Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3

Review

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Evidence for the physiological functions of UCP2 and UCP3 is critically reviewed. They do not mediate adaptive thermogenesis, but they may be significantly thermogenic under specific pharmacological conditions. There is strong evidence that the mild regulated uncoupling they cause attenuates mitochondrial ROS production, protects against cellular damage, and diminishes insulin secretion. Evidence that they export fatty acids physiologically is weak. UCP2 and UCP3 are important potential targets for treatment of aging, degenerative diseases, diabetes, and perhaps obesity.

Introduction

Uncoupling proteins (UCPs) have become prominent in the fields of thermogenesis, obesity, diabetes, and free radical biology following the discovery of a family of novel UCPs and have recently started to make an impact in the areas of degenerative, neurological, circulatory, and immunological diseases and aging. The new UCPs are closely related to UCP1, which catalyzes adaptive thermogenesis in mammalian brown adipose tissue by greatly increasing the proton conductance of the mitochondrial inner membrane (Cannon and Nedergaard, 2004). UCP1 is present at huge concentrations, up to 10% of the membrane protein, to accomplish this. The leak of protons through UCP1 uncouples substrate oxidation from phosphorylation of ADP to ATP, leading to fast oxygen consumption and heat production. The proton conductance of UCP1 is under tight control: it is strongly inhibited by purine nucleotides at physiological concentrations, and this inhibition is overcome by fatty acids released from intracellular triacylglycerol stores following adrenergic activation in response to cold.

The discovery of UCP1 orthologs (UCP2 and UCP3) in mitochondria from several mammalian tissues prompted initial speculation that these UCPs are also thermogenic and involved in regulation of energy expenditure and obesity. These reasonable speculations were based on the conservation of residues thought to be involved in proton transport and nucleotide binding. However, it is now clear that UCP2 and UCP3 are present at tiny concentrations, 0.01% to 0.1% of the membrane protein, and only transport protons when they are specifically activated (Esteves and Brand, 2005). These observations, together with the discovery of related UCPs in ectothermic fish and plants that do not require thermogenesis, raised the possibility that the UCPs have some different, more general function. In this review, we consider the evidence for different models of the physiological functions of UCP2 and UCP3. For general background, see the many reviews that are available (Boss et al., 2000; Brand et al., 2004a, 2004b; Casteilla et al., 2001; Dulloo and Samec, 2001; Harper et al., 2001a, 2001b; Harper and Himms-Hagen, 2001; Horvath et al., 2003; Jezek et al., 2004; Kozak and Harper, 2000; Nedergaard and Cannon, 2003; Ricquier and Bouillaud, 2000a, 2000b; Saleh et al., 2002).

The reaction catalyzed by UCP2 and UCP3

There is a broad consensus that UCP2 and UCP3 do not transport protons in the absence of specific activators. Evidence for this comes from the lack of effect of UCP2 or UCP3 knockout on the basal proton conductance of isolated mouse mitochondria (Cadenas et al., 2002; Couplan et al., 2002; Echtay et al., 2002b, 2003; Krauss et al., 2003), and the very low proton conductance in the absence of fatty acids of proteoliposomes containing UCP2 or UCP3 (Echtay et al., 1999, 2001; Jaburek and Garlid, 2003; Jaburek et al., 1999).

However, it is now clear that UCP2, UCP3, avian UCPs, and plant UCPs will transport protons and increase the net proton conductance of mitochondria in the presence of specific activators (Brand et al., 2004a, 2004b; Considine et al., 2003; Talbot et al., 2003), including reactive alkenals such as hydroxynonenal, which is produced by peroxidation of membrane phospholipids (Echtay et al., 2003). The proton conductance in the presence of these activators is inhibited by purine nucleotides such as ATP and GDP. Fatty acids are probably required for activation (Echtay et al., 2001, 2002b; Rial et al., 2004), possibly to relieve inhibition by nucleotides. Thus these UCPs catalyze an inducible proton conductance in the presence of activators, but they do not increase basal proton conductance in the absence of activators.

Measurement of the transport of fatty acid and other anions in proteoliposomes shows that UCP2 and UCP3 can transport anions across the mitochondrial inner membrane in a reaction that is sensitive to nucleotide inhibition (Echtay et al., 1999; Jaburek et al., 1999). Whether fatty-acid (or fatty-acid peroxide) cycling across the membrane explains net proton transport is controversial for UCP1 (Echtay et al., 2001; Garlid et al., 2001; Gonzalez-Barroso et al., 1998; Jaburek et al., 2001; Klingenberg and Echtay, 2001; Klingenberg and Huang, 1999; Rial et al., 2004; Skulachev, 1998). By extension, it is also controversial for UCP2 and UCP3.

