Regulation of insulin secretion by uncoupling protein

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Abstract
UCPs (uncoupling proteins) can regulate cellular ATP production by uncoupling oxidative phosphorylation. UCP2 is expressed in islet \( \beta \)-cells and its induction reduces glucose-stimulated insulin secretion. Under physiological conditions, superoxide, formed as a by-product of respiration, activates UCP2. This leads to reduced ATP production, which impairs closure of the ATP-dependent \( K^+ \) channels to prevent insulin secretion. It is suggested that the physiological role of UCP2 is to prevent excessive superoxide generation through a feedback loop. UCP2 induction may also alter fatty acid metabolism by altering NAD/NADH or by facilitating cycling of fatty acid anions. Recently, UCP2 has been proposed to keep insulin secretion low during starvation, a function under the control of the transcription co-repressor, surtin-1, which has been shown to bind to the UCP2 promoter. Pathological UCP2 expression or activation may suppress glucose-stimulated insulin secretion to the extent that diabetes onset is hastened. In \( ob/ob \) mice, induction of UCP2 at age 5 weeks precedes development of insulin secretion defects and hyperglycaemia. Activating protein kinase A-dependent pathways can normalize insulin secretion in UCP2-overexpressing islets. Conversely, lowering UCP2 expression may promote increased insulin secretion. UCP2 knockout mice were protected from the diabetogenic effects of a high-fat diet and their islets exhibited increased sensitivity to glucose and elevated ATP/ADP. These results support a role for UCP2 as a gene contributing to the pathogenesis of Type 2 diabetes.

Introduction
UCP2 (uncoupling protein-2) was identified in 1997 and a role in energy balance was quickly postulated based on its strong homology with UCP1, which regulates thermogenesis in rodents and neonatal mammals. However, it now appears that UCP2’s primary physiology is tissue-dependent and may include protection from oxidative stress (particularly in the brain) [1] and participation in innate immunity [2], in addition to regulation of insulin secretion. The importance of UCP2 as a modulator of insulin secretion is underscored by genetic analyses as well as studies of physiological and pathophysiological processes altered by UCP2.

Genetic studies
Recent studies of several populations indicate that a polymorphism in the UCP2 promoter (–866G/A) (A/A allele) associates with higher UCP2 expression, Type 2 diabetes mellitus and decreased insulin secretion (reviewed in [3]). The A/A allele may also modulate plasma lipids [4] and blood pressure [5]. The A55V mutation also associates with Type 2 diabetes mellitus in some studies [6].

Expression of UCPs in islets and its regulation
Protein expression of UCP2 is tightly regulated and mRNA detection is not indicative of protein expression [7]. However, UCP2 is detected in pancreatic \( \beta \)-cells by immunohistochemistry [8] and immunoblotting [9,10]. Transcriptional up-regulation in \( \beta \)-cells is mediated by SREBP-1c (sterol-regulatory-element-binding protein-1c) [11], PPAR-\( \gamma \) (peroxisome-proliferator-activated receptor-\( \gamma \)) [12] and PPAR-\( \alpha \) [13,14]. These transcription factors are activated upon exposure of islets to either elevated glucose or NEFAs [non-esterified (‘free’) fatty acids] and result in decreased glucose-stimulated insulin secretion. Cold temperature exposure, acting via the sympathetic nervous system, has also been shown to increase UCP2 expression via activation of PPAR-\( \gamma \) co-activator-1 [15]. Our understanding of how UCP2 expression is decreased is less complete but exposing \( \beta \)-cells to interleukin-1\( \beta \) is reported to lower UCP2 mRNA [16]. Recently, Sirt1 (surtn-1), which regulates the metabolic response to caloric restriction, was shown to repress UCP2 by binding to its promoter, thereby increasing insulin secretion [17,18].

