the mitochondrial factor from the TCA cycle intermediates. Experimental evidence gathered in permeabilised cells largely supports this model.

1. Introduction

Blood glucose homeostasis is mainly controlled by insulin through its secretion by the pancreatic β-cell and its action on target tissues ([47], and references therein). The most important physiological nutrient stimuli of insulin release are glucose and leucine [35]. The action of these secretagogues is potentiated by gastrointestinal hormones, which activate either adenylate cyclase or phospholipase C [47]. In contrast to the receptor-mediated signal transduction triggered by the hormones, glucose and leucine generate signals following their metabolism by the β-cell. Glucose enters the β-cell by facilitated diffusion and undergoes glycolysis before its final oxidation in the mitochondria, whereas leucine is directly metabolised by the mitochondria.

Non-insulin-dependent-diabetes-mellitus (NIDDM), or type II diabetes, is a polygenic disease characterised by impaired insulin secretion [32] often combined with insulin resistance [17]. A rare subform of NIDDM referred to as maternally inherited diabetes and deafness (MIDD) has been linked to mutations in the mitochondrial genome. These mutations involve deletions or point mutations such as that in tRNA\(^{Leu(UUR)}\) at position 3243 also giving rise to mitochondrial encephalomyopathy, lactic acidosis and stroke-like syndromes (MELAS) [22]. In MIDD there is a progressive impairment of insulin secretion, which may eventually lead to severe insulin deficiency reminiscent of type I (insulin-dependent) diabetes.

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2. Signal generation in insulin secretion

In the pancreatic β-cell, mitochondrial metabolism plays a pivotal role in the generation of signals coupling glucose recognition to insulin secretion [11,27,30,35]. Metabolism of nutrient stimuli in the β-cell causes the closure of ATP-sensitive K⁺ (K_ATP) channels and depolarisation of the plasma membrane potential [38]. This leads to Ca²⁺ influx through voltage-gated Ca²⁺ channels and a rise in cytosolic Ca²⁺ ([Ca²⁺]_c) [45,47]. The increase in [Ca²⁺]_c is the main trigger of exocytosis, the process by which the insulin containing secretory granules fuse with the plasma membrane [4,38,47]. Ca²⁺, the universal second messenger, controls numerous cellular functions including mitochondrial metabolism [15,28]. An increase in [Ca²⁺]_c is relayed to the mitochondria with the consequent rise in matrix Ca²⁺ concentration ([Ca²⁺]_m) [36,37]. This elevation in [Ca²⁺]_m leads to stimulation of Ca²⁺-sensitive NADH-generating dehydrogenases [6,14,28,34,39,43]. The latter process potentiates the generation of NADH which transfers reducing equivalents to the respiratory chain, thereby ensuring adequate ATP synthesis to balance the augmented energy needs of cell activation [28]. Incubation of islet β-cells in the absence of extracellular Ca²⁺ results in a decrease of glucose oxidation without an apparent change in the ATP/ADP ratio [43]. This emphasises the observation that a rise in [Ca²⁺]_m is not necessary for the initial signal generation by the mitochondria in response to glucose, i.e., the increases in NAD(P)H [33] and in the ATP/ADP ratio [31]. The ensuing elevation in [Ca²⁺]_m augments [Ca²⁺]_m which in turn exerts a positive feed-back on the mitochondrial metabolism, thereby ensuring maintenance of the metabolism-secretion coupling beyond the initiating events.

