Uncoupling Protein 2 and Islet Function

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Stressors such as chronic hyperglycemia or hyperlipidemia may lead to insufficient insulin secretion in susceptible individuals, contributing to type 2 diabetes. The molecules mediating this effect are just beginning to be identified. Uncoupling protein (UCP)-2 may be one such negative modulator of insulin secretion. Accumulating evidence shows that β-cell UCP2 expression is upregulated by glucolipotoxic conditions and that increased activity of UCP2 decreases insulin secretion. Mitochondrial superoxide has been identified as a post-translational regulator of UCP2 activity in islets; thus, UCP2 may provide protection to β-cells at one level while simultaneously having detrimental effects on insulin secretion. Interestingly, the latter appears to be the dominant outcome, because UCP2 knockout mice display an increased β-cell mass and retained insulin secretion capacity in the face of glucolipotoxicity. Diabetes 53 (Suppl. 1):S136–S142, 2004

Healthy pancreatic β-cells are poised to respond rapidly and efficiently to acute changes in circulating nutrient availability to maintain metabolic homeostasis. However, it is well recognized that chronic exposure to overnutrition, such as what occurs in obesity, results in a blunting of the insulin response to an acute stimulus. The mechanisms by which this adaptation occurs are hotly debated but, based on recent analyses of gene expression using oligonucleotide microarray technology, include multiple enzymes involved in glucose and fat metabolism (1,2). Key rate-limiting enzymes, such as carnitine palmitoyl transferase (CPT)-1, have been identified as crucial mediators of altered metabolism of fat and glucose leading to impaired insulin secretion (3). Until recently, regulatory proteins that participate specifically in downregulation of insulin secretion have received little attention. The discovery that uncoupling protein (UCP)-2 is present in pancreatic islets and β-cell lines (4) has led to the suggestion that such molecules can participate in the long-term adaptation of the β-cell to increased nutrient availability and contribute to the suppression of glucose-stimulated insulin secretion (GSIS) (5).

UCP2

The existence of UCP2 was first described in 1997 in multiple tissues (6–8), including the pancreas (6). It was subsequently localized in rat (4,5) and human pancreatic (9,10) islets. In general, UCPs function to decrease metabolic efficiency by dissociating substrate oxidation in the mitochondrion from ATP synthesis. This is thought to be accomplished by promoting net translocation of protons from the intermembrane space, across the inner mitochondrial membrane to the matrix, thereby dissipating the potential energy available for conversion of ADP to ATP despite continued oxidation of fuels (11). This uncoupling effect then leads to homologue- and tissue-specific functions such as thermogenesis for UCP1 (12), regulation of free fatty acid (FFA) metabolism and transport for UCP2 and UCP3 (11,13,14), decreasing reactive oxygen species (ROS) formation (UCP1 and UCP2) (15,16), and inhibition of insulin secretion for UCP2 (9). Delineation of the common versus unique properties of the UCP family members continues to fuel much research.

UCP2 NEGATIVELY MODULATES INSULIN SECRETION

Initially controversial because of a dissenting report (17), the bulk of evidence now shows that an increase in UCP2 expression results in suppression of GSIS (5,9,18), whereas absence of Ucp2 enhances GSIS (19,20). To test the hypothesis that UCP2 would inhibit insulin secretion, we created an adenovirus vector containing the full-length human Ucp2 cDNA to overexpress the protein (5). Such upregulation completely suppressed GSIS (5), an effect confirmed by others using similar technology (18). The effects could be partially overcome by adding calcium ionophore to a stimulatory glucose concentration (5) or normalized with sulfonylurea or KCl (9). Upregulation of UCP3 did not inhibit GSIS (18), whereas induction of UCP1 was suppressive (21). These studies provided the first direct evidence that UCP2 could negatively regulate insulin secretion but could be criticized because the degree of upregulation was likely supraphysiological. This criticism was difficult to refute because of the continuing lack of a suitable antibody for unambiguous immunoblotting of UCP2. Development of the UCP2 knockout mouse provided another method for directly assessing the role of UCP2 in insulin secretion. We showed that isolated cultured islets from UCP2 knockout mice had increased responsiveness to glucose characterized by a left-shift in the concentration-response curve (20). The knockout mice demonstrated an increase in the insulin-to-glucose ratio in
We examined the function of K<sub>ATP</sub> channels using <sup>86</sup>Rb<sup>+</sup> as a marker for K<sup>+</sup> flux. As predicted, when UCP2 was induced and cellular ATP content reduced by 50%, K<sub>ATP</sub> channels failed to close in response to elevated glucose, although sulfonylurea was effective (9). Likewise, an increase in K<sub>ATP</sub> channel activity of UCP1-overexpressing β-cells has been reported (21), leading to a reduction in voltage-dependent Ca<sup>2+</sup> influx. The reduction in insulin secretion secondary to the decrease in Ca<sup>2+</sup> entry was only partially overcome by use of calcium ionophore A23187 (5), suggesting that ATP depletion affects regulatory sites other than K<sub>ATP</sub> channels. Other sites modulated by ATP or ATP/ADP have been less well characterized after UCP2 overexpression. However, our unpublished data suggest that cAMP formation is not altered, and elevation of cAMP does not potentiate GSIS to the same extent in UCP2 knockout as in wild-type mice (T.S. McQuaid, J.W. Joseph, M.C.S., M.B.W., C.B.C., unpublished data). cAMP is important in distal events of signaling (26); coincidently, the energy requirement for such distal steps is estimated to be lower than that for K<sub>ATP</sub> channel closure (27).