For a broader critical review of the reactions catalyzed by UCP2 and UCP3, see Esteves and Brand (2005).

The functions of UCP2 and UCP3

What functions do UCP2 and UCP3 carry out? All models have to accommodate the following observations:

- Fatty acids increase the expression of UCP2 and UCP3 mRNA, implying that these UCPs are somehow involved in fatty-acid metabolism (Nedergaard and Cannon, 2003).
- UCP2 and UCP3 catalyze net proton conductance, but only when activated by fatty acids and free radical-derived alkenals (Brand et al., 2004a, 2004b).
- UCP2 and UCP3 can probably export fatty acids and other anions (Echtay et al., 1999; Jaburek et al., 1999).
- Mice in which UCP2 or UCP3 are knocked out show only weak phenotypes in the laboratory (Harper and Himms-Hagen, 2001).

Model 1: UCP2 and UCP3 increase thermogenesis and protect against obesity

When UCP2 and UCP3 were first identified, their function was postulated to be thermogenesis and body weight regulation (Boss et al., 1997b; Fleury et al., 1997; Gimeno et al., 1997; Gong et al., 1997; Vidal-Puig et al., 1997), perhaps by catalyzing a constitutive basal proton leak in widespread tissues that complemented the specialized adaptive thermogenic function of UCP1 in brown adipose tissue (Figure 1, Model 1A). However, evidence against a thermogenic function quickly appeared. UCP2 mRNA and UCP3 mRNA and protein concentration rise in muscle of starved rats, whereas thermogenesis falls (Boss et al., 1997a, 1998; Cadenas et al., 1999; Samec et al., 1998), and UCP homologs are found in tissues of fish (Stuart et al., 1999) and plants (Laloi et al., 1997), ectotherms in which adaptive thermogenesis does not occur. The most persuasive argument comes from UCP2 and UCP3 knockout mice, which have normal responses to cold exposure and are not obese (Arsenijevic et al., 2000; Gong et al., 2000; Vidal-Puig et al., 2000; Zhang et al., 2001).

There is now a consensus that the primary function of UCP2 and UCP3 is not to promote gross thermogenesis or energetic inefficiency. However, thermogenic models should not be completely abandoned since activation of UCP3 in muscle may cause significant thermogenesis in particular conditions. The concentrations of UCP2 in spleen, lung, and stomach mitochondria (Pecqueur et al., 2001) and of UCP3 in skeletal muscle mitochondria (Harper et al., 2002) are tiny: 0.1% to 1% of the concentration of UCP1 in brown adipose tissue mitochondria. However, considering the much greater mass of muscle and its large contribution to metabolic rate, the maximum thermogenic capacity of UCP3 may still be significant. The stimulation of metabolic rate and decreased weight gain caused by UCP3 overexpression in mouse muscle (Clapham et al., 2000) is caused by artifactual uncoupling, but the extent of this artifact in isolated muscle mitochondria (Cadenas et al., 2002) happens to be similar to the extent of stimulation of UCP3-mediated proton conductance achieved by addition of superoxide or alkenals (Echtay et al., 2002b, 2003), suggesting that activation of UCP3 in vivo by physiological activators or pharmacological intervention might have the capacity to be significantly thermogenic (Figure 1, Model 1B). In support of this suggestion, UCP3 knockout mice have diminished muscle hyperthermia in response to the recreational drug ecstasy (MDMA) (Mills et al., 2003).

Birds lack brown adipose tissue and UCP1 but still have adaptive thermogenesis. Avian UCP is 70% identical in sequence to mammalian UCP2 and UCP3 and is restricted to skeletal muscle (Raimbault et al., 2001; Vianna et al., 2001). Like these proteins, avUCP does not catalyze the basal proton leak of mitochondria, and its proton conductance can be activated by superoxide (Talbot et al., 2003). AvUCP mRNA is increased by cold exposure in hummingbirds, ducklings, and chickens (Raimbault et al., 2001; Toyomizu et al., 2002; Vianna et al., 2001). Its mRNA and the superoxide-inducible proton conductance are upregulated by cold water immersion of penguins (Talbot et al., 2004a). These observations are consistent with a major role of avUCP in thermogenesis in birds (Figure 1, Model 1B). Equally, they are consistent with roles in other thermogenesis-associated functions (Figure 1), such as fatty-acid metabolism or attenuation of free radical generation.