3. Role of the mitochondria in signal generation

Although an increase in [Ca²⁺]_c is a necessary event in insulin secretion, glucose is also capable of stimulating secretion in a manner not involving K_ATP channels and Ca²⁺ influx. This is observed at permissive and constant [Ca²⁺]_c [9,10,40]. This effect could be explained by an activation of the mitochondrial metabolism triggered by an increase in [Ca²⁺]_m. We reported that glucose induces [Ca²⁺]_c and [Ca²⁺]_m rises which are associated with the stimulation of insulin secretion [18]. A pivotal role of the mitochondria has also been highlighted by the observations that insulin secretion-deficient diabetes melitus can be associated with mutations in the mitochondrial genome which encodes several subunits of the respiratory chain complexes [22]. Elimination of the mitochondrial DNA in β-cell lines after culture with mutagenic compounds results in the complete inhibition of glucose induced insulin secretion. This was associated with impaired oxygen consumption [44] and defective ATP production [20,46]. These studies emphasise the crucial role of mitochondrial metabolism for the glucose mediated [Ca²⁺]_c rise and insulin release and corroborate earlier investigations using mitochondrial poisons or reduced oxygen tension (reviewed in [8]). Transfer of reducing equivalents to the electron transport chain increases the mitochondrial membrane potential (ΔΨ_m), which enhances the driving force for mitochondrial Ca²⁺ uptake mediated by a low affinity uniporter [13], the threshold of which is approximately 300–400 nM [15]. This ΔΨ_m-dependent Ca²⁺ entry permits an amplification of [Ca²⁺]_m, relative to [Ca²⁺]_c, further favouring the stimulation of the Ca²⁺-sensitive dehydrogenases [18,37]. Stimulatory glucose concentrations have indeed been shown to promote hyperpolarization of ΔΨ_m in β-cells [7,12] and in the differentiated β-cell line INS-1 [24]. As expected, ΔΨ_m was depolarised and insensitive to glucose application in cells deficient in mitochondrial DNA [20].

To characterise the role of mitochondrial activation in metabolism-secretion coupling, ΔΨ_m, [Ca²⁺]_m and insulin secretion were measured in intact and permeabilised cells. In order to study the link between
Fig. 1. Effects of the TCA cycle intermediate succinate (Suc) on $[\text{Ca}^{2+}]_c$, $[\text{Ca}^{2+}]_m$ and insulin secretion in permeabilized INS-1 cells. Cells expressing the $\text{Ca}^{2+}$-sensitive photoprotein aequorin in the cytosol (a) or in the mitochondria (b) were permeabilized with $\alpha$-toxin and perfused with an intracellular buffer containing 500 nM free $\text{Ca}^{2+}$ and 10 mM ATP. The effects of Suc on $[\text{Ca}^{2+}]_m$ (b) and insulin secretion (c) were measured simultaneously. The efficient clamping of $[\text{Ca}^{2+}]_c$ is demonstrated in panel (a). Reprinted with permission from Maechler et al. [24].

$[\text{Ca}^{2+}]_c$, mitochondrial activation and the exocytosis of insulin, we have permeabilised cells with staphylococcus $\alpha$-toxin, generating small holes of only 2–3 nm in the plasma membrane and, therefore, largely preserving cellular integrity. This approach renders possible the clamping of cytosolic [ATP] and $[\text{Ca}^{2+}]_c$ at permissive concentrations, as well as the direct stimulation of mitochondrial metabolism by various non-lipophilic substrates. The preparation permits the on-line monitoring of $[\text{Ca}^{2+}]_m$ and the simultaneous measurement of insulin secretion [24]. As illustrated in Fig. 1(a), the $[\text{Ca}^{2+}]_c$ could be clamped to the permissive concentration of 500 nM. This was monitored as photons emitted by the $\text{Ca}^{2+}$-sensitive photoprotein aequorin stably expressed in INS-1 cells, and still retained after $\alpha$-toxin permeabilisation. The tricarboxylic acid (TCA) cycle intermediate succinate transfers reducing equivalents to the electron transport chain in a $\text{Ca}^{2+}$-independent manner on complex II at succinate dehydrogenase. In permeabilised INS-1 cells, succinate hyperpolarises the $\Delta\psi_m$ [24,26]. As a consequence $[\text{Ca}^{2+}]_m$ is rapidly increased in a biphasic fashion (Fig. 1(b)). This mitochondrial activation was associated with a marked stimulation of insulin release (Fig. 1(c)). The importance of the $[\text{Ca}^{2+}]_m$ rise for the secretory response to succinate was demonstrated by the inhibition of secretion when $\text{Ca}^{2+}$ entry into the mitochondria was suppressed with ruthenium red, a blocker of the uniporter [24]. Furthermore, an increase of $[\text{Ca}^{2+}]_c$ from 100 to 500 nM markedly enhances succinate oxidation, which underscores the importance of the rise in $[\text{Ca}^{2+}]_m$ [26].
Fig. 2. Model for the role of mitochondria in metabolism secretion coupling in the pancreatic β-cell (see text for description). Glc – glucose; GAP – glyceraldehyde3-phosphate; 1,3DPG – 1,3-diphosphoglycerate; Pyr – pyruvate; LDH – lactate dehydrogenase; TCA – tricarboxylic acid cycle; red. equ. – reducing equivalents; GP – glycerophosphate; met-Suc – methyl-succinate; Leu – L-leucine; KIC – α-ketoisocaproate; ΔΨm – mitochondrial membrane potential; ΔΨc – plasma membrane potential; [Ca2+]c – cytosolic [Ca2+]; [Ca2+]m – mitochondrial [Ca2+]; mt-factor – mitochondrially-derived factor.