\( \beta \)-Cell UCP in diabetes
In addition to the genetic studies cited above, a recently published prospective study of nearly 3000 non-diabetic men provides strong evidence that UCP2 –866A allele (and also UCP3 –55T allele) predicts early development of Type 2
Type 2 diabetes mellitus is a state in which both plasma lipids (triacylglycerol and NEFA) and glucose are elevated, which leads to increased expression of UCP2 in islets. Animal models in which UCP2 has been shown, at the protein level, to be increased include ob/ob mice [9] and the diet-induced obesity (using a diet high in fat) mice [10]. However, these studies examined UCP2 expression only after diabetes was established. Recently, a longitudinal study of diabetes development and UCP2 expression was conducted in ob/ob mice [20]. At 5 weeks of age, UCP2 protein expression was increased in islets from ob/ob mice. Metabolically, the mice were normal with respect to fasting glucose, triacylglycerol and NEFA. However, they were already insulin-resistant and had increased insulin secretion in compensation. By 8 weeks, UCP2 remained elevated and was associated and islet ATP reduced when the mice are challenged with a high-fat diet [29]. Conversely, infecting β-cells with a vector encoding carnitine palmitoyltransferase-I, the rate-limiting enzyme for fatty acid oxidation, leads to reduced glucose-stimulated insulin secretion but no change in UCP2 expression [30]. The difference in UCP2 expression between the latter two studies is likely to be due to the availability of NEFA as signalling molecules. With induction of hormone-sensitive lipase also develop diabetes with impaired glucose-stimulated insulin secretion. Although islet triacylglycerol content is lower, UCP2 is up-regulated and islet ATP reduced when the mice are challenged with a high-fat diet [29]. Conversely, infecting β-cells with a vector encoding carnitine palmitoyltransferase-I, the rate-limiting enzyme for fatty acid oxidation, leads to reduced glucose-stimulated insulin secretion but no change in UCP2 expression [30]. The difference in UCP2 expression between the latter two studies is likely to be due to the availability of NEFA as signalling molecules. With induction of hormone-sensitive lipase, liberation of NEFA from triacylglycerol would increase the intracellular concentration of ligands for PPAR-α and -γ, to induce UCP2 mRNA expression. In the case of carnitine palmitoyltransferase-I overexpression, increased oxidation of NEFA would decrease ligands for the PPARs. In agreement with this, induction of PPAR-γ expression in islets increases UCP2 protein and impairs glucose-stimulated insulin secretion [12].

The puzzle of a physiological role(s) for islet UCP

Significant up-regulation of UCP2 is clearly detrimental to the function of β-cells. The question is whether UCP2 plays an important physiological role in regulating insulin secretion. Three hypotheses have been considered for β-cells. The first is that the cellular content of coenzymes and thus the metabolic activity of cells may be regulated by mild alterations in UCP2 activity. Increased uncoupling permits respiration to continue without creating additional ATP, thus allowing for unimpeed respiration. Such an effect would maintain higher amounts of oxidized coenzymes (e.g. NAD+) [31], thereby modulating processes such as lipid metabolism [32]. Interestingly, both starvation and Sirt1 knockout in β-cells induces UCP2 and reduces cellular NADH generation in response to glucose [17], which would increase the NAD/NADH ratio. Therefore, in periods of fasting, increasing UCP2 expression and activity may limit insulin secretion. The elevation of circulating NEFA that occurs during fasting may also contribute by activating PPAR-α-dependent pathways [33] to increase UCP2. This hypothesis is summarized in Figure 1. It suggests that the true
The physiological role of UCP2 is to help prevent hypoglycaemia in the fasting state, when calories are restricted and circulating NEFAs are elevated. It then follows that over-nutrition, which also increases circulating NEFA, could inappropriately induce UCP2 and contribute to insufficient insulin secretion in response to glucose.