Increasing mitochondrial uncoupling is predicted to promote glucose metabolism by virtue of releasing the back pressure on the electron transport chain caused by a buildup in the proton gradient. While this phenomenon of increased glucose usage and oxidation has been reported in ZDF rat islets overexpressing UCP2, thus leading to an increase in insulin secretion (17), it has not been reproduced in either UCP2-overexpressing (18; C.B.C., M.C.S., unpublished data) or UCP1-overexpressing (21) β-cells. Moreover, incubating islets with chemical uncouplers has classically been shown to inhibit insulin secretion (28).

**REGULATION OF UCP2 EXPRESSION BY NUTRIENTS**

Emphasis in current investigations of the regulation of UCP2 expression focuses on the two culprits of glucolipotoxicity: glucose and FFA. In the case of glucose, there are conflicting results. Using isolated islets or INS1 cells, exposure to high glucose for at least 48 h had no effect on (29), reduced (30), or increased UCP2 mRNA and/or protein expression (10,31). When rats were made hyperglycemic by means of partial pancreatectomy (32) or glucose infusion (33), Ucp2 mRNA expression was upregulated—an effect reversed by normalizing the plasma glucose concentrations with phlorizin (32). Conversely, hyperglycemic Zucker diabetic fatty rats were reported to have low expression of islet Ucp2 mRNA (4).

The hypothesis that Ucp2 mRNA expression could be induced by FFA was quickly established in nonislet tissues, such as skeletal muscle and adipocytes (34). That FFAs also upregulate islet UCP2 has now been shown both by effects of in vivo feeding of high-fat diets on mRNA (9,20) and in vitro exposure of isolated islets or clonal β-cells to palmitic or oleic acid on UCP2 (29,31,35,36).

Despite consensus on the final outcome, the mechanism by which FFAs stimulate UCP2 transcription in islets is unclear. Conflicting evidence has been presented regarding the necessity for fat oxidation (29,35). Support for induction of UCP2 secondary to fat oxidation is demonstrated by use of etomoxir to inhibit CPT-1 and block entry of fatty acyl-CoA into the mitochondria. In this study, it was shown that oleic acid upregulated Ucp2 mRNA only in...
the presence of submaximal glucose concentrations (29). Because high glucose inhibits CPT-1 by generating malonyl CoA (37), the importance of FFA oxidation seems apparent. These results would imply that the mediator regulating UCP2 transcription is a metabolite of FFA. On the other hand, studies showing similar results of nonmetabolizable versus metabolizable analogs of FFAs (e.g., bromopalmitic acid) provide evidence that FFA oxidation is not necessary for Ucp2 gene transcription (35).

Interestingly, the UCP2 gene promoter region contains peroxisome proliferator response elements that are activated by FFAs. When islets from Zucker diabetic fatty rats were treated with the peroxisome proliferator–activated receptor (PPAR)-γ activator troglitazone, Ucp2 mRNA was increased (38), supporting the idea that nonmetabolized FFAs directly induce UCP2 via PPARs. Unfortunately, these results were not replicated with another PPAR-γ activator, rosiglitazone (29). Laybutt et al. (32) showed concomitant induction of PPAR-γ and UCP2 but had no direct evidence of a causal link between the two. However, culture of rat islets for 72 h with supraphysiological concentrations of FFAs (2 mmol/l) induced UCP2 and suppressed GSIS by mechanisms that were decreased with a PPAR-γ antagonist (31). A study specifically examining the role of PPAR-α by use of the ligand clofibrate demonstrated concomitant induction of CPT-1 and UCP2 in islets. Whereas inhibition of FFA oxidation with etomoxir reversed the negative effects of clofibrate on insulin secretion, the effects of etomoxir on UCP2 expression were not reported (39).