Citrate, another TCA cycle intermediate which does not increase [Ca2+]m in the permeabilised INS-1 cells, is unable to elicit insulin release unless [Ca2+]m is raised during its application [24]. The latter effect can be achieved with α-glycerophosphate which hyperpolarises ΔΨm through activation of complex II by the FAD-linked glycerophosphate dehydrogenase. It is noteworthy that α-glycerophosphate alone, while raising [Ca2+]m, fails to trigger insulin secretion [24]. Taken together, these results suggest that insulin secretion caused by mitochondrial substrates involves the generation of factors which require both a rise of [Ca2+]m and a supply of carbons for the TCA cycle. The experimental conditions allow the conclusion that the activation of mitochondrial metabolism generates factors other than Ca2+ and ATP, which are capable of inducing insulin exocytosis.

ATP has been considered to be the principal metabolic coupling factor in glucose-induced insulin secretion [27,30]. The overwhelming amount of ATP is generated through oxidative phosphorylation in the mitochondria not only by glucose but also by other metabolisable insulin secretagogues such as leucine, its deamination product α-ketoisocaproic acid, and methyl-succinate [1,25]. The initiating event in stimulus-secretion coupling is the closure of KATP channels in the β-cell plasma membrane, resulting in membrane depolarisation and voltage gated Ca2+ influx [45,47]. In the mitochondria, the generation of reducing equivalents by substrates and their subsequent transfer to the respiratory chain causes hyperpolarisation of the ΔΨm. This permits the rise in [Ca2+]m to concentrations sufficient for the activation of NADH-generating dehydrogenases [28,39]. This feed forward effect of Ca2+ depends on permissive levels of [Ca2+]c and on the availability of substrates for the TCA cycle, ensuring anaplerotic input [6,35] (Fig. 2). The enzymatic profiles of the β-cell are uniquely poised to achieve optimal entry of glucose carbons into the TCA cycle. Thus, escape of carbons in the form of lactate is minimised through low
lactate dehydrogenase activity [41,42] coupled with high expression of the mitochondrial FAD-linked α-glycerophosphate dehydrogenase which insures reoxidation of cytosolic NADH [5,42]. Moreover, the β-cell mitochondria display high activity of pyruvate carboxylase, explaining why approximately 50% of the pyruvate entering the mitochondria is converted to the TCA cycle intermediate oxaloacetate, the remainder being decarboxylated by pyruvate dehydrogenase [23,41].

Insulin secretion can be stimulated with potassium, causing membrane depolarisation, followed by [Ca²⁺]c and [Ca²⁺]m rises [18], but potassium does not reproduce the prominent and long-lasting secretory response to glucose [10,47]. This difference can be explained by the lack of carbon supply to the TCA cycle, as is also the case for α-glycerophosphate in permeabilised cells [24]. We have suggested that an increase in both [Ca²⁺]m and TCA cycle activity govern the production of the putative coupling factor of mitochondrial origin distinct from ATP and Ca²⁺ itself. The messenger molecule could be either one of the TCA cycle intermediates or a derived metabolite. Cytosolic citrate is the precursor of the putative second messenger malonyl-CoA [35]. This pathway is not likely to be implicated, as citrate failed to cause insulin secretion in permeabilised cells [24]. The nature of the mitochondrial factor, which should cooperate with ATP and Ca²⁺ to ensure the normal and complete secretory response to glucose, remains to be established.

4. Role of the mitochondria in the desensitisation of metabolism-secretion coupling

We sought to probe further the existence of the mitochondrial factor by studying desensitisation of insulin secretion. It has been shown that exposure of β-cells to short successive pulses of glucose resulted in the inhibition of the insulin secretory response to the second stimulus [29]. Desensitisation could occur at any one of the steps of the metabolism-secretion coupling including insulin exocytosis itself [16].