The second hypothesis is that UCP2 regulates fatty acid metabolism by promoting transport of fatty acid anions out of the mitochondrial matrix, as has been proposed for UCP3 [34,35]. Generally speaking, UCP2 expression is elevated by increased fatty acid concentrations [36], creating a need for increased fatty acid oxidation in order to prevent build-up of intracellular triacylglycerol or ceramide, which can be toxic to β-cells [27]. Although fatty acid transport into the mitochondrial matrix is normally regulated by carnitine palmitoyltransferase-I, under conditions of excess there may be diffusion of neutral fatty acids across the membranes. Increased egress of fatty acid anions via UCP could prevent peroxidation of mitochondrial lipids [34] and increase the efficiency of β-oxidation by allowing cycling and preventing overload [35]. Although there is experimental evidence to support this hypothesis with respect to UCP3 in muscle cells, the hypothesis has not been tested for UCP2 in β-cells. However, it has been noted that overexpression of UCP2 decreases palmitate oxidation [26], whereas null expression increases palmitate oxidation [10]. These results are consistent with UCP2 regulating the supply of fatty acyl-CoA by transporting fatty acid anions out of the mitochondria.

The third hypothesis is that UCP2 protects cells from the damaging effects of reactive oxygen species by limiting their production. Fuel-induced hyperpolarization of the mitochondrial inner membrane predicts the rate of mitochondrial metabolism and insulin secretion [37]. However, increased fuel oxidation also increases the production of oxygen radicals. Mild uncoupling shortens the half-life of molecular oxygen and decreases its availability to react with stray electrons to create superoxide by increasing the reduction of O₂ to H₂O [32]. UCP2 knockout mice generate more superoxide in islets and other tissues [2,10,21,38] and studies of neural tissues suggest that UCP2 decreases susceptibility to damage from stroke and other neurodegenerative conditions [1]. Overexpression of UCP2 protects clonal β-cells from death caused by exposure to exogenous H₂O₂ and such exposure increases endogenous UCP2 expression [39]. β-Cells express relatively low levels of protective enzymes such as glutathione peroxidase, catalase and superoxide dismutase [40]; perhaps UCP2 activity provides alternative protection. However, UCP2 knockout mouse islets do not appear to be damaged by oxidative stress. Paradoxically, evidence suggests that although β-cell apoptosis is higher in UCP2 knockout than wild-type mice fed a chow diet, there was no further increase after feeding a high-fat diet. Furthermore, there was increased proliferation of the β-cells of UCP2 knockout mice [10] and the deleterious effects of high-fat diet on glucose-stimulated insulin secretion were reduced [10]. Thus the ability of UCP2 to play an important role in regulating oxidative stress in the β-cell is not established.

Circumventing UCP2 in diabetes

Working from the premise that increased UCP2 expression or activity (alone, or in concert with other regulators) in diabetes can suppress insulin secretion sufficiently to cause hyperglycaemia, then strategies that reduce UCP2 should be helpful. However, the strategy is complicated by the fact that UCP2 is protective in the central nervous system; therefore tissue specificity is required.

Alternatively, bypassing UCP2 effects without affecting gene expression could be useful. Recently, we showed that elevating cAMP concentrations in β-cells can normalize K<sub>ATP</sub> channel activity, Ca<sup>2+</sup> influx and insulin secretion by a PKA-dependent pathway in UCP2-overexpressing cells [23]. Agents that act via adenylyl cyclase and cAMP, such as gua-482-guayalike peptide-1, have already been shown to improve insulin secretion in diabetes by acting on a number of cellular pathways [41]. These recent results suggest another means by which these compounds may act therapeutically.

The amino acid taurine has both antioxidant [42] and Ca<sup>2+</sup> mobilizing properties [43]. Treatment with taurine of UCP2 overexpressing β-cells normalized the ATP/ADP ratio and K<sub>ATP</sub> channel activity. The mechanism was shown to be dependent on Ca<sup>2+</sup> entry into the mitochondria [43], which would then improve dehydrogenase activity of electron transport chain enzymes. Glucose-stimulated insulin secretion was also improved by taurine supplementation of the culture medium [43]. Thus two distinct mechanisms exist for bypassing UCP2 activity to improve insulin secretion.

Conclusion

UCP2 is a negative regulator of insulin secretion. Its induction in Type 2 diabetes in both humans and rodents may contribute
to insufficient insulin secretion. Strategies to reduce UCP2 need to be carefully devised, given its protective role in the brain. However, it may be possible to bypass the negative effects of UCP2 by several means, such as was demonstrated by increasing cAMP.

References


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