Another region of the Ucp2 promoter contains the sterol regulatory element (SRE) (36). Deletion of the SRE eliminated the effects of oleic acid on Ucp2 gene transcription (36), whereas overexpression of the SRE binding protein 1 caused a marked induction of Ucp2 promoter activity and expression (36,40). Therefore, it appears that FFAs may activate UCP2 gene transcription via SRE and possibly peroxisome proliferator response elements, but there may also be potentiating effects exerted by FFA metabolites.

When consensus is reached as to what nutrients or their metabolites regulate islet UCP2 expression, the outcome may well be that, for maximal expression (and therefore negative effects on insulin secretion), both glucose and FFAs need to be elevated. When high-fat diets were fed to normal Wistar versus hyperglycemic Goto-Katazaki (GK) rats, only the GK rats exhibited enhanced islet UCP2 expression concomitant with impaired GSIS (41). More-
long-chain CoA catalyzed by mitochondrial thioesterases (14). Increased oxidation of long-chain CoA is expected to increase ROS production, leading to activation of UCP2 by superoxide. In addition, both UCP2 and DIC mRNA transcription is upregulated by exposure of β-cells to chronic FFAs.

**INTERACTION WITH ROS**

Because FFA treatment of islets has been shown to induce UCP2 expression, the upregulation of UCP2 may be a protective mechanism against excessive lipid exposure. The concept of lipotoxicity in pancreatic islets is supported by evidence that high FFA levels induce a variety of genes that influence fat metabolism (1,2,47a). One key modulator that undergoes upregulation after FFA exposure (1) or high-fat diet (20) is CPT-1, the rate-limiting enzyme that facilitates transfer of long chain acyl-CoA into the mitochondrial matrix, thus promoting β-oxidation. High rates of FFA oxidation generate oxygen radicals (48) that are potentially damaging to β-cells, leading to so-called lipoapoptosis. In our investigation of uncoupling in permeabilized β-cells, we found that oleate treatment for 72 h increased succinate-dependent ROS production by nearly twofold. Moreover, acute application of oleate caused a fivefold increase in ROS production in both control cells and β-cells chronically exposed to oleate. When β-cells were induced to produce superoxide, the mitochondria became more depolarized only in the presence of FFAs. Depolarization was greater in cells preincubated with FFAs for 72 h (44). Thus, concomitant induction of UCP2 by FFAs would at least partially negate the potential to increase ROS production.

Less physiological induction of oxidative stress by use of exogenous hydrogen peroxide elevated UCP2 mRNA expression in clonal β-cells. Furthermore, when UCP2 was transiently overexpressed, cell survival was enhanced after peroxide exposure (49). These and other data showing that UCP2 knockout mice have increased ROS production from macrophages (15) whereas UCP2 overexpressing transgenic mice have protection from lesions inducing apoptosis (50) suggest that a physiological role of UCP2 might be to modulate free radical production. Because superoxide induces UCP2 expression and activity, its modulatory effect is expected to be enhanced when most needed, i.e., when free radical production is elevated.

**UCP2 AND TYPE 2 DIABETES**

Initially, the discovery of UCP1 homologues spurred interest in their potential energy-wasting capabilities, a function associated with leanness and thus antidiabetic status. Therefore, the finding that UCP2 suppressed insulin secretion from islets and the hypothesis that an increase in UCP2 could participate in insulin insufficiency in type 2 diabetes was difficult to accept at first. The “yin-yang” of

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**FIG. 2.** Model of UCP2 and DIC catalyzed transport of FFA anions out of the mitochondrial matrix. Under conditions of FFA excess, concentrations of FFA anions in the mitochondria are projected to rise. The mechanism may either be through the flip-flop of neutral FFAs across the mitochondrial membranes (11) or through deacylation of long-chain CoA by means of mitochondrial thioesterases (14). Increased oxidation of long-chain CoA is expected to increase ROS production, leading to activation of UCP2 by superoxide. In addition, both UCP2 and DIC mRNA transcription is upregulated by exposure of β-cells to chronic FFAs.

**FIG. 3.** Palmitate oxidation by islets. Islets were cultured for 48 h in the absence (fatty acid-free BSA, □) or presence of 0.5 mmol/l palmitate (with 1% fatty acid-free BSA carrier, ■) and 8.3 mmol/l glucose. Overexpression of UCP2 in the absence of culture FFAs reduced palmitate oxidation in the presence of 2.8 mmol/l glucose. In the presence of culture FFAs, palmitate oxidation was enhanced in all islet groups, but UCP2-overexpressing islets still oxidized significantly less palmitate than control islets at both 2.8 and 25 mmol/l glucose. The number of experiments performed for each condition is 8–15. *P < 0.05 compared with control islets exposed to the same glucose concentration during palmitate oxidation assessment. §P < 0.05 comparing 25 mmol/l glucose with 2.8 mmol/l glucose condition.
UCP2 and diabetes was reviewed (51,52) after the publication of the UCP2 knockout mouse article (19). Other commentators have listed UCP2 as a potential "diabetes gene" because of its negative effects in β-cells (53). Of interest is a recent report of a functional polymorphism in the UCP2 promoter that increases the risk of obesity but decreases the risk of type 2 diabetes (54).

What is the functional evidence, at least in the rodent β-cell, that UCP2 is associated with type 2 diabetes? UCP2 expression has been quantified in islets of several rodent models of type 2 diabetes. Ucp2 mRNA or protein is elevated in fa/fa rats (33) and ob/ob mice (19) but decreased in Zucker diabetic fatty rats (4,17). Induction of type 2 diabetes by high-fat feeding also upregulates UCP2 gene expression, which is associated in all cases with elevated plasma lipids (9,20,41). Likewise, hyperglycemic models also display an increase in islet UCP2 transcripts, at least temporarily (32,33). These animal models differ in the degree of insulin insufficiency and thus hyperglycemia. None of these studies demonstrated a strong causal effect of UCP2 induction on insulin insufficiency. Also, no systematic studies following the development of diabetes and correlating changes in UCP2 expression have been published.

The development of the UCP2 knockout mouse provided an important model for studying causal relationships of UCP2 and various phenomena related to regulation of insulin secretion. When the UCP2 gene was knocked out in the ob/ob mouse model by cross-breeding, the progeny were not less obese than the parent ob/ob animals, but they displayed a reduction in plasma glucose that was associated with an improvement in GSIS (19). Recently, we studied the effects of a chronic high-fat diet on glucose homeostasis and insulin secretion in the UCP2 knockout mice compared with wild-type controls (20). Both groups of mice gained the same amount of weight after 4.5 months on the lard-based diet. The UCP2 knockout mice displayed partial resistance to the diet, in that they maintained lower fasting and fed blood glucose levels. Both in vivo and in vitro GSIS were enhanced. The maintenance of a high capacity for insulin secretion was at least partially achieved. However, the number of apoptotic nuclei was similar in both UCP2 knockout and wild-type mice. Indeed, UCP2 knockout mice fed the normal diet had twofold more apoptotic nuclei per islet area than wild-type mice. However, after the high-fat diet, the number of apoptotic nuclei was similar in both UCP2 knockout and wild-type mice (20). We speculated that the higher energy level maintained in the UCP2 knockout islets allows proliferation and other protective processes to proceed at a rate that not only preserves, but enhances, the insulin secretory capacity. The mechanisms of this putative protection have yet to be determined. However, one interesting finding was that the UCP2 knockout mice fed the high-fat diet maintained normal fasting plasma FFAs and fed plasma triglycerides along with significantly lower plasma glucose concentrations in both fed and fasted states (20). This provides additional evidence to support the contention that lipotoxic effects are contingent upon the coexistence of hyperglycemia (56).

Upregulation of UCP2 expression and activity leading to suppression of GSIS as a contributor to diabetes development is relatively straightforward to rationalize. However, it becomes more difficult to reconcile an increase in β-cell UCP2 with the fasting hyperinsulinemia observed in obese and obese-diabetic rodents such as fa/fa and ZDF rats. This hypersecretion of insulin has been shown to be dependent on mitochondrial ATP production and KATP channel inactivation (57) and may be related to altered kinetics of glucokinase or other metabolic enzymes (58), which strongly depends on the deranged hypothalamic-pituitary-adrenal axis in genetically obese rodent models (59). The increase in overall metabolism is sufficient to induce KATP channel closure at lower than normal glucose (60). We propose that when fuel levels in β-cells are relatively low, the mitochondrial membrane potential will be commensurately reduced. Because activation of UCP2 depends on generation of superoxide radicals, which depends on mitochondrial membrane hyperpolarization, uncoupling will be minimized (44).

SIGNIFICANCE

The overwhelming majority of research on β-cell metabolism-secretion coupling has concentrated on stimulatory pathways and their modulation. UCP2 represents a novel negative modulator of insulin secretion that has the potential to play a role in the pathogenesis of diet-related type 2 diabetes. By determining how its endogenous expression and activity is regulated, new methods for improving insulin secretion in diabetes may be realized.

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