Using INS-1 cells stably expressing aequorin in the cytosol or in the mitochondria, we have previously shown that desensitisation of insulin secretion is associated with a parallel reduction of the [Ca²⁺]c and [Ca²⁺]m responses, the latter being more pronounced [18]. When the glucose evoked [Ca²⁺]m rise is prevented by either a protonophore or an inhibitor of complex I, the second response to glucose (in the absence of inhibitors) is not desensitised [19]. To monitor cytosolic [ATP] in living cells we used an INS-1 subline expressing high levels of cytosolic luciferase [25]. The cells were exposed to two successive 5 min pulses of 12.8 mM glucose separated by a 5 min recovery period at 2.8 mM glucose. In contrast to the desensitisation of [Ca²⁺]m and insulin secretion, the 23% increase in [ATP] was the same during both stimulation periods [26]. This result is taken as evidence for the existence of the mitochondrial coupling factor generated under conditions of increased [Ca²⁺]m and distinct from ATP.

In order to investigate the role of the mitochondria in the desensitisation process, glycolysis was bypassed by the use of the TCA cycle intermediate succinate. In intact cells, two applications of the cell permeant methyl-ester form of succinate reproduced the results obtained with glucose, with respect to insulin secretion, [Ca²⁺]m and ATP generation. To study the dissociation between the [Ca²⁺]m signal and ATP generation, experiments were performed in α-toxin permeabilised INS-1 cells which permit the control of the mitochondrial environment in terms of cytosolic [Ca²⁺] and [ATP]. Under identical conditions as those described in Fig. 1, i.e., 500 nM free Ca²⁺, the [Ca²⁺]m response was completely inhibited during the second exposure to succinate [26]. This inhibitory effect could be reproduced with α-glycerophosphate which also transfers reducing equivalents to complex II of the electron transport chain, in this case originating from the cytosol. The site of desensitisation is not the respiratory chain,
since succinate was able to induce similar hyperpolarisation of $\Delta V_m$ during the two consecutive applications. This is in agreement with the absence of desensitisation of the ATP response observed in intact cells.

The desensitisation of the $[\text{Ca}^{2+}]_m$ response induced by KCl in intact cells is indirect evidence for the inhibitory effect of $\text{Ca}^{2+}$ on its own handling [18]. KCl (20 mM) evokes increases in $[\text{Ca}^{2+}]_c$ up to 2 $\mu$M. In permeabilised cells, direct applications of $\text{Ca}^{2+}$ in this concentration range clearly caused desensitisation of the $[\text{Ca}^{2+}]_m$ response to the second pulse [26]. Although the molecular nature of the mitochondrial $\text{Ca}^{2+}$ uniporter has not been identified, it appears to have properties similar to those of plasma membrane $\text{Ca}^{2+}$ channels [21]. It could well be that the desensitisation evoked by an increase in $[\text{Ca}^{2+}]_m$ involves a mechanism similar to that described for L-type $\text{Ca}^{2+}$ channels [48]. In the present discussion the role of $\text{Ca}^{2+}$ as an activator of mitochondrial metabolism has been emphasised. An additional function of mitochondria in cellular $\text{Ca}^{2+}$ homeostasis has been proposed. Indeed, $\text{Ca}^{2+}$ mobilised from the endoplasmic reticulum was shown to be transferred to the mitochondria [3] and $\text{Ca}^{2+}$ buffering by mitochondria has been implicated in the regulation of $[\text{Ca}^{2+}]_c$ microdomains [2].

5. Conclusion

A key role has been attributed to ATP as a coupling factor in the consensus model of nutrient-induced insulin secretion [27,30]. There is accumulating evidence that an increase in the concentrations of $\text{Ca}^{2+}$ and ATP in the cytosol is a necessary though not sufficient condition for an optimal secretory response [10,24,26]. The existence of a mitochondrial factor capable of eliciting insulin exocytosis has been demonstrated with several experimental paradigms described in this review. The generation of this factor depends on an increase in $[\text{Ca}^{2+}]_m$ resulting in the activation of the $\text{Ca}^{2+}$-sensitive dehydrogenases in the mitochondrial matrix. However, a $[\text{Ca}^{2+}]_m$ rise per se is ineffective but when combined with a carbon supply to the TCA cycle, insulin secretion occurs. In cells depleted of mitochondrial DNA, neither glucose in intact cells nor succinate in the permeabilised cell preparation elicits insulin release [20]. Furthermore, mitochondrial desensitisation in the $\beta$-cell underlies the desensitisation of the secretory response, despite normal ATP generation. Under these conditions, the mitochondrial factor is missing due to insufficient elevation of $[\text{Ca}^{2+}]_m$. Current work aims at defining the mitochondrial factor(s) coupling metabolism to secretion in the $\beta$-cell.

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