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Comprehensive Invited Review

The Emerging Functions of UCP2 in Health, Disease, and Therapeutics

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ABSTRACT

The uncoupling proteins (UCPs) are attracting an increased interest as potential therapeutic targets in a number of important diseases. UCP2 is expressed in several tissues, but its physiological functions as well as potential therapeutic applications are still unclear. Unlike UCP1, UCP2 does not seem to be important to thermogenesis or weight control, but appears to have an important role in the regulation of production of reactive oxygen species, inhibition of inflammation, and inhibition of cell death. These are central features in, for example, neurodegenerative and cardiovascular disease, and experimental evidence suggests that an increased expression and activity of UCP2 in models of these diseases has a beneficial effect on disease progression, implicating a potential therapeutic role for UCP2. UCP2 has an important role in the pathogenesis of type 2 diabetes by inhibiting insulin secretion in islet beta cells. At the same time, type 2 diabetes is associated with increased risk of cardiovascular disease and atherosclerosis where an increased expression of UCP2 appears to be beneficial. This illustrates that therapeutic applications involving UCP2 likely will have to regulate expression and activity in a tissue-specific manner. *Antioxid. Redox Signal.* 8: 1–38.

I. INTRODUCTION

THE FULL ARRAY of actions of uncoupling proteins (UCPs), and UCP2 in particular, in the functioning body are currently not well known. However, it is clear that these novel mitochondrial proteins are present in several tissues of the body, and that they may have an important role in the development and/or treatment of several major diseases, including the metabolic syndrome, diabetes, obesity, cardiovascular and neurodegenerative disease. One of the current challenges and obstacles of UCP research is the lack of a broad understanding of how to manipulate endogenous substances to activate UCPs and, perhaps more importantly, what pharmacological compounds could selectively activate different UCPs. The aim of this article is to review the current knowledge about UCP2, with a focus on the role of UCP2 in development and treatment of disease. The introduction contains sections about the function, regulation of expression, and activity of UCP2. The introduction is followed by a section that reviews what is known about the role of UCP2 in specific diseases and organ systems. After this section, the potential therapeutic possibilities using UCP2 are addressed.

A. The function of uncoupling proteins

Uncoupling proteins (UCPs; thermogenins) are encoded by nuclear DNA and are located in the inner membrane of the mitochondria. Their primary function is thought to be to translocate protons from the intermembrane space to the matrix of the mitochondria (16, 38, 120, 233, 337, 390). In the individual mitochondrion, these proteins, through this process, may reduce the driving force of ATP synthase from catalyzing ATP synthesis, dissipate energy in the form of heat, diminish the production of superoxide anion, and decrease the likelihood of calcium entry to the mitochondrial matrix (11, 230, 272). Also, UCPs appear to be important to several metabolic processes (192).

The most well-characterized UCP (UCP1) was first discovered in the 1960s when researchers, focusing their attention on the thermogenic capacity of brown adipose tissue (BAT) (38, 277), were looking more specifically at the mitochondria in BAT to determine the mechanism of fat storage and mobilization in response to both dietary restrictions and temperature (275). Experiments done during this time recognized the ability of BAT to provide the mitochondria with enough oxygen to allow them to function; also its stores of lipid substrates could be mobilized by lipases activated in response to the sympathetic nervous system (SNS) (275).

BAT consists of specialized fat cells that function in heat generation and energy balance, particularly through nonshivering thermogenesis. Hibernating and cold-adapted animals have significant stores of such tissue. The evidence indicates that UCP1 functions to maintain the core body temperature of hibernating mammals and other cold-adapted animals by rais-

ing the resting metabolic rate (38, 277), is necessary for nonshivering thermogenesis in mice (142, 271), and plays an important role in cold- and diet-induced thermogenesis [reviewed in (34, 275)]. While humans have a UCP1 gene that is active in brown fat, these fat deposits disappear shortly after birth (38). Therefore, until recently, little attention has been paid to this mechanism with regard to other tissues. Nonetheless, measurements showing that 25% to 30% of the oxygen which humans and other animals utilize to metabolize food is used to compensate for mitochondrial proton leaks suggested the presence of other UCPs in humans. In fact, in the last few years several human UCPs, starting with UCP2 (120, 136) have now been identified (UCP1-4, BMCP1) (34, 37, 109, 233, 337, 390). The five putative UCPs have been found to promote partial uncoupling of oxidation from phosphorylation *in vitro*, but differ greatly in tissue distribution and regulation which indicate they have distinct physiological roles, although these roles are still very much debated (109).

As the name indicates, UCPs serve an uncoupling function, specifically by uncoupling proton flux through the mitochondrial membranes and ATP synthesis (36, 233, 337, 390). The mitochondrial oxidation of metabolites (e.g., pyruvate and fatty acids) is accompanied by proton transport out of the mitochondrial matrix, thereby generating a transmembrane proton gradient. The protons re-enter the mitochondria through the ATP synthase and drive the synthesis of ATP, thereby coupling oxidation of fuel to energy production. The UCPs, however, provide a route for the re-entry of the protons that is uncoupled to ATP synthesis. Consequently, instead of the proton gradient resulting in the generation of ATP, UCPs act to convert the proton gradient into heat energy and increase the rate of respiration (Fig. 1).

It is likely that the uncoupling activity of the UCPs is under tight control within the cell, since activation may have substantial effects on cell metabolism. Extensive characterization of function has been performed on UCP1, and also to some extent on the analogues UCP2 and UCP3 (58% and 55% sequence similarity, respectively). Importantly, 10 or 11 of the 12 residues identified in UCP1 so far as critical to regulation of UCP1 function are conserved in UCP2 (103), suggesting that data on UCP1 function may be relevant also for UCP2. The mechanism of mitochondrial uncoupling by UCPs is not completely known, but there are two main hypotheses:

i) UCPs transport protons directly (193)
ii) UCPs transport nonesterified fatty acid anions out of the matrix in a process called fatty acid cycling (174).

Both process i) and ii) would reduce the proton gradient across the inner mitochondrial membrane and uncouple mitochondrial ATP production. UCP2 is activated by free fatty acids (FFA) (Figs. 2 and 3) (102, 363, 422, 423), and there are two main lines of argumentation for how this occurs. The first model is similar to i), and suggests that FFA donate H^+ directly to the uncoupling protein, which translocates the proton to the matrix. The donation has been suggested to occur by direct interaction between the FFA and the UCP (194), or through interaction between Coenzyme Q (CoQ) and FFA, where FFA in combination with CoQ donates H^+ to UCP, that transports H^+ across the membrane (104) (see Fig. 2). The second line of argumentation is similar to (ii) in which protonated and electroneutral FFA flip-flop across the inner mitochondrial membrane, release H^+ in the mitochondrial matrix, and the monovalent, negatively charged fatty acid is transported to the outside of the mitochondrial membrane by the uncoupling protein, where the cycle repeats (Fig. 2). UCP1 can transport fatty acid anions (135), which supports the fatty acid cycling model (ii). However, it has been shown that also fatty acid derivatives that are unable to flip-flop through the mitochondrial membrane successfully activate H^+ transport by UCP1, suggesting that fatty acid cycling is not required for H^+ to occur (194, 409).

Mitochondrial respiration is detected as rate of oxygen consumption in the presence of substrate. Respiration is in-

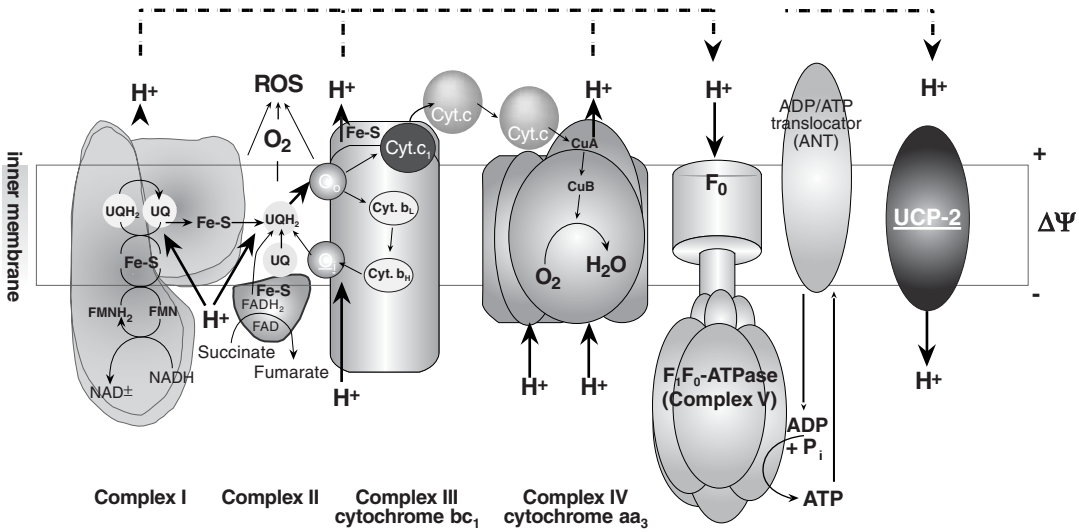

FIG. 1. Uncoupling action of UCP2. The electron transport chain builds up a proton gradient across the inner mitochondrial membrane (IMM), which provides energy for the conversion of ADP to ATP through the ATP synthase (complex V). The uncoupling proteins provide an alternative pathway for proton re-entry to the mitochondrial matrix, thereby generating heat instead of ATP.

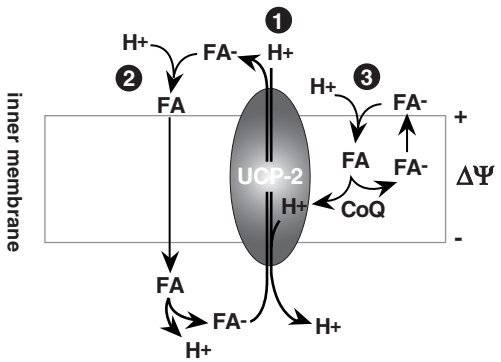

FIG. 2. Schematic drawing of three hypothetic mechanisms for UCP2-mediated uncoupling: 1) UCP2 transport protons directly; 2) Electroneutral, protonated fatty acids diffuse across the inner mitochondrial membrane and the proton is released in the matrix. The fatty acid anion is transported back to the intermembrane space by UCP2, and the cycle repeats; 3) Electroneutral, protonated fatty acids donate protons to UCP2 with help of Coenzyme Q (CoQ). UCP2 transport the protons to the matrix side, and the fatty acid anion returns to the intermembrane space, and the cycle repeats.

creased in the presence of ADP, which induces a transient depolarization as ADP is converted to ATP, and thereby an increased flow of electrons and consumption of oxygen to maintain the proton gradient over the inner mitochondrial membrane. The conversion of ADP to ATP is blocked by addition of oligomycin (an inhibitor of ATP synthase), meaning that the remaining respiration is a result of proton leaks across the inner mitochondrial membrane, mediated, for example, by UCPs. Addition of FFAs increase respiration in isolated mitochondria (Fig. 3), especially in mitochondria from animals overexpressing UCP2 (244).

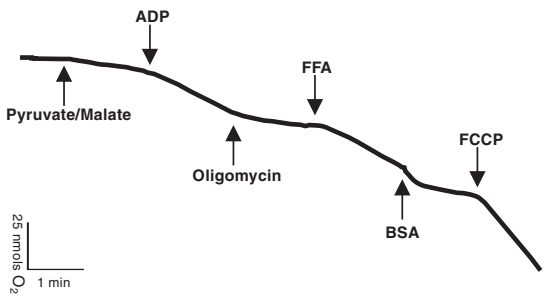

FIG. 3. Free Fatty Acids (FFA) increase respiration in brain mitochondria. Respiration is detected as a decrease in oxygen in the mitochondrial suspension. The rate of oxygen consumption is increased following addition of ADP (*arrow*), and decreased when the conversion of ADP to ATP is inhibited by the addition of oligomycin (*arrow*). After addition of FFA (*arrow*), the respiration rate increases again, presumably through FFA-mediated activation of UCPs. The increase in respiration is reversed by the addition of BSA (*arrow*), which binds FFA. Maximum respiratory rate is detected following addition of FCCP (*arrow*), a protonophore that strongly depolarizes the inner mitochondrial membrane.

B. Expression of UCP2; tissue distribution, and physiological function

UCP1 and UCP3 are expressed in peripheral tissues (UCP1 only in brown adipose tissue and UCP3 solely in skeletal muscle and the heart in humans) and UCP4 and brain mitochondrial carrier protein 1 (BMCP1/UCP5) are predominantly expressed in the central nervous system (233, 337). It is of significance to note, however, that UCP4 and BMCP1/UCP5 have only 30% similarity to UCP1 in amino acid sequence (233, 337). In addition, these proteins were not proven to be mitochondrial uncouplers either *in vivo* or *in vitro* in knockout animals.

The UCP2 gene maps to human chromosome 11 and UCP2 mRNA is found in many tissues with a relatively larger amount in spleen, thymus, pancreatic β-cells, heart, lung, white adipose tissue, stomach, and testis and a lesser amount in brain, kidney, liver, and muscle (36, 102, 120, 121, 167, 315) (Fig. 4). The expression of UCP2 will be discussed in greater detail below when the role of UCP2 in specific diseases/organ systems is considered. UCP2 was first cloned and identified in humans in 1997 and subsequently in rodents (34) and shares about 58% amino acid identity with UCP1. However, UCP2 does not appear to be solely involved in thermogenesis, but has proposed roles in modulating generation of reactive oxygen species and in lipid handling (75). The UCP2 amino acid sequence has high homology across species: rat UCP2 is 99% and 95% identical to mouse and human UCP2, respectively (159). The strong conservation of the sequence across species and its widespread expression among organs indicate that UCP2 is a physiologically important protein. The expression pattern of UCP2 is consistent with a wide variety of proposed roles for UCP2. Variations in activity or regulation of UCP2 in these tissues could contribute to regulation of cell death, obesity, and associated diseases, suggesting an important role

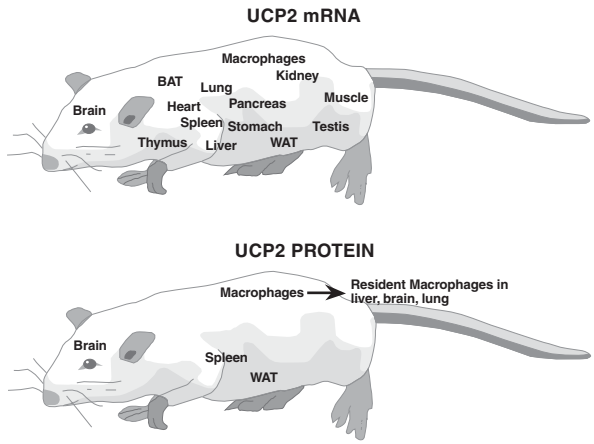

FIG. 4. Schematic drawing of UCP2 mRNA and protein expression. UCP2 mRNA is expressed in a number of tissues, but translation to detectable amounts of protein has not been verified in parenchymal cells of all these tissues, largely due to a lack of reliable UCP2 antibodies. Analysis of UCP2 protein expression is also complicated by the fact that UCP2 mRNA is expressed in macrophages, including resident macrophages in several types of tissue (e.g., microglia in the brain), meaning that it is often difficult to determine if protein expression occurs in macrophages or in parenchymal cells of the tissue.

for UCP2 in a number of diseases with a major socioeconomic impact, which explains the large and growing research effort within the field.

To date, several hypotheses have been put forth concerning possible physiological roles of the UCPs, including energy partitioning, energy balance and control of metabolism which may be important for metabolic disorders such as obesity and diabetes [for review see (7, 173)]. The primary physiologic and pathophysiologic functions of UCP2 are probably related to regulation of ATP synthesis and ATP/ADP ratio (43), the regulation of glucose and fatty acid metabolism and fatty acid anion export (135), ROS production (11, 272, 314, 363, 368) and handling of ROS and cellular redox changes (57, 244). The physiological functions of UCP2 are still debated, but it has been implicated in hyperinsulinemia (176), obesity (69, 120), aging (41, 259), and beneficial cardiac effects of exercise (361). UCP2 may also have a role in inhibiting cell death (23, 129, 244, 252, 363, 377) and in modulating inflammation (11, 98). UCP2 may be induced as an endogenous protective response to cellular stress, for example, following sublethal cerebral (244) and cardiac (252) ischemia or radiation damage (393). In addition, UCP2 may have a role in hypermetabolic states such as those associated with sepsis (371), cancer cachexia (27, 49, 336), and hyperthyroidism (239, 430).

C. Control of UCP2 expression and activity

To date, limited information is available on the regulation of UCP2 mRNA expression and translation, as well as protein function. Importantly, due to translational regulation of UCP2, changes in mRNA levels do not necessarily have to be reflected as changes in UCP2 protein, and conversely, changes in UCP2 protein level does not have to be preceded by changes in mRNA levels (299). *In vitro* and *in vivo* data that are available on peripheral UCP2 expression show varying levels of UCP2 regulation by metabolic rate and FFA, as well as different hormones, including leptin (430) and thyroid hormone (239, 430). It appears, however, that the regulation of UCP2 by these hormones is tissue-specific and most likely involves indirect mechanisms of action. Transcriptional regulation of UCP2 is further complicated by the robust promoter polymorphism of the UCP2 gene (111). FFA and metabolic rate as measured by oxygen consumption may change as a result of injury (244), diet, and during perinatal development (45, 116, 363, 367). Other biologically active substances were also shown to affect transcription, translation, and/or activity of UCP2, including retinoic acid (54, 313), lipopolysaccharides (78), coenzyme Q (104, 105), superoxide anion (101, 102) and free fatty acids (FFA) (363, 367, 378). The expression of UCP2 also varies during postnatal development, and has been suggested to have a role in the maturation of lung structure, energy expenditure, and lipid metabolism (98, 144, 264, 345).

A number of physiological and pathological states lead to increased expression of UCP2 mRNA. These include fasting (35, 52, 185, 256, 333, 352, 385), high-fat diets (120, 143, 243, 268, 367, 373, 381), suckling of newborn pups (412), sepsis (114), acute endurance exercise (80, 341), neurodegenerative disease (23, 244, 363), and hyperthyroidism [reviewed in (211)], as well as experimental manipulations such as lipid infusion (186, 285), streptozotocin-induced diabetes (160), and treatment with PPAR agonists (15). Considerable effort has gone into determining how the expression of UCP2 is regulated. It turns out that several of the various physiological and pathological states that are associated with raised levels of UCP2 mRNAs are characterized by elevated plasma free fatty acid (FFA) levels. This is the case following brain ischemia (22, 90, 91, 96, 407), obesity and the metabolic syndrome with associated insulin resistance (236, 322). However, UCP2 can be up-regulated in the absence of increased levels of FFA (49), and FFA are often not the only potential activators of UCP2 expression that are present following, for example, acute brain injury or neuronal stress, where UCP2 expression is increased in the brain (23, 244, 363). Typically, neurodegenerative disease is associated both with an increased production of reactive oxygen species (ROS) and elevated levels of FFA. Similarly, diabetes is associated with hyperglycemia in addition to hyperlipidemia, as well as an increased production of ROS, which makes it complicated to dissect the activation pathways *in vivo*. In spite of this, it is clear that FFA are important molecular signals that induce UCP2 expression, and better understanding of how FFA could induce UCP2 expression has the potential to provide new information about how processes related to energy metabolism are controlled in health and disease (378). It is also possible that different combinations of inducers of UCP2 will produce a tissue-specific response, which may be related to distinct physiological roles of UCP2 in different tissues.

a. Free fatty acids and UCP expression

The hypothesis that UCP2 mRNA expression could be induced by FFA was quickly established in skeletal muscle and adipocytes (332). In preadipocyte cell lines, unsaturated FFA markedly induced UCP2 mRNA (14, 311). A number of other cultured cell systems representing heart, liver, and pancreatic islets also responded to addition of various FFA to the culture medium with increased levels of UCP2 mRNA (8, 209, 254, 385). Islet β-cells are the only cells so far to show responsiveness to a saturated FFA (209). The response to FFA is most likely due to increased transcription, since addition of actinomycin D, an inhibitor of transcription, prevented the increase of UCP2 mRNA (311). From these studies, it is clear that all classes of unsaturated FFA and/or their metabolism can directly bring about the up-regulation of UCP2 mRNA in cultured cells. This apparent lack of specificity for the FFA that causes induction seems surprising. It suggests that FFAs per se are the regulators rather than a specific secondary metabolite derived from a particular pathway. The response is dependent upon the cell type and supports the likelihood of tissue-specific differences in the signaling mechanisms regulating UCP2 expression in response to FFA.

Regulation of UCP2 expression by FFA could occur through direct interaction of FFA with the UCP2 promoter region. Some regions that confer FFA responsiveness within the 50-upstream regulatory region (50-USR) of the UCP2 genes have been identified. If FFA works via peroxisomal proliferator-activated receptors (PPARs) (268) or sterol responsive element binding protein (SREBP), then binding sites for these factors might be expected within this region. Such binding sites have been found, and were determined to consist of one Sp1 site, a putative SRE motif, and two E-box motifs. Mutations of the SRE

site or either of the E-boxes eliminated the response of the UCP2 promoter to FFA. If FFA are regulating UCP2 expression via this region, then transcription factors that can bind to them would in turn need to be influenced by FFA, and several such transcription factors have been suggested [SREBP1, upstream stimulatory factor 1 (USF1) and USF2] (254), and also PPARγ, which appears to act indirectly via this same region (255). Overexpression of SREBP-1c in islet β-cells led to an increased expression of UCP2 (376).

There is already good evidence that FFA induce transcription of a cohort of genes involved in lipid oxidation in liver and adipose tissue by acting as ligands of PPARs (99). It is possible that UCP2 belong to this cohort of genes as its increased expression could potentially increase FFA oxidation. Unsaturated FFAs that have been shown to induce UCP2 have also been shown to act both as ligands and activators for all three main isoforms of PPARs (125). Other known synthetic PPAR ligands, such as the thiazolidinediones, are able to upregulate UCP2 (15). The pattern of up-regulation by selective PPAR ligands in cultured cells matches the predominant tissue-selective expression of the PPAR isoforms: via PPARγ in adipose tissue (14, 318, 392, 418), via PPARδ in muscle (265) and via PPARα in liver (8, 267). All these observations are consistent with, although not definitive proof of, FFA acting via PPARs to upregulate UCP2, and also suggest that this may occur in a tissue-specific manner via different PPAR isoforms.

An alternative pathway for regulation of UCP2 expression by FFA is the sterol responsive element (SRE) binding protein (SREBP) family of transcription factors, which are known for their role in regulating lipid metabolism (162). SREBP1 induces the expression of genes involved in lipid synthesis in liver and adipose tissue. Polyunsaturated FFA decrease the expression of SREBP1 mRNA and decrease the level of the proteolytic fragment of SREBP1 that enters the nucleus as the active transcription factor, thus decreasing the ability of SREBP1 to activate transcription of the SREBP responsive lipogenic genes in liver (241, 415). UCP2 is unlikely to belong to this group of lipogenic genes because all conjecture so far gives UCPs a role in lipid oxidation. Interestingly, polyunsaturated FFA repress the expression of these same genes (61, 99), and this appears to occur via a mechanism involving SREBP1. SREBP1 levels are inversely associated with UCP2 mRNA levels in adipose tissue (157, 187) and skeletal muscle (28, 149), respectively. This suggests that SREBP1 could repress UCP2 (perhaps in a tissue-specific way), so that when FFA decreases SREBP1 activity, repression would be relieved and the expression of UCPs would be increased. However, conflicting data showing increased UCP2 expression in the presence of increased levels of the active form of SREBP1 have been reported (254, 397).

b. Regulation of UCP expression by glucose In the case of glucose regulating the expression of UCP2, there are conflicting results. Hyperglycemia (> 48 hours) has been reported to have no effect on (225), to reduce (323), or to increase UCP2 mRNA and/or protein expression (44, 297). When rats were made hyperglycemic by means of partial pancreatectomy (213) or glucose infusion (179), UCP2 mRNA expression was up-regulated—an effect reversed by normalizing the plasma glucose concentrations with phlorizin (inhibits renal reuptake of glucose) (213).

c. Regulation of UCP2 expression by ROS Expression of UCP2 in hepatocytes have been suggested to be increased as a response to increased ROS production *in vivo* and *in vitro* (78, 79, 306), suggesting that UCP2 could be part of an endogenous response to oxidative stress. Further, it has also been suggested that superoxide anion regulate UCP2 transcriptionally and posttranslationally (299). Superoxide anions and lipid peroxidation products, including hydroxyalkenals such as hydroxynonenal, are potent activators of proton conductance by mitochondrial uncoupling proteins such as UCP2 and UCP3, although the mechanism of activation has yet to be established (42). The accumulating evidence suggests that the main physiological role of UCP2 is to lower the production of ROS, rather than participating in energy expenditure or metabolic control (42, 110).

D. UCP2 activity

As discussed above, UCP2 acts as a protonophore, similar to UCP1, is activated by FFA and is nucleotide-dependent (101, 102, 104, 105, 120, 299). In addition, *in vitro* evidence emerged to suggest the critical involvement of coenzyme Q (104, 105) and superoxide anion (101, 102) in the activation of UCP2 as an uncoupler in peripheral tissues. Further, it has been suggested that it is superoxide anions from within the matrix of the mitochondria that are required for the activation of UCP2 (101). These findings are, however, equivocal (81). An alternative hypothesis to explain increased uncoupling by superoxide anion is that it is protonated to hydroperoxyl radicals ($HO_2^{\bullet}$) by combining with protons in the intermembrane space. $HO_2^{\bullet}$ then diffuses across the inner mitochondrial membrane to the matrix, where it dissociates back into H^+ and superoxide anion (228). However, the hydroperoxyl radical has a high reactivity, and has been shown to react with fatty acids, making it unlikely that the hydroperoxyl radical is a significant contributor to uncoupling mediated by superoxide anions. Whether these findings apply *in vivo*, and whether tissue specificity and other endogenous activating substances will emerge, remains to be determined.

The activity of UCP1 is inhibited by purine nucleoside tri- and diphosphates (GTP, GDP, ATP, ADP) (277, 360), which bind to UCP1 on the cytosolic side of the inner mitochondrial membrane. This inhibition is pH-dependent, and decreases with increased pH (168, 191, 274, 305). Also, UCP2 activity is stimulated by retinoic acid in a pH-dependent manner (313). The pH-dependent activity of UCP2 suggests that it may be involved in metabolic regulation and avoidance of ATP overproduction under conditions of high fatty acid synthesis (313). However, although the regulatory effects of FFA and purine nucleotides are well documented *in vitro*, the role of these regulators *in vivo* is either unproven or unknown.

The activity of UCP2 may, like UCP1 (317) be posttranslationally regulated in a variety of models (100, 244), including in pancreatic β-cells (202).

E. Effect of UCP2 on cell function

The uncoupling of mitochondrial respiration by UCPs raise several intriguing possibilities regarding their impact on

cell function, ranging from thermogenesis and effects on energy production, to calcium homeostasis, generation and handling of reactive oxygen species and control of cell death.

a. ATP The mitochondrial $\Delta\Psi$ is generated by the translocation of protons across the inner mitochondrial membrane via the electron transport chain, culminating in the oxidation of substrates and the reduction of O_2 to H_2O. This store of potential energy (the electrochemical gradient) is then coupled to ATP production as protons flow back through the ATP synthase where ADP is phosphorylated to generate ATP. UCPs dissociate oxidation from phosphorylation and dissipate energy in the form of heat (Fig. 1). By this, UCPs can decrease mitochondrial ATP production, which could affect cellular activity. Indeed, observations on UCP2 knockout animals revealed increased pancreatic ATP and ADP ratios, which were temporarily associated with increased insulin secretion by pancreatic β-cells (425). In contrast to this theory, it has been observed that induction of mitochondrial uncoupling by UCP2 in the brain and UCP3 in muscle cells led to elevated ratios of ATP and ADP (133, 165). One possible explanation may be that UCPs induce mitochondrial proliferation (411); both in the brain (165) as well in adipose tissue (326), and that mitochondrial proliferation may provide increased levels of ATP and ADP for a given cell.

b. Calcium Calcium is a key regulator of cell signaling cascades as well as in induction of cell death, particularly during excitotoxicity in neurons. Therefore, maintenance of low intracellular [Ca^{2+}] is necessary for proper cell function, while brief pulses of increased intracellular calcium levels are needed to initiate second-messenger pathways, the basis for intracellular communication. Since Ca^{2+} cannot be metabolized like other second-messenger molecules, the intracellular levels must be tightly regulated by other means. Numerous intracellular proteins and some organelles have adapted to bind or sequester Ca^{2+} to ensure that homeostasis is maintained. Mitochondria are one such organelle (169, 319, 320), and mitochondria are major sites for calcium cycling in cells. Calcium influx or efflux in the mitochondria is dependent upon the inner mitochondrial membrane potential (46, 58, 60, 278), leading to an increased uptake via the electrogenic uniporter when cytosolic levels increase (150). When cytosolic levels of calcium decrease, or mitochondrial membrane potential decreases, mitochondria pump calcium out to regulate cytosolic calcium homeostasis precisely.

Because UCP2 may affect mitochondrial membrane potential, it is then reasonable to suggest that it will have an influence on mitochondrial calcium cycling dynamics. Mitochondrial calcium uptake is Nernstian, suggesting that a drop in mitochondrial membrane potential of, for example, 30 mV would reduce the amount of calcium in mitochondria by ten-fold. However, the magnitude of mitochondrial membrane depolarization due to UCP2 activation under physiological conditions is not known, but is probably not as large as 30 mV. Therefore, the UCP2-mediated depolarization may not be large enough to affect the total amount of calcium sequestered in mitochondria, but may reduce the rate of uptake (276). Although seemingly small, such alterations in cellular calcium dynamics may be very important to initiation of cell death cascades (e.g., following transient cerebral ischemic attacks) (244). In addition, UCP2 may function to locally increase the temperature (132, 165), which may negatively affect the calcium storage capability of mitochondria. This, in turn, could enhance calcium-dependent presynaptic mechanisms. In the case of glutamate excitotoxicity, when there is a rapid elevation of cytosolic calcium due to the opening of ionotropic glutamate receptors at the plasma membrane, UCP2-induced lowering of mitochondrial membrane potential could limit the overloading of mitochondria with calcium, and hence decrease the potential for cell death (244, 363, 368).

c. Reactive oxygen species (ROS) Free radical production is a byproduct of electron flow through the respiratory chain in mitochondria. It is generally believed that during normal cell respiration, 1–6% of the oxygen reduced by mitochondria is converted to superoxide anion at the level of complex I or at the level of ubiquinone (39, 67, 203), and the daily yield of $O_2^{\bullet -}$ could reach 3×10^7 molecules per mitochondrion (321). Normally cells convert $O_2^{\bullet -}$ to H_2O_2 utilizing both manganese superoxide dismutase (MnSOD), which is localized to the mitochondria, and copper-zinc superoxide dismutase found in the cytosol. Superoxide anions do not readily cross membranes (130), but may be transported by anion channels (199). Superoxide anions rapidly react with NO, forming peroxynitrite ($ONOO^-$), which is a mediator of neurodegeneration (112, 342, 375) that may damage and kill cells by induction of lipid peroxidation and protein tyrosin nitration (24, 25). Hydrogen peroxide easily penetrates lipid bilayers, acts as an oxidizing agent, and is relatively stable, although it is not a free radical. Hydrogen peroxide helps modulate signaling systems in the cell, such as kinases and phosphatases (88, 404) and transcription of genes (245), and is not toxic except in high concentrations (134). However, hydrogen peroxide is a precursor in the formation of hydroxyl radicals ($O_2^{\bullet -} + H_2O_2 \rightarrow OH^{\bullet} + OH^- + O_2$), particularly in the presence of ferrous ions (Fe^{2+}; the Fenton reaction), that will be present in the brain parenchyma (e.g., after trauma and intracerebral hemorrhage). Hydroxyl radicals are extremely reactive, and rapidly attack unsaturated fatty acids in membranes causing lipid peroxidation and the production of 4-hydroxynonenal (HNE) that conjugates to membrane proteins, impairing their function (182, 183). Such oxidative injury results in significant alterations in cellular function, and is an important cause of cell death. During homeostasis, the production of ROS is balanced by anti-oxidant systems such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, maintaining the levels of $O_2^{\bullet -}$ and H_2O_2 *in vivo* at about 10^{-11} and 10^{-9} *M*, respectively (40, 126).

Mitochondrial ROS production is intimately linked to $\Delta\psi$ such that hyperpolarization (high $\Delta\psi$) increases and promotes ROS production (353, 355). The underlying mechanism is the altered redox potential of electron transport chain carriers (reduced) and an increase in semiquinone anion half-life time (high $\Delta\psi$ prevents b_h oxidation of cytochrome b_1 in the Q cycle). In other words, at a high $\Delta\psi$, protons can no longer be pumped out of the matrix (against the electrochemical proton gradient) by the electron transport chain, so electron transport slows/stalls resulting in intermediates staying reduced longer and increasing the chance that the electrons escape from these

intermediates, reduce O_2 and increase ROS production. Since the magnitude of ROS production is largely dependent on—and correlates with—$\Delta\psi$, even a modest reduction via increased proton conductance (decreases $\Delta\psi$, the electrochemical proton gradient) across the mitochondrial inner membrane (uncoupling) reduces ROS formation (188, 353, 395).

Skulachev was the first to hypothesize that mild uncoupling could be beneficial since it causes a decrease in ROS production (353). Several studies have now demonstrated roles for UCPs in modulating ROS production (11, 272, 363, 367, 368). In UCP2 knockout animals, increased free radical production by monocytes has been attributed to strengthening the innate immune system and preventing *Toxoplasma gondii*-induced lethality (11). UCP3 knockout animals exhibited increased levels of ROS in muscle (391). Leptin-deficient mice have decreased levels of UCP2 and increased ROS production in macrophages (215). Overexpression of UCP2 (224) or UCP5/BMCP1 (188) decrease cell death following H_2O_2 exposure and ROS production, respectively. This aspect of UCP function further strengthens the proposition that UCPs can modulate mitochondrial ROS production and activity, and, thus, may participate in cell protection (Figs. 5 and 6). Also, cellular redox status influences a number of signaling systems in the cell, which may induce protective responses (see below in the CNS section; Fig. 12).

d. Thermogenesis In relation to neuronal functions, one very exciting and provocative aspect of controlled mitochondrial uncoupling by UCPs is the potential to affect the temperature in the microenvironment. There has been a great debate regarding the thermogenic capacity of UCPs other than that of UCP1. However, this debate is focusing on thermogenesis as it pertains to core body temperature rather than energy dissipation in the form of heat at the mitochondrial level. The

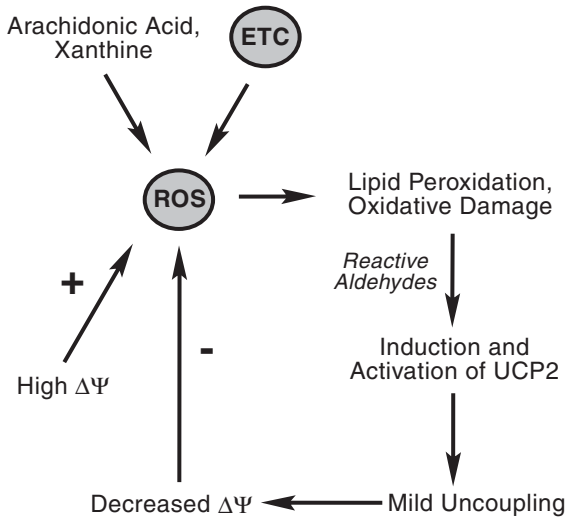

FIG. 5. Schematic drawing of the antioxidative function of UCP2. ROS are produced as byproducts in the metabolism of xanthine and arachidonic acid, which is activated particularly following ischemic events. The main source of ROS is however the mitochondrial electron transport chain (ETC). During normal respiration, superoxide is formed as a consequence of leakage of electrons from the ETC. Following many forms of cellular injury, the generation of superoxide from the ETC increases, which leads to oxidative damage and peroxidation of lipids, resulting in the formation of reactive aldehydes. Reactive aldehydes activate UCP2, which induces a mild uncoupling of the ETC. The mild uncoupling results in a tighter coupling of the ETC with less leakage of electrons and a reduced formation of superoxide, which reduces the cellular oxidative damage. Similarly, a high membrane potential of the ETC leads to an increased formation of ROS and increased oxidative damage. Consequently, the activation of UCP2 constitutes a negative feedback loop that attenuates the production of mitochondrial ROS.

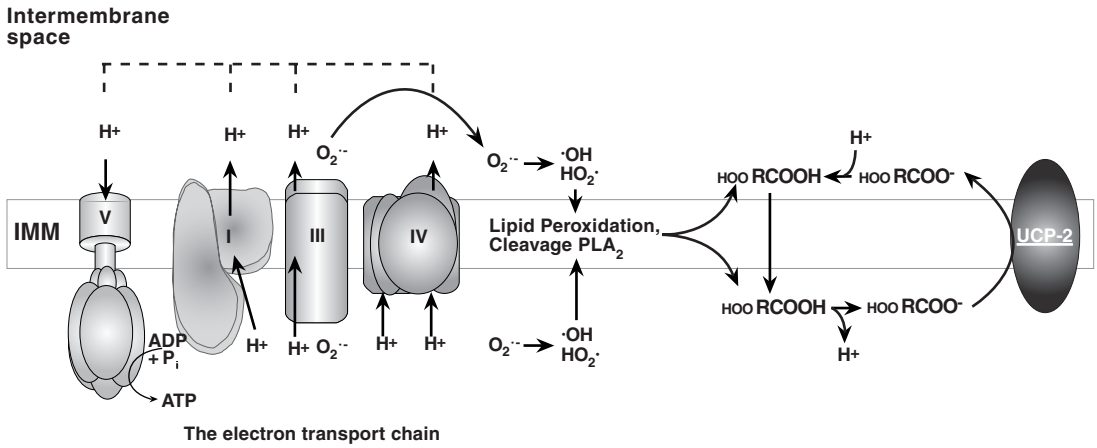

FIG. 6. Model of feedback down-regulation of mitochondrial ROS production by lipoperoxidation products that activate UCP2. In mitochondria, superoxide anions are released both at the matrix- and intermembrane side of the inner mitochondrial membrane. The superoxide anions are hydrated to hydroperoxyl ($HO_2^{\bullet}$) or via H_2O_2 and the Fenton reaction to hydroxyl radicals ($^{\bullet}OH$). Both these compounds are highly reactive and initiate lipoperoxidation, resulting in the formation of carbon-centered radicals, which react with oxygen, forming peroxyl radicals that in turn react with neighboring fatty acid side chains, forming hydroperoxides. The peroxidation product can be cleaved off from the phospholipids by PLA_2, and hydroperoxide-FA (HOOR-COOH) can cycle, and is transported in its anionic form from the matrix side to the intermembrane space, where it may bind a proton and diffuse across the membrane, resulting in a mild uncoupling. This type of uncoupling can attenuate the production of superoxide anions.

distinction between these is critical. It has been suggested that UCP2 and 3 are not thermogenic, because they do not appear to contribute to the generation of core body temperature (11, 425), but Horvath and co-workers have proposed a microenvironmental thermogenic function of UCP2 in the synapses of the brain (165) (see also below in the CNS section). On a similar note, Mizuno and colleagues observed a cold-induced increase in expression of UCP2 in the spinal cord, and suggest that this may play a role in modulating synaptic activity as a response to cold exposure (259).

e. Control of cell death Several studies have suggested that the UCPs, including UCP2, have a role in diminishing cell death by acting on mitochondrial function. The protective functions are related to decreases in production of ROS as discussed above, and also to a decreased activation of mitochondria-mediated cell death. These cell death mechanisms are central in several important clinical conditions, for example, neurodegenerative and cardiovascular diseases.

Based on morphological criteria, cell death has traditionally been divided into two distinct types: apoptosis and necrosis. Necrosis is accompanied by a breakdown of transmembrane ionic pumps caused by a lack of ATP, whereas apoptosis requires ATP and active protein synthesis. However, it has been more and more accepted that necrotic and apoptotic cell death cannot be separated as two totally different entities, and that cell death that has morphological and biochemical features of both apoptosis and necrosis may occur (e.g., following ischemic events). Furthermore, depending on the cellular environment and energy supply, a switch between the two types of cell death within a single cell is possible.

Several mechanisms have been proposed to explain mitochondrial involvement in cell death; for example, increased oxidative stress, altered calcium homeostasis and impairment of respiratory chain complexes. During the last decade, it has become clear that mitochondria are key players in the initiation and control of cell death (208, 234, 424) through activation of the mitochondrial permeability transition pore (mPTP) (154, 219), and release of apoptogenic factors, leading to activation of cell death cascades (Fig. 7). The mPT may be regarded as a checkpoint in cell death similar to the checkpoints in the cell cycle (367). The role of the mPTP in normal cell physiology is still not known. It has been suggested that the mPTP may regulate mitochondrial calcium (151), that it is important for mitochondrial turnover by means of autophagy (218), or elimination of mitochondria that produce excessive amounts of ROS (354), or that the mPTP may be important for the formation of mitochondrial networks within the cell, thereby allowing energy transfer between different parts of the cell (82). Recently, it was also suggested that the mPT regulates synaptic plasticity in the hippocampus, through its influence on mitochondrial calcium storage and intracellular calcium concentrations (220).

Respiration-dependent mitochondrial Ca^{2+}-uptake is important for normal cellular Ca^{2+} homeostasis, but can also contribute to Ca^{2+}-induced cell death. In neurons, mitochondria actively accumulate most of the excess Ca^{2+} that enters

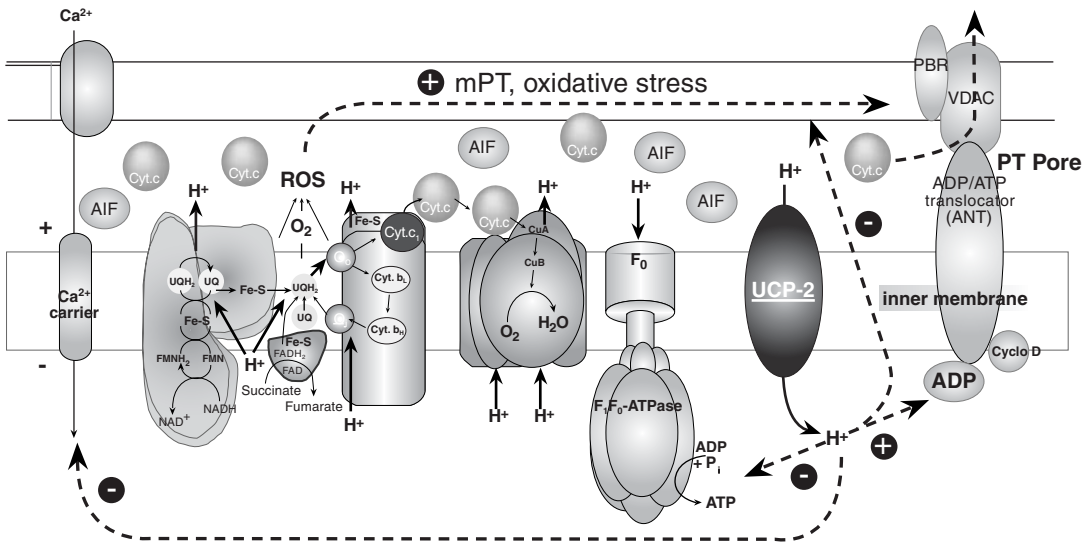

FIG. 7. Schematic drawing of the two mitochondrial membranes including the electron transport chain and the mPT complex. Reactive oxygen species (ROS) are produced by the ETC (complexes I and III). In the outer membrane, the pore protein VDAC is shown interacting with the peripheral benzodiazepine receptor (PBR), and the ADP/ATP translocator (ANT). The latter interaction is represented as forming the mitochondrial permeability transition pore (mPTP), sometimes associated with release of cytochrome c (Cyt.c) and apoptosis inducing factor (AIF) and loss of inner membrane potential during cell death. Activation of the mPTP is stimulated by high levels of intramitochondrial calcium and increased levels of ROS, and inhibited by ADP and cyclophilin D (Cyclo D) binding to the matrix side of the ANT. UCP2 may inhibit mitochondria-mediated cell death by inducing a slight depolarization of the inner membrane, leading to decreased calcium uptake, reduced ROS formation and increased ADP binding to ANT. A more detailed discussion of the depicted mechanisms can be found below.

neurons following excitotoxic stimulation. By depolarizing mitochondria prior to increased levels of intracellular calcium, it has been demonstrated that excessive mitochondrial Ca^{2+} accumulation, rather than increased cytosolic Ca^{2+}, is the primary cause of excitotoxic cell death (46, 60, 359). Excessive mitochondrial accumulation of calcium cause disturbances in oxidative phosphorylation (97, 340, 414), resulting in a decreased capacity for ATP production at a time when the ATP requirement is increased due to the need of restoration of ion gradients and cell repair (414).

Excess mitochondrial calcium sequestration may also trigger mPT, and the consequences of mPT to cellular function and homeostasis are profound. The opening of a large pore in the mitochondrial membrane, and equilibration of solutes < 1500 Da, leads to dissipation of electrochemical gradients (432), uncoupling of oxidative phosphorylation, and triggers mitochondrial ATP hydrolysis due to reversal of the ATP synthase. Furthermore, because of high protein colloidal osmotic pressure in the mitochondrial matrix, mPT triggers mitochondrial osmotic swelling of the matrix and rupture of the outer membrane, since it cannot expand as much as the extensively folded inner membrane, leading to release of proteins from the intermembrane space, as well as calcium from the mitochondrial matrix. Opening of the mPTP in a subpopulation of mitochondria may lead to an increased calcium load on the remaining mitochondria, leading to a propagation of mPT and eventually a collapse of cellular metabolism. Proteins such as cytochrome c (195, 229, 426), AIF (374), smac/DIABLO (95, 388) are released from the intermembrane space into the cytosol (307, 386), where they participate in the activation of cell death cascades, such as the caspases.

Pathophysiologic activation of the mPTP under conditions of increased mitochondrial calcium accumulation and oxidative stress can lead to irreversible mitochondrial dysfunction, and constitutes a critical event in cell death following acute brain injury (119, 128, 368, 405). The protective effect of the mPT-blocker Cyclosporin A (CsA) in several models of acute neurodegeneration (47, 127, 197, 226, 289, 290, 339, 346, 366, 369, 370, 382, 383) lends further support to this conclusion. Also, antioxidant enzymes may be released during mPT (298), suggesting a relative deprotection of mitochondrial membranes from oxidative reactions following mPT.

Although calcium is necessary for mPT to occur, it is perhaps not sufficient. Other activators of the mPT are oxidative stress (59, 115, 217), and low levels of adenine nucleotides (214). Similarly, activation of mPT is inhibited by ADP in the matrix binding to the ANT (154), antioxidants, high membrane potential, magnesium and CsA.

UCP2 may inhibit the activation of mPT by slightly depolarizing mitochondria, leading to a decreased uptake of calcium, a decreased production of ROS and an altered ATP/ADP ratio (244). Given the central role of mitochondria and cell death in a number of common conditions and diseases, such as neurodegeneration, myocardial infarction, HIV and cancer, it is clear that research within this field has the potential to make a substantial impact on several important diseases.

II. ROLE OF UCP2 IN SPECIFIC DISEASES/TISSUES

As mentioned in the introduction, UCP2 is expressed in several different tissues, and seems to have physiological and pathophysiological roles that could be important targets for the treatment of several clinically important conditions. Below, the potential role of UCP2 in different organ systems and diseases will be outlined. Potential therapeutic implications are summarized in a separate section below.

A. Central nervous system

Accumulating evidence suggests that UCP2 could be involved in neuroprotection (23, 74, 92, 244, 259, 363, 367, 394), including global cerebral ischemia (244), traumatic brain injury (244), Parkinson's disease (6, 77), and seizures (92, 363, 367). Below, the experimental findings, and possible explanations of the protective role of UCP2 in the CNS, will be reviewed.

a. UCP2 expression in the brain To date, three of the uncoupling proteins, UCP2, UCP4, and BMCP1/UCP5 have been described in the central nervous system (11, 93, 120, 167, 188, 233). UCP2 is expressed in various parts of the brain; including the hypothalamus (suprachiasmatic, paraventricular, dorsomedial, ventromedial nucleus, and arcuate nuclei), brainstem, and limbic system, suggesting that UCP2 may play a role in neuroendocrine, behavioral, autonomic functions and metabolic processes (93, 167, 314). There has been some debate about differences in expression between species (299), and this debate largely seems to be driven by methodological problems in detecting UCP2 expression. However, Horvath and co-workers demonstrated that brain UCP2 is present in the inner membranes of mitochondria in neuronal profiles in several brain regions in both rodents and primates (93, 167). The expression has been found to be both neuronal and microglial (74), although the identity of the type of neuron that is participating is somewhat contentious.

b. Neuroprotection by UCP2 Excitotoxic cell death is the fundamental process responsible for many human neurodegenerative disorders, yet the basic mechanisms involved are not fully understood. Below is a short review of the cell death mechanisms that are the most relevant with respect to the neuroprotective roles of UCP2, including excitotoxicity, mitochondria-mediated cell death and reactive oxygen species in the development and prevention of secondary injuries following acute (e.g., cerebral ischemia or trauma) (244) (Fig. 8) and excitotoxic brain injury (e.g., epileptic seizures) (363, 367) (Figs. 9 and 10). Following this short introduction is a review of activation of endogenous protective pathways in the brain, including up-regulation of UCP2. Finally, the role of UCP2 in neuroprotection is reviewed.

Acute brain injury causes a depolarization of cells and a disturbance of membrane ion homeostasis with K^+ efflux and Ca^{2+} influx (180, 280, 282). The increase in intracellular Ca^{2+} may persist for up to several days following injury (117, 414).

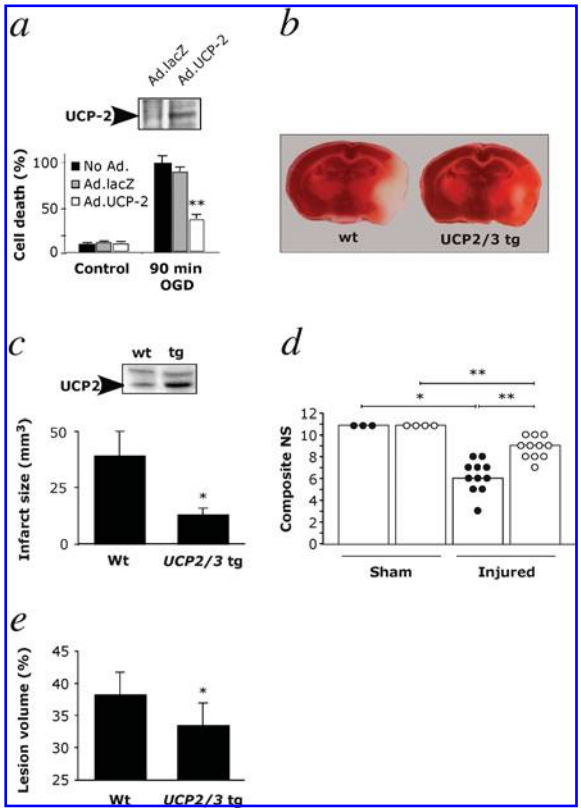

FIG. 8. UCP2 is neuroprotective in models of neuronal damage *in vitro* and *in vivo*. (**a**) Overexpression of UCP-2 (Ad.Ucp2) in neuronal cultures protects against oxygen-glucose deprivation (90 min OGD). (**b, c**) Damage by middle cerebral artery occlusion (MCAO) is diminished in UCP2/3-transgenic mice, as shown by TTC-stained sections. *White area* indicates damage (**b**). Infarct size in wt and UCP2/3-transgenic mice, as well as a western blot of UCP2 protein from the corresponding brain regions is shown in (**c**). (**d, e**) Functional deficits and damage due to brain trauma are diminished in transgenic mice. (**d**) Composite neuroscore (NS) in transgenic (○) and wildtype (●) mice. (**e**) Cortical lesion volume in wt and transgenic animals following controlled cortical impact. Figure adapted from Mattiasson *et al.* (244).

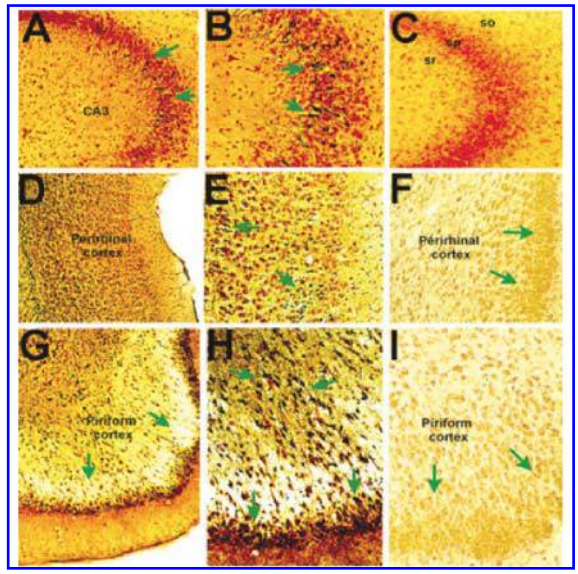

FIG. 9. Altering UCP2 expression and activity. Changing dietary fat content alters mitochondrial reactive oxygen species production and increases neuronal sensitivity to seizure-induced damage in immature animals. Neuronal injury in seizure-sensitive limbic regions of mature and postnatal day 10 (P10) rats after systemic administration of kainic acid. (**A**, **B**) Hippocampal CA3 from adult rats demonstrates neurons with silver affinity within the pyramidal cell layer (*green arrows*), indicating their injury. Such cells are not found in the P10 rat CA3 (**C**). (**D**, **E**) Low and higher magnification views of the perirhinal cortex of mature rat show excitotoxicity in both deep and superficial layers of this seizure-vulnerable limbic region, whereas the corresponding region from a P10 rat (**F**) is free of silver-stained cells. (**G**–**I**) The piriform cortex, a highly seizure-vulnerable region demonstrates seizure-injured neurons in adult (**G**, **H**) but not immature (**I**) brain.

In the uninjured brain, glutamate levels are regulated by energy-dependent uptake into astrocytes (119), but following acute brain injury, excitatory amino acids, particularly glutamate, are released from presynaptic vesicles into the extracellular space due to lack of energy (431), and contribute to excitotoxic neuronal cell death through overstimulation of glutamate receptors such as the *N*-methyl-D-aspartate (NMDA) receptors. Activation of NMDA-receptors cause calcium- and sodium influx with a concomitant passive influx of water and chloride ions, which results in cell swelling (431).

Ca^{2+} is the most common signal transduction element in neurons, and is instrumental to the life and function of neurons. Paradoxically, prolonged high levels of intracellular [Ca^{2+}] leads to cell death (71). The influx of Ca^{2+} is a key event in acute brain injuries, affecting signaling cascades within the cell, such as second messenger systems and protein kinases (406), as well as mitochondrial function, integrity and production of reactive oxygen species (119, 365, 370, 405), activation of caspase cascades (270, 364, 416) and changes in gene expression (251).

The increased intracellular calcium levels and increased formation of reactive oxygen species (106, 137, 155, 222, 281, 288, 293, 300, 350), may lead to neuronal death (97). The brain is very vulnerable to oxidative stress due to its high metabolic rate, high production of ROS, relatively low antioxidant activity and postmitotic nature of cells (350). Superoxide anions, hydrogen peroxide, nitric oxide, peroxynitrite, and hydroxyl radicals are generated, causing oxidative stress with damage to mitochondrial DNA (331), changes in genes and gene expression (122, 253), alterations in protein structure (51) and membrane phospholipid degradation (13, 222). The contribution of excitotoxicity and oxidative stress to acute brain injury (66) is further supported by the neuroprotective effect of NMDA-receptor blockers (113, 221) and free radical scavengers (235).

As discussed in the introduction, it has during the last decade become clear that mitochondria may induce cell death

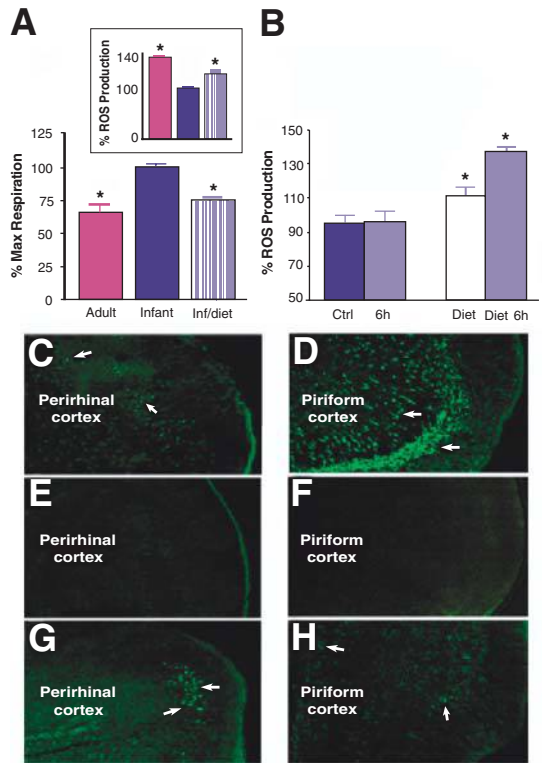

FIG. 10. Reduction of dietary fat. Substitution of an isocaloric, low-fat diet to immature rats reduces UCP function and promotes ROS production as well as seizure-induced excitotoxicity. (**A**) UCP function, measured as fatty acid–induced respiration, is significantly reduced in neonatal rats fed low-fat diet compared with maternal milk–fed littermates and resembles those in adult mitochondria. (*inset*) Basal ROS production in the presence of oligomycin (to maximize membrane potential) in isolated mitochondria from the group fed a low-fat diet is significantly increased compared with milk-fed littermates, approaching the basal levels found in adult mitochondria. (**B**) Energetic demand induced by severe seizures provokes striking increases in ROS production in UCP-suppressed (low-fat diet fed) neonatal rats, but not in those maintained on maternal milk. (**C**–**H**) Seizures provoke neuronal injury (visualized using Fluro-Jade) in several highly seizure-vulnerable regions of infant rats with suppressed UCP function. In adults, both perirhinal (**C**) and piriform (**D**) cortex demonstrated excitotoxic injury (arrows), whereas none was evident in the corresponding limbic regions of P10 rats on a "normal" high-fat diet (**E**, **F**). In striking contrast, excitotoxic injury occurred in perirhinal (**G**) and piriform (**H**) cortex of the low-fat diet fed infant rats (*arrows*). Figure adapted from Ref. (363).

following mitochondrial permeability transition (mPT), which leads to mitochondrial swelling and release of apoptogenic factors that activate cell death cascades (mitochondria-mediated cell death). Mitochondrial permeability transition may, as previously mentioned, be induced by high levels of intracellular calcium as well as increased levels of ROS.

Experimental data over the last decades have demonstrated neuroprotective effects of a number of often very different treatment regimens after acute brain injury. To understand this, it may be useful to think of secondary brain damage as composed of a number of individual factors. The cells are able to cope with stress up to a certain threshold, and when the sum of secondary brain injury components exceeds this level, cell death ensues. The objective of neuroprotective strategies is thus to reduce the total level of stress to below the threshold, where cells eventually may regain homeostasis. Also, a therapeutic intervention may affect more than one mechanism, for example, an inhibition of mitochondrial permeability transition also leads to a decreased activation of proteolytic enzymes and lower production of ROS. This model of brain injury may be visualized as "the sandwich model" (406) (Fig. 11).

c. Endogenous neuroprotective pathways It is known that short, sublethal ischemic insults may render the brain resistant to subsequent, longer and normally lethal ischemic episodes. The phenomenon is called ischemic tolerance or ischemic preconditioning (IPC), and the development is time- and protein-synthesis dependent (20, 33), suggesting that changes in gene expression are involved. Ischemic tolerance represents the mobilization of endogenous neuroprotective pathways, and includes changes in the expression of a large number of genes. Using differential cloning, subtraction-suppression hybridization and microarray analysis, we found that UCP2 was up-regulated in the cornu ammonis 1 (CA1) region of the hippocampus (two-fold) following ischemic preconditioning (244). Microarray analysis and *in situ* hybridization showed that preconditioning did not increase hippocampal expression of the other mitochondrial uncoupling proteins (UCP1, 3, 4, and BMCP1/ UCP5). Increased protein levels were found also following IPC in cell cultures. These findings are supported by other recent studies (74, 363) that clearly demonstrated the ability of the excitotoxic molecule kainic

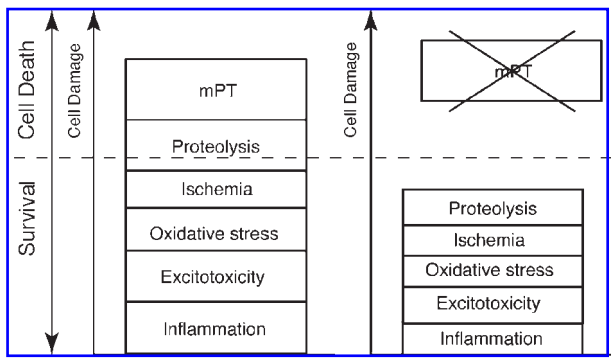

FIG. 11. The "sandwich model" of acquired brain injuries. Secondary brain damage following acute brain injury is composed of a number of individual detrimental factors. The total cell- and tissue stress can be derived from the summation of the individual factors. Through endogenous protective systems, cells are able to cope with stress up to a certain threshold. When the sum of secondary brain injury components exceeds this level, cell death ensues (*left*). The objective of neuroprotective strategies is to reduce the total level of stress to below the threshold, where cells eventually may regain homeostasis (*right*). Since many processes of secondary brain injury influence each other, inhibiting one process will to some extent diminish the impact of other processes.

acid to strongly induce UCP2 in the CA1 region of the mouse hippocampus, emphasizing the importance of glutamate excitotoxicity in the expression of UCP2.

The mechanism of induction of UCP2 by ischemic preconditioning is not completely known. However, as discussed in the introduction, transcription could be activated by the PPARs (268), which induce transcription of genes associated with fat metabolism, and can be stimulated by synthetic analogues such as the thiazolidinediones (15). Free fatty acids are potent inducers of PPARs (206, 212), and following ischemic preconditioning in the brain, levels of free fatty acids (FFA) increase (22, 96), which may induce UCP2 (209, 255). Also, expression of UCP2 have been suggested to be increased as a response to increased ROS production *in vivo* and *in vitro* (78, 79, 306), suggesting that UCP2 could be part of an endogenous response to oxidative stress.

To investigate if increased levels of UCP2 were related to neuroprotection, the injury response following overexpression *in vitro* and *in vivo* was studied. When UCP2 was overexpressed *in vitro* by adenovirus transfection prior to OGD, a substantial neuroprotective effect was demonstrated. Similarly, transgenic mice overexpressing UCP2 (UCP2/3tg) (131, 163, 164) showed improved outcome following focal ischemia and controlled cortical impact traumatic brain injury (244). The level of overexpression of UCP2 in the brains of these animals was about two-fold, meaning that the level is similar to that of the physiological response after ischemic preconditioning. The neuroprotective potential of UCP2 was also demonstrated in primary cell cultures, and based on experiments with the mitochondrial uncoupling agent dinitrophenol in similar cultures, it was concluded that mitochondrial uncoupling was inherently linked to the neuroprotective effects of UCP2 (244).

These findings are in line with other studies demonstrating neuroprotection by UCP2 against excitotoxicity *in vitro* (92). We have recently reported a neuroprotective role for UCP2 in excitotoxic cell death *in vivo* (363), where a reduced UCP2 expression and UCP activity increased kainic acid-induced mitochondrial ROS production and neuronal cell loss in p12 rat pups, which are normally resistant to excitotoxic insults (363). Additionally in animals maintained on a ketogenic diet, UCP2 expression and activity is significantly increased in the hippocampus (367). Since the ketogenic diet is clinically very effective at reducing intractable pediatric seizures by a yet undetermined mechanism(s), UCP2 may play a potential role in the modulation and control of seizure activity. All in all these findings lend support to the idea that UCP2 expression and its up-regulation following neuronal injury/insult is an endogenous, neuroprotective response to protect neurons in the CNS from increased ROS production, calcium overload, and subsequent cell death.

d. Neuroprotective effect of UCP2 Our experimental data indicate that the mechanisms behind the ability of UCPs in general and UCP2 in particular to protect against excitotoxic injury is related to a slight depolarization of the inner mitochondrial membrane, with decreased production of ROS, decreased uptake of calcium, and reduced induction of mitochondrial membrane permeability transition with the associated release of cell death-inducing factors (mitochondria-mediated cell death) (244, 363) (Fig. 7). UCP2 is clearly activated by free fatty acids (FFA) and possibly also by superoxide anions (101, 102). Both FFA (22, 96) and superoxide anions (222) are increased after acute brain injury, leading to activation of UCPs, and a limited depolarization of the inner mitochondrial membrane (118). In addition, the uncoupling activity of UCPs is inhibited by adenonucleotides (ATP, ADP) binding to the cytosolic side of the UCP (277, 360), suggesting that decreased levels of ATP and ADP (e.g., during ischemia) may release inhibition, and facilitate activation.

In isolated mitochondria, activation of UCP2 by palmitic acid is detected as an increase in state 4 respiration (244, 363, 367) (see Fig. 3), similar to what is seen after treatment with low concentrations of 2,4-dinitrophenol (158). In the present context, a slight depolarization of mitochondria could lead to a decrease in the electrophoretic movement of calcium ions into mitochondria, preventing mitochondrial calcium overload and cytotoxicity (58). Also, a reduction in membrane potential decreases the generation of ROS (353, 363, 367), presumably by increasing the flow of electrons through the electron transport chain, thereby decreasing the time of interaction between electrons and molecular oxygen, lowering the formation of ROS (201, 353). Even a slight depolarization (approx. 10 mV) is sufficient to significantly lower the production of ROS (228), suggesting that the depolarizing activity of UCP2 may have important physiological effects.

The role of UCP2 in control of ROS production is also evidenced by the fact that inhibition of UCP2 by GDP led to an increased production of ROS (272). The decrease in intramitochondrial calcium and ROS release as a consequence of UCP2 activation decreases the probability of mPT, the release of apoptogenic factors such as cytochrome c and AIF (405), and activation of caspase-3 will be attenuated (153). This hypothesis is supported by experimental data, demonstrating that in cortical neurons overexpressing UCP2, membrane potential was preserved, and caspase-3 activation following oxygen-glucose deprivation (OGD) was prevented (244), suggesting that mPT was inhibited.

A slight depolarization of mitochondria using the mitochondrial uncoupler 2,4-dinitrophenol in primary neuronal cultures (244), as well as in mice subjected to excitotoxic brain injury (296), was neuroprotective, suggesting that the neuroprotective effect of UCP2 was related to a slight depolarization of mitochondria (74, 314). Moreover, adenonucleotides decrease the sensitivity of the mPTP complex to calcium, specifically by matrix ADP binding to ANT (154). Therefore, UCP2-mediated mitochondrial uncoupling may also influence the ATP/ADP ratio in mitochondria, and thereby directly the sensitivity to mPT (341). Also, UCP2 interacts with CoQ of the electron transport chain (104, 105), and CoQ may directly inhibit mPT (123, 124).

An additional, potentially neuroprotective pathway is related to microglial expression of UCP2. Excitotoxic insults, including ischemia, are accompanied and exacerbated by the activation of the innate immune system, represented by activated astrocytes and phagocytic microglial cells. Activated microglial cells produce ROS, which are known to cause serious damage to surrounding neurons when produced in excess. UCP2 expression is up-regulated in microglial cells following

exposure to kainic acid (74), but the role of this overexpression is currently unknown. Given the role of UCP2 in reducing ROS production in phagocytes (190), one might assume that this occurs to limit or terminate the production of ROS by phagocytic cells (284).

In relation to brain functions, the effect of UCP2 will probably involve all of the aforementioned possibilities, but most likely to a various extent. For example, during degenerative processes, if UCP2 was expressed prior to the initiation of cellular stress [which can be achieved either in transgenic animals, changes in dietary fat or by subclinical stressors before a large insult (244, 363, 367)], cell death could be inhibited through decreased production of ROS, as well as a decreased activation of mitochondria-mediated cell death (244). Mitochondria-mediated cell death leads to the activation of caspase-3 (207), and an inverse relationship between UCP2 expression levels and activation of caspase-3 during acute brain injury has been demonstrated (23).

Interestingly, a recent study in UCP2 -/- animals that were subjected to permanent middle cerebral artery occlusion demonstrated that UCP -/- animals had smaller lesions compared to the wt animals. This effect is explained by the fact that the UCP2 -/- animals had higher levels of antioxidant enzymes in the brain, which limited the amount of oxidative damage following the ischemic insult (84).

Oxidative stress is implicated in the death of dopaminergic neurons in sporadic forms of Parkinson's disease. Conti and co-workers (77) have demonstrated a neuroprotective effect of UCP2 in a mouse model of Parkinson's disease. They used transgenic mice overexpressing UCP2 in catecholaminergic neurons under the control of the tyrosine hydroxylase promoter (TH-UCP2). In these mice, dopaminergic neurons of the substantia nigra showed a two-fold elevation in UCP2 expression, elevated uncoupling of their mitochondria, and a marked reduction in indicators of oxidative stress, an effect also observed in the striatum. Upon acute exposure to 1,2,3,6-methyl-phenyltetrahydropyridine (MPTP, a toxin that selectively kills dopaminergic cells of the substantia nigra), TH-UCP2 mice showed neuroprotection and retention of locomotor functions, suggesting that UCP2 may represent a drug target for slowing the progression of Parkinson's disease (77). Because most of the neurodegenerative disorders involve free radical production, it is very reasonable to propose that UCP2 induction will have a potential therapeutic role in the treatment of these disorders, including epilepsy, Parkinson's disease, Alzheimer's disease, as well as hypoxia and stroke.

e. Neuroprotective signaling by UCP2 As discussed above, uncoupling proteins 1–3 may directly influence ROS production in various tissues (11, 363, 391). A decrease in the level or activity of UCPs could be used to increase ROS production [e.g., in macrophages during infection (11)], whereas increased levels of UCP2 or UCP3 in other tissues in response to injury could be a tissue-specific physiological response to prevent excessive oxidative stress, which is an important mediator of secondary brain injury. In addition, we have found evidence that under conditions of sublethal injury, such as ischemic preconditioning, UCP2 may support protective cellular redox signaling by promoting a shift of hydrogen peroxide release from an intramitochondrial to an extramitochondrial site (244), possibly through direct interaction with the electron transport chain (104, 105).

Increasing evidence point to the role of reactive oxygen species as second messengers and signaling molecules in the brain (2, 53, 156, 245, 374). Cellular redox states influence intracellular signaling pathways in several ways. ROS activate protein tyrosine kinases, followed by activation of downstream cascades (e.g., MAP kinase and PLCγ), which in turn increase the intracellular levels of Ca^{2+}, influencing a number of signaling systems. Oxidation inactivates protein tyrosine and protein serine threonine phosphatases, activate protein serine threonine kinases, small G-protein (RAS), and lipid signaling (PLC, PLD, PLA_2, PI3-kinase) (177). Increased levels of cytoplasmic oxidants will stimulate mitochondria-mediated cell death, but will inhibit the activity of caspases (156) and apoptosis inducing factor (AIF) (374), the effectors of cell death. Also, the activity of transcription factors is influenced by cellular redox state. Most transcription factors are inactivated by increased levels of ROS, but it seems that some (e.g., NF-κB and AP1) can be activated by increased levels of ROS (177), leading to expression of neuroprotective genes such as MnSOD (246, 362) (Figs. 12 and 13). Indeed, NF-κB activation and increased levels of MnSOD have been reported following preconditioning in the heart (33), and in brains preconditioned by sublethal ischemic insults (181). In UCP2 -/- mice, ROS production in macrophages is increased compared to wild type animals (11). In support of the hypothesis that NF-κB activity is regulated through ROS-mediated signaling in macrophages, it was recently shown that NF-κB activity was increased in macrophages from UCP2 -/- mice, leading to an increased production of inflammatory mediators (18). Consequently, it appears that an increased expression of UCP2 may inhibit the generation of ROS and/or promote a translocation of ROS from mitochondria to the cytoplasm where expression of protective genes is activated. In UCP2 -/- mice, ROS production is increased, leading to activation of a different set of genes, which may be proinflammatory (18) or

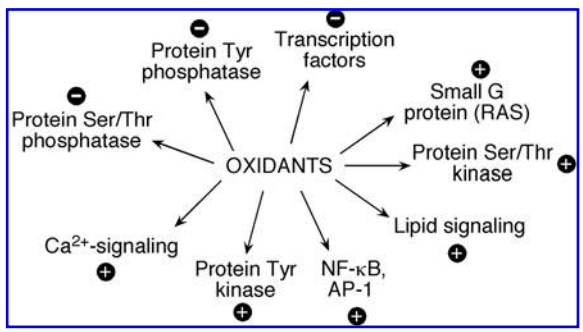

FIG. 12. Schematic drawing of effects of cellular redox status on intracellular signaling systems. Increased levels of intracellular ROS (oxidants) activate calcium signaling, protein tyrosine and serine/threonine kinases, small G protein (RAS), lipid signaling, and the transcription factors NF-κB and AP-1. Similarly, protein serine/threonine and tyrosine phosphatases as well as several transcription factors are inactivated by increased levels of intracellular ROS.

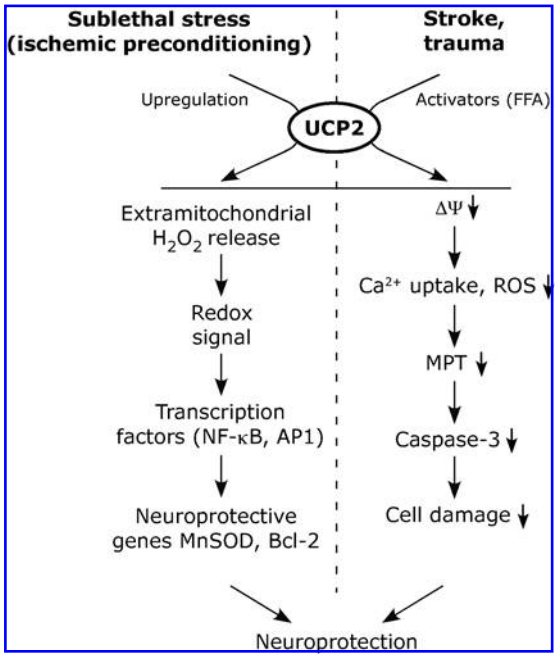

FIG. 13. Schematic drawing of the neuroprotective effects of UCP2 in acute brain injury. Sublethal stress (e.g., ischemic preconditioning) up-regulates UCP-2, which leads to increased extramitochondrial release of ROS that signals neuroprotection by inhibiting caspases or activating transcription factors. After acute brain injuries, levels of FFA increase, activating UCP-2 that depolarize the mitochondrial membrane, resulting in less Ca^{2+}-uptake and ROS-generation, decreasing the probability for mPT, activation of caspase-3 and cell death. Adopted from Ref. (244).

neuroprotective (84). This apparent contradiction could probably be explained by the fact that a constantly increased production of ROS (such as in UCP2 -/- mice) will lead to an up-regulation of antioxidant defenses, which will be protective following ischemic injuries with the associated increases in ROS production. An increased expression of UCP2 will lower the production of ROS and inhibit mitochondria-mediated cell death following the ischemic episode (244).

f. Thermogenic modulation Horvath and co-workers have suggested that the thermogenic features of UCPs may be important in regulating the activity of presynaptic terminals in neurons located in homeostatic centers, hence providing a basis for temperature as a neuromodulator (167). They hypothesized that if UCP2 in neuronal circuits is a functional uncoupler in a manner similar to what was found in a yeast model (120) and in cardiomyocytes (377), the proton leak of mitochondria in UCP2-containing brain regions should be increased. In support of this hypothesis, they found that the mitochondrial respiratory control ratio (RCR) in rat extracts from regions with abundant UCP2 expression (hypothalamus) was significantly higher than that measured in regions that lack UCP2 expression. Furthermore, they also found that UCP2-containing brain regions had a significantly higher local temperature when compared to other sites or to the core body temperature (167). UCP2 is expressed in neuronal mitochondria, which are frequently accumulated in axon terminals in close proximity to synaptic vesicles and synaptic membranes. Since activation of UCP2 may lead to heat generation, this change in presynaptic temperature may have an impact on synaptic transmission as synaptic function and plasticity is temperature dependent (132, 419, 427). Mizuno and co-workers suggested a similar role for UCP2 after they observed increased UCP2-expression in the spinal cord following cold exposure (259). Further, the development of brain damage is inhibited by hypothermia and aggravated by hyperthermia (292), suggesting that increased temperature in neuronal populations with a high expression of UCP2 may make the neurons more vulnerable to acute brain injury, for example, whereas at the same time a slight increase in temperature may be beneficial for synaptic plasticity and functional recovery. The regional and temporal specificity of this mechanism may be determined by the selective brain distribution of different UCPs and their availability for activating substances such as circulating free fatty acids and cofactors such as coenzyme Q.

B. UCPs and metabolic disorders

Because UCPs may have a role in the regulation of metabolism and energy expenditure, they have attracted substantial interests as potential targets for treatment of obesity and diabetes. Obesity and diabetes are increasing in the western world today, and both are central features of the so-called metabolic syndrome, which is a large risk factor for a number of major diseases. We will therefore begin the discussion about potential roles of UCP2 in the context of metabolic disorders with a review of the metabolic syndrome, obesity, and diabetes. Finally, the role of UCP2 as a potential target for the treatment of these conditions will be addressed.

a. The metabolic syndrome The metabolic syndrome is caused by disturbed energy homeostasis, leading to increased plasma FFA and glucose levels as well as redistribution of body fat. The syndrome is common in the western world today, and leads to an increasing risk of developing several major diseases, and consequently represents an increasing health risk (260). The most important features are (central) obesity, insulin resistance or glucose intolerance, hyperglycemia, and high plasma triglyceride and FFA levels, as well as a prothrombotic proinflammatory state and raised blood pressure. People with the metabolic syndrome are at increased risk of coronary heart disease, other diseases related to atherosclerotic plaque buildups in artery walls (e.g., stroke and peripheral vascular disease) and type 2 diabetes mellitus (T2DM)—all common causes of death and morbidity in the western societies, and therefore diseases with major socioeconomic impact. It is currently estimated that about 24% of adults in the United States have the metabolic syndrome, and the numbers appear to be increasing. The safest, most effective, and preferred way to reduce the effects of the syndrome is weight loss and increased physical activity. However, there may also be a therapeutic opportunity in the intervention in the dysregulated energy homeostasis, suggesting a possible role for genes with a metabolic control function, such as the UCPs.

The biologic correlates at the molecular level that lead to the metabolic syndrome are both complex and inter-related, and

are currently not known in detail. The syndrome is closely associated with insulin resistance, which appears to have a hereditary component. However, acquired factors such as excess body fat and physical inactivity can also promote development of insulin resistance and the metabolic syndrome. The discussion below will provide a background of the pathogenesis and molecular mechanisms underlying the development of the metabolic syndrome, and will delineate the possible roles of UCP2 in the development (and possibly therapies against) the metabolic syndrome, including obesity, T2DM and (briefly) cardiovascular disease.

b. Obesity Energy balance in animals is a metabolic state that exists when total body energy expenditure equals dietary energy intake. The precise regulation of energy homeostasis is very complex and involves a number of systems. When energy intake exceeds expenditure, the excess is stored as fat in fat cells in adipose tissue, resulting in weight gain and eventually obesity (Body Mass Index > 30). The rapidly increasing worldwide incidence of obesity and its association with major diseases means it is becoming the most significant contributor to ill health in the developed world (200). Fat cells are only able to store a certain amount of fat. As this limit is reached, fat overflows to other tissues and leads to ectopic accumulation of triglycerides in muscle and liver, as well as increased levels of plasma FFA. Ectopic fat accumulation contributes to the development of hepatic and muscle insulin resistance, glucose intolerance, and overt diabetes (83). Increased plasma FFA also contributes to the development of cardiovascular disease.

Weight loss, induced by dieting, is successful in reducing the health consequences of obesity, but unfortunately > 90% of individuals who lose weight through dietary control eventually return to their original weight (396). Pharmacological treatment may therefore be desirable for those patients with associated comorbid conditions who have been unable to control their obesity through diet and exercise. Any treatment for obesity has to reduce energy intake, increase energy expenditure or combine both effects. Exercise is the most practical and potentially easiest way to increase energy output. The main benefit of exercise is to increase resting metabolic rate, and overall energy expenditure, by a greater amount than that resulting directly from the exercise (302). Current pharmacological therapies for obesity predominantly lead to decreased energy intake either by acting at satiety centers in the brain or by reducing the efficiency of intestinal absorption. Pharmacological agents that increase metabolic rate by increasing uncoupling of mitochondrial oxidative phosphorylation are likely to mimic the beneficial effect of exercise on resting metabolic rate and could provide a useful addition to agents acting to induce satiety. Pharmacological induction of mitochondrial uncoupling by 2,4-dinitrophenol (DNP) has successfully been used to this end in humans in the past, and some of the effects of thyroid hormone treatment to induce weight loss may also be due to mitochondrial uncoupling. Diet can alter the pattern of phospholipid fatty acyl groups in the mitochondrial membrane, and this may also be a route to uncoupling *in vivo* (158). However, there are problems associated with several of the chemical mitochondrial uncouplers (e.g., DNP). The problems are related to a narrow therapeutic range and undesirable side effects; doses of DNP outside the (narrow) therapeutic range may even result in death (158). Also, using pharmacological agents to uncouple all mitochondria throughout the body may be a high-risk treatment, because it might compromise energy homeostasis in critical tissues such as heart and brain. On the other hand, active tissues like these may be less susceptible to mild uncoupling than less active ones like resting muscle or resting BAT because proton conductance has much less control over respiration rate in active mitochondria (152). The small difference between the effective and the fatal doses of DNP, as well as side-effects resulting from its nonselective actions, means that it is not itself a suitable antiobesity drug.

Consequently, activation of physiological uncoupling mechanisms such as the UCPs has recently attracted considerable interest as therapeutic targets for the treatment of obesity. It has been suggested that the side effects of pharmacological uncoupling could be reduced by selective overexpression of uncoupling proteins in target tissues. One such target could be skeletal muscle, since it has a large mass, and a slight uncoupling of skeletal muscle mitochondria may increase energy expenditure without producing adverse side effects. In small animals and newborn humans, the activity of uncoupling protein 1 (UCP1) in brown adipose tissue (BAT) is responsible for nonshivering thermogenesis. Indeed, expression of UCP1 in mouse skeletal muscle led to improvements in insulin sensitivity and resistance to obesity on a high fat diet (223). Similarly, an increased expression of human UCP3 in mouse skeletal muscle decreases weight gain despite increased food intake (72), demonstrating that uncoupling of mitochondria remains a viable and attractive target for the development of drugs for the treatment of obesity. However, the amount of UCP3 expressed in this study was very large, and it is possible that the uncoupling effect observed was an artifact, and also unlikely that similar levels of UCP3 expression could be obtained by a pharmacological approach (316). UCP2 may be involved in regulation of energy expenditure and nutrient partitioning, particularly that of fats, possibly by switching metabolism from a state of enhanced lipid utilization during starvation to one of reduced lipid utilization during refeeding (172, 334, 335). UCP2 has also been suggested to be related to energy metabolism and obesity in rodents and humans (166), but there is also conflicting evidence. The expression of UCP2 is increased (35, 257) or unaltered (338, 357) during food restriction, at a time when whole-body energy expenditure is reduced (232, 327), suggesting that it has other physiological functions than control of metabolism. Using mice deficient in UCP2 (UCP2-/-), it has been demonstrated that UCP2 is not required for body-weight regulation or control of metabolism (11, 107, 341). Further, alterations in expression of UCP2/3 have been suggested to have a role in the development of anorexia nervosa, but this hypothesis was not supported in a Japanese case-control study (5). In conclusion, the experimental evidence supporting a potential role for UCP2 and UCP3 as pharmacological targets for the treatment of obesity is currently not clear. However, experimental data on the role of UCP1 in energy expenditure suggests that induction of UCP1 in adipose tissue could be an attractive target for the development of anti-obesity drugs (316).

c. Diabetes The role of UCP2 in the development and potentially in the treatment of diabetes is complex. As discussed previously, UCP2 has a role in directing metabolism towards increased use of lipids, which is generally good for diabetes. However, expression of UCP2 in pancreatic β-cells will lead to a decreased insulin release and decreased insulin sensitivity, which promotes the development of insulin resistance and diabetes. To understand this complex relationship, we begin with a review of the pathophysiology of development of diabetes.

The pathophysiology of type 2 diabetes mellitus (T2DM) is generally thought to be multifactorial, involving both genetic susceptibility