How does this model accommodate the requirements listed above? Fatty acids and alkenals might activate UCP2 and UCP3 when thermogenesis is required; anion transport might be part of the uncoupling cycle, or a side reaction. The lack of a strong cold-sensitive or obese phenotype in knockout mice argues strongly against thermogenesis being a major function. However, it could be important under specific conditions that were not applied in the phenotyping experiments; the lack of a thermogenic response to MDMA in UCP3 knockout mice supports this argument.

Thus UCP2 and UCP3 are not normally thermogenic or involved in the adaptive response to cold, but UCP3 might still be significantly thermogenic under specific conditions and it remains an attractive drug target for the treatment of obesity.

Model 2: UCPs attenuate mitochondrial production of free radicals and protect against oxidative damage, degenerative diseases, and aging

Mitochondria are the major source of reactive oxygen species (ROS) in cells. ROS damage is thought to underpin several degenerative diseases, including diabetes, Parkinson's, Alzheimer's, and Friedrich's ataxia, and aging itself (Brand et al., 2004a, 2004b). Mitochondrial ROS production is very sensitive to the protonmotive force set up across the inner membrane by electron transport, so the mild uncoupling caused by activation of UCP2 or UCP3 might lower protonmotive force slightly, attenuate mitochondrial ROS production, and protect against ROS-related cellular damage (Figure 1, Model 2) (Arsenijevic et al., 2000; Brand et al., 2002; Casteilla et al., 2001; Cortez-Pinto et al., 1999; Echtay et al., 2002b; Kowaltowski et al., 1998; Negre-Salvayre et al., 1997; Nicholls and Budd, 2000; Rolfe and Brand, 1997; Skulachev, 1996, 1998; Vidal-Puig et al., 2000). "Mild" uncoupling implies a limited increase in proton conductance so that protonmotive force is slightly lowered and respiration rate is slightly increased, but ATP can still be made. It differs from full uncoupling, where protonmotive force and ATP synthesis are abolished and respiration is maximal.

The fullest elaboration of this model (Figure 2) proposes a specific pathway of UCP activation in response to mitochondrial radical production. Superoxide production from the electron transport chain is high during fatty-acid oxidation (St-Pierre et al., 2002). The model proposes that superoxide directly (or indirectly, through formation of hydroxyl radicals) attacks *n*-6 polyunsaturated fatty acyl chains of membrane phospholipids, chiefly arachidonic acid chains, forming carbon-centered fatty acyl radicals. Oxidation of these carbon-centered radicals to peroxyl radicals initiates a well-known chain reaction (Halliwell and Gutteridge, 1999) that leads to massive production of

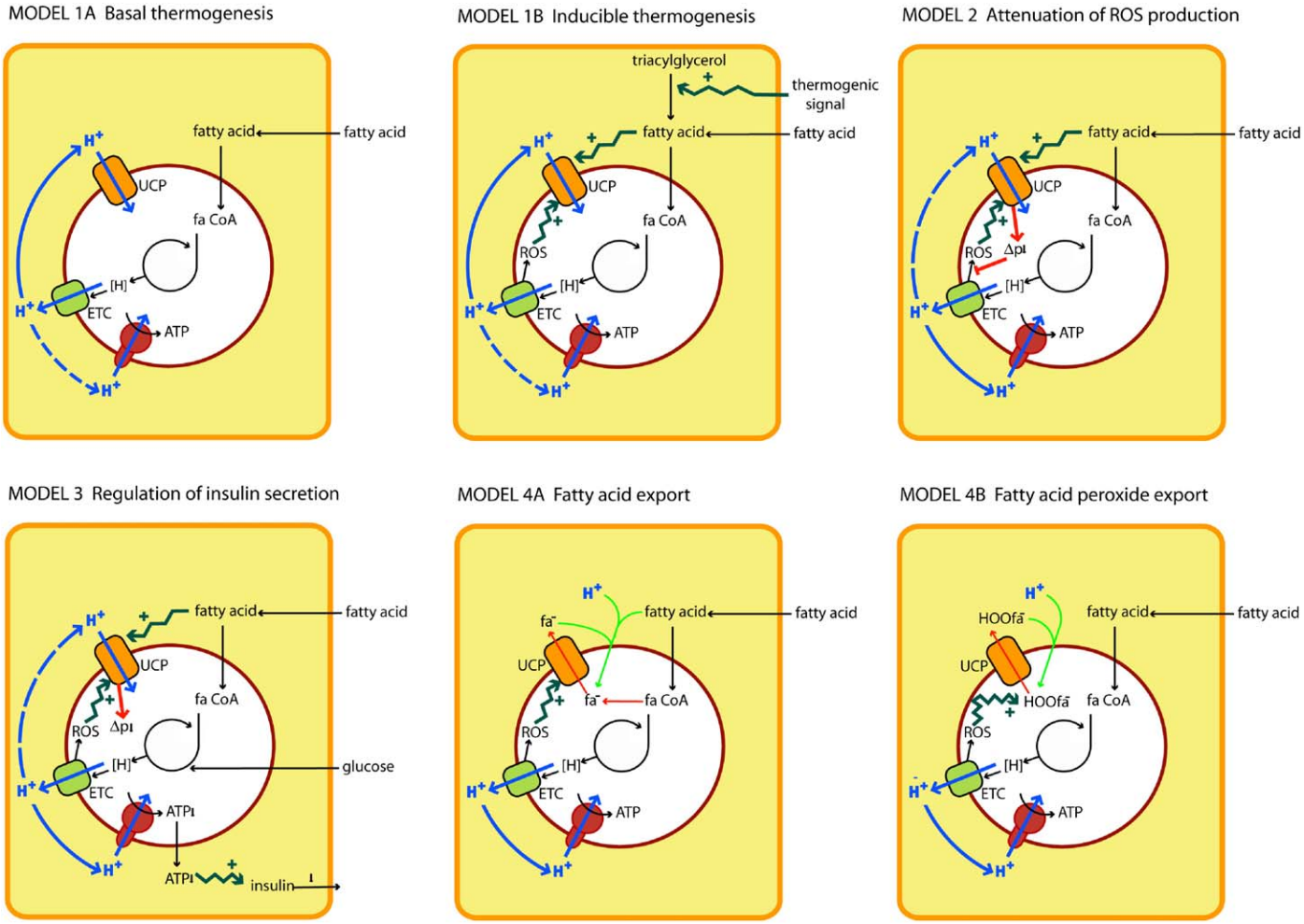

Figure 1. Models of the physiological functions catalyzed by UCP2 and UCP3

The yellow rectangle represents a cell; the red circle represents the inner membrane of mitochondria within it. Fatty acids are converted to fatty acyl CoA (fa CoA) in the matrix, then reducing equivalents ([H]) are removed by β oxidation and passed to the electron transport chain (ETC) where they are oxidized, causing proton pumping. The resultant protonmotive force drives ATP synthesis or is dissipated by UCPs. Model 1A: in the presence of UCPs, oxidation of fuels is significantly uncoupled from ATP synthesis, leading to increased fuel oxidation and thermogenesis. Model 1B: by analogy with activation of UCP1 in brown adipose tissue, an appropriate signal triggers triacylglycerol breakdown, releasing fatty acids. The fatty acids are oxidized, leading to ROS production by the electron transport chain. The ROS and fatty acids activate the proton conductance of UCP2 or UCP3, causing thermogenesis. Model 2: during fatty-acid oxidation, ROS production activates UCPs, lowering the protonmotive force (Δp). ROS production is very sensitive to Δp, so it decreases, setting up a negative feedback loop of mild uncoupling that attenuates ROS production when it rises too high, protecting the cell against oxidative stress. Model 3: the mild uncoupling lowers Δp and therefore decreases ATP synthesis. In pancreatic β cells, this lowers ATP and raises ADP in the cytoplasm, leading to decreased insulin release. Model 4A: the primary function of UCPs is to export fatty-acid anions generated when fatty acyl CoA is hydrolyzed by thioesterases to release the CoA needed for continued rapid fatty-acid β oxidation (red arrows). Alternatively, the fatty-acid anions are generated by uncatalyzed entry of excess protonated fatty acids from the cytosol (green arrows) and the cycle of protonated fatty-acid entry and fatty-acid anion export causes net proton conductance as a side reaction. Model 4B: ROS cause peroxidation of membrane phospholipids and the resulting fatty-acid peroxide anions ($HOOfa^-$) are exported by UCPs (red arrow). If the peroxides can protonate and return (green arrows), the cycle catalyzes net proton conductance.

4-hydroxynonenal and other reactive alkenals. The alkenals activate the proton conductance of UCPs, lowering protonmotive force and attenuating the original superoxide production. This provides a local feedback by which UCP2 and UCP3 control the mitochondrial production of ROS (Brand et al., 2004a, 2004b; Echtay et al., 2002a, 2002b, 2003; Murphy et al., 2003; Talbot et al., 2004b).

The evidence for this model is extensive. Superoxide, products of oxidation of membrane phospholipids such as hydroxynonenal, and analogs of these products all activate the GDP-sensitive proton conductance of UCP2 and UCP3, causing mild uncoupling (Brand et al., 2004a). Activation of proton conductance occurs when the effects of ROS are mimicked by addition of a carbon-centered radical generator, AAPH, and activation by superoxide or AAPH is prevented by a spin trap that quenches carbon-centered radicals (Murphy et al., 2003), suggesting that a carbon-centered radical is an intermediate in the activatory pathway. Mild uncoupling strongly decreases ROS production (Brand, 2000; Korshunov et al., 1997; Lambert and Brand, 2004; Liu, 1997; Miwa et al., 2003; Papa and Skulachev, 1997). Inhibition of UCPs by GDP in mitochondria increases membrane potential and mitochondrial ROS produc-

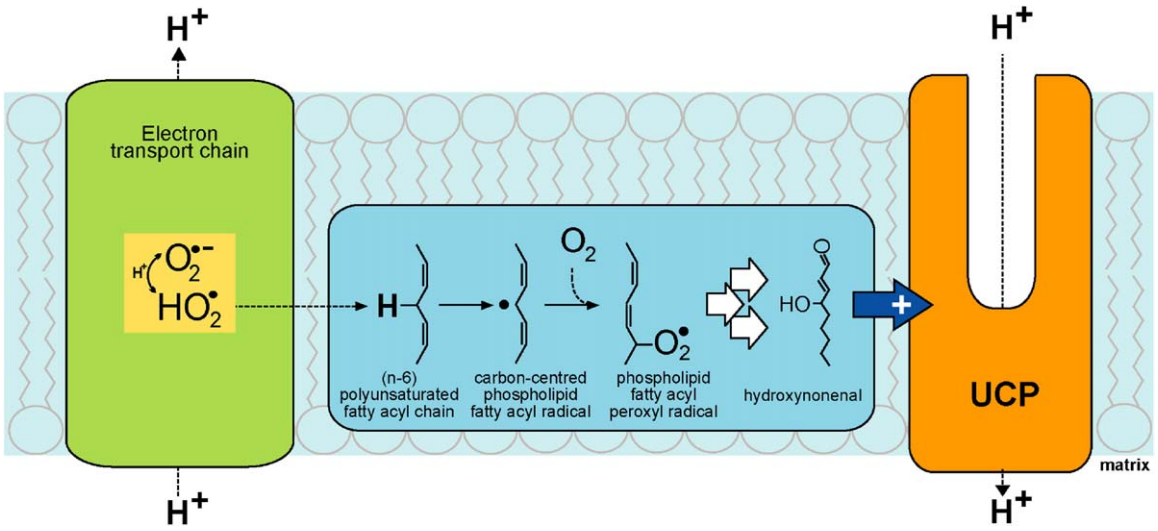

Figure 2. A model for the physiological activation of UCP2 or UCP3 by superoxide

The mitochondrial electron transport chain pumps protons, setting up a high protonmotive force that backs up the electrons and allows them to spill onto molecular oxygen, forming superoxide. Superoxide, or its more lipid-soluble protonated form, hydroperoxyl radical, diffuses into the inner membrane, where it attacks phospholipid *n*-6 polyunsaturated fatty acyl groups such as arachidonate, extracting a hydrogen atom and leaving behind a carbon-centered fatty acyl radical. This can react with molecular oxygen to form a lipid peroxyl radical, which initiates another round of carbon-centered radical production and breaks down to fragments including 4-hydroxynonenal. In this way, a single hydroperoxyl radical can initiate a cascade that generates a large number of hydroxynonenal molecules. The hydroxynonenal activates the UCP, causing it to transport protons and lower the protonmotive force, leading to attenuation of superoxide production by the electron transport chain. Based on Brand et al. (2004a).

tion (Kowaltowski et al., 1998; Negre-Salvayre et al., 1997; Talbot et al., 2004b), consistent with a role of the UCPs in keeping ROS production low by causing mild uncoupling. Similarly, mitochondria from UCP3 knockout mice show evidence of higher ROS production (Brand et al., 2002; Vidal-Puig et al., 2000). Thus there is strong experimental support for several different aspects of the alkenal feedback mechanism in isolated mitochondria.

Supporting evidence comes from phenotyping UCP2 knockout mice. These animals are resistant to infection by the intracellular parasite *Toxoplasma gondii* through a mechanism proposed to involve increased macrophage ROS production (Arsenijevic et al., 2000; Richard et al., 2001). Their livers have elevated markers of ROS production and delayed liver regeneration after partial hepatectomy, suggesting that UCP2 protects against ROS (Horimoto et al., 2004). Similarly, LDL receptor knockout mice with UCP2 knockout bone marrow have more oxidative stress and are more susceptible to atherosclerosis than controls, suggesting that UCP2 protects against ROS-mediated atherosclerosis in vivo (Blanc et al., 2003), and endothelial cells treated with UCP2 antisense oligonucleotides (Duval et al., 2002) and pancreatic islet cells from UCP2 knockout mice (Krauss et al., 2003) have increased ROS production.

How does this model accommodate the requirements listed above? ROS production may be particularly high when fatty acids are oxidized (St-Pierre et al., 2002), explaining the need for fatty-acid upregulation of gene expression. Superoxide and alkenal activation would be signals to activate mild uncoupling in the face of excessive ROS production. Anion transport might be part of the uncoupling cycle, or a side reaction. The weak phenotype may reflect low ROS-related damage in animals in laboratory settings. However, the phenotypes that are seen implicate ROS and are consistent with the model. Note that the effects of UCP2 and UCP3 knockout on development of degenerative diseases and aging have not been reported and might be significant.

Thus there is good evidence that a major function of UCP2 and UCP3 is to attenuate mitochondrial production of free radicals in mitochondria, in cells, and in vivo and to protect against oxidative damage.

Model 3: UCPs mediate ROS signaling and insulin secretion

The mild uncoupling caused by activation of UCP2 may have a signaling role. Activation of UCP2 may attenuate glucose-stimulated insulin secretion by pancreatic β cells. In the consensus model of the major route of glucose-stimulated insulin secretion by pancreatic β cells (Rutter, 2001), glucose catabolism increases the mitochondrial protonmotive force, the cytoplasmic ATP/ADP ratio rises, and plasma membrane K_{ATP} channels close, leading to depolarization, opening of voltage-sensitive calcium channels, calcium influx, and insulin secretion. UCP2 could short-circuit this pathway by mild uncoupling, blunting the rise in protonmotive force caused by raised glucose, and attenuating insulin secretion (Chan et al., 1999). A plausible regulator of UCP2 function is fatty-acid oxidation, which would raise β cell ROS production, activate the proton conductance of UCP2, and attenuate insulin secretion (Figure 1, Model 3) (Brand et al., 2004a; Green et al., 2004; Lameloise et al., 2001), while hyperglycemia may do the same pathologically (Zhang et al., 2001).

There is considerable indirect evidence that UCP2 regulates

insulin secretion, perhaps through ROS produced during fatty-acid oxidation. UCP2 mRNA is expressed in pancreatic islets (Zhou et al., 1997), and β cell mitochondria show a large GDP-sensitive superoxide-activated proton conductance (Echtay et al., 2002b), showing that UCP2 is present and can be activated by ROS. In β cells, fatty acids increase mitochondrial ROS production and decrease glucose-stimulated increases in mitochondrial membrane potential, cellular ATP content, cytoplasmic calcium, and insulin secretion (Carlsson et al., 1999; Joseph et al., 2004; Koshkin et al., 2003; Lameloise et al., 2001). Completing the circumstantial case, ROS (Sakai et al., 2003) and hydroxynonenal (Miwa et al., 2000), activators of UCP2 (Echtay et al., 2003), also decrease glucose-stimulated insulin secretion.

Overexpression of UCP2 is subject to gain-of-function artifacts (Esteves and Brand, 2005). With this important caveat, the effects of UCP2 overexpression (Chan et al., 1999, 2001; Hong et al., 2001; Li et al., 2001) support the suggestion that increased UCP2 lowers mitochondrial membrane potential, mitochondrial coupling, mitochondrial ROS production, cytoplasmic ATP content, and plasma membrane potassium fluxes and attenuates glucose-stimulated insulin secretion. Even if these overexpression effects reflect an uncoupling artifact rather than any native function of the overexpressed UCP2, they still show that mild uncoupling of β cells can modulate insulin secretion by antagonizing the K_{ATP} channel pathway.

Strong and convincing evidence for this model comes from investigations of loss of function in UCP2 knockout mice. UCP2-deficient mice have higher islet ATP levels and increased glucose-stimulated insulin secretion, establishing that UCP2 negatively regulates insulin secretion (Zhang et al., 2001). Fat feeding and fatty acids attenuate glucose-stimulated increases in mitochondrial potential, mitochondrial ROS production, ATP/ADP ratio, cytosolic calcium, and insulin secretion. These effects of fatty acids are abolished in UCP2 knockouts, strongly implicating UCP2 in the fatty-acid attenuation of glucose-stimulated insulin secretion (Joseph et al., 2002, 2004). In wild-type islets, endogenous superoxide activates mitochondrial proton conductance and removal of endogenous superoxide enhances glucose-stimulated insulin secretion. Hyperglycemia diminishes the insulin response to glucose through a superoxide-dependent mechanism. These effects are abolished in islets from UCP2 knockouts, showing that superoxide activation of UCP2 proton conductance in islets attenuates insulin secretion and mediates the suppression of insulin secretion by hyperglycemia (Krauss et al., 2003).

How does this model accommodate the requirements listed above? Activation of UCP2 may mediate some of the effects of fatty acids on insulin secretion, providing a rationale for fatty-acid upregulation of gene expression. Superoxide and alkenal activation would be signals to activate mild uncoupling when fatty acids are high. Anion transport might be part of the uncoupling cycle, or a side reaction. The apparently weak phenotype is not weak at all: it is marked when the effects on insulin secretion of high fatty acids and hyperglycemia are investigated.

Thus activating the proton conductance of UCP2 by ROS attenuates insulin secretion in pancreatic β cells. This may be a pathological side effect of a protective mechanism that limits ROS production and islet damage during high-fat feeding or hyperglycemia (Model 2) and leads to type II diabetes (Joseph et al., 2004; Krauss et al., 2003; Zhang et al., 2001). In addition, it may be a physiological mechanism to regulate the use of fatty acids and glucose as fuels (Model 3) (Brand et al., 2004a).

Model 4: UCPs mediate the export of fatty acids and fatty-acid peroxides

Model 4A. UCP3 exports fatty acids allowing high fatty-acid oxidation rates or protecting against lipotoxicity

When fatty acids are the predominant substrate for muscle and brown fat, they are converted to fatty acyl CoA for oxidation. If fatty-acid supply exceeds the oxidation rate, fatty acyl CoA might accumulate in the mitochondria and limit further fatty-acid β oxidation because of lack of free CoA. To circumvent this, acyl CoA is hydrolyzed within the mitochondria to fatty-acid and free CoA. In this model, the fatty-acid anion is exported to the cytosol by UCP3, allowing continued rapid fatty-acid β oxidation in the face of any oversupply (Figure 1, Model 4A) (Himms-Hagen and Harper, 2001).

A related hypothesis suggests that when fatty-acid supply is high, fatty acids protonate and flip into mitochondria, where they accumulate up to ten-fold because of the pH gradient. They cannot be metabolized because of the lack of matrix acyl CoA synthases and may be toxic. UCP3 would export the fatty-acid anions, which in principle could lower the matrix concentration more than 1000-fold. The cycle of protonated fatty-acid entry and deprotonated fatty-acid anion export would cause net proton influx as a secondary side reaction rather than as the primary function (Figure 1, Model 4A) (Schrauwen and Hesselink, 2004).

In our opinion, the evidence for these hypotheses is entirely circumstantial. The hypotheses are consistent with observations that UCP3 can transport anions (Esteves and Brand, 2005). They are supported by observations that UCP3 expression is upregulated by long-chain fatty acids and tends to correlate with the oxidation of fatty acids and the expression of other genes involved in fatty-acid oxidation (Himms-Hagen and Harper, 2001; Schrauwen and Hesselink, 2004). However, the correlations with fatty-acid metabolism are equally well explained by Model 2 (attenuation of ROS production), so the correlations cannot be taken as evidence in favor of or against either model.

How do these models accommodate the requirements listed above? They explain the fatty-acid upregulation of gene expression and the anion transport by UCP3, but not why superoxide and alkenals activate the proton conductance of UCP3. They predict inhibition of fatty-acid oxidation or enhanced lipotoxicity in UCP knockouts, but this has not been observed despite specific tests of high-fat diets. However, it may be that some particular aspect of the conditions under which this function is recruited was not in place in these experiments.

Thus these hypotheses are consistent with physiological data correlating fatty-acid oxidation and UCP3 expression, but they lack the much stronger evidence that comes from knockout phenotypes, and they fail to explain the ROS-associated properties of UCP3 discussed above. They need to be supported by more direct tests if they are to be serious contenders.

Model 4B. UCP2 and UCP3 catalyze the export of fatty-acid peroxides

The polyunsaturated fatty-acid side chains of membrane phospholipids are very sensitive to oxidation and can form aggressive oxidants such as hydroxynonenal that damage mitochondrial proteins and DNA. If UCP2 and UCP3 were to export

fatty-acid peroxide anions following their release from phospholipids by phospholipases, they could rid the inner leaflet of the mitochondrial membrane of damaging peroxides, leaving them more safely in the outer leaflet (Figure 1, Model 4B) (Goglia and Skulachev, 2003).

In an extension of this hypothesis, if the exported fatty-acid peroxides could protonate and flip back across the membrane, UCPs would catalyze net proton transport (Figure 1, Model 4B) (Jaburek et al., 2004).

The first model does not explain the observed net proton transport. It could explain changes in ROS production by mitochondria as the result rather than the cause of oxidative damage. The hypothesis that fatty-acid peroxide cycling across the membrane causes net proton transport is a variant of the fatty-acid cycling model that provides an alternative mechanistic explanation for the activation of UCPs by ROS; the evidence for the function of net proton transport in attenuating ROS production and ROS-related damage is discussed above. These models explain anion transport by UCPs, but whether they are correct awaits further experimentation.

Conclusions

It is clear that UCP2 and UCP3 increase the proton conductance of the mitochondrial inner membrane, but only when they are activated by products of ROS metabolism such as hydroxynonenal, and perhaps by fatty acids. Most importantly, they do not affect the basal proton conductance of the membrane in the absence of these activators. Many studies on the function of UCPs measure only mRNA levels, but it follows that changes in mRNA or even protein levels for UCPs cannot predict the functional effect (as this will depend on the activation state of the protein in situ) but can only predict the capacity of the protein for activation.

UCP2 and UCP3 are not generally responsible for adaptive thermogenesis, but nonetheless they may be significantly thermogenic when fully activated by endogenous or exogenous effectors. There is strong evidence that the mild regulated uncoupling they cause is responsible for attenuating mitochondrial ROS production and protecting against ROS-induced cellular damage, and for diminishing insulin secretion by pancreatic β cells. The effect on insulin secretion may be a pathological side reaction of the ROS-attenuating function, but a more attractive hypothesis is that it represents an important physiological regulator of insulin secretion in β cells. In an extension of this idea, UCP2 may have a general role in attenuating secretory or excitatory function in cells that use ATP or ADP as internal signals of energy supply, for example, in specific cells in the brain, such as hypothalamic neurons, which display glucose-dependent excitation and neuropeptide secretion. It could also modulate ROS production by mitochondria, which may be used as a signal for regulating gene expression. The evidence that UCP2 and UCP3 are involved physiologically in fatty-acid (peroxide) anion export is weak and circumstantial and needs to be improved before hypotheses based on this model can be accepted.

Now that at least some of the physiological functions of UCP2 and UCP3 have been identified, their importance in human physiology, pathology, and medicine is becoming clearer. They remain important targets for activation to treat obesity or for inhibition to treat weight loss (or to improve feed efficiency in domesticated animals). Enhancing their expression and their activity by nontoxic ROS analogs may become an important way to attenuate mitochondrial ROS production and the consequent oxidative damage that may underlie normal aging and contribute to a variety of degenerative diseases. Inhibiting the expression or activity of UCP2 may protect against inadequate insulin secretion caused by hyperglycemia or lipotoxicity in pancreatic β cells in diabetes. Clearly, some of these prospects are mutually incompatible, so further understanding of the conditions under which the proteins operate and careful design and targeting of activators and inhibitors will be required to ensure that only the beneficial effects are enhanced in any therapies that may be developed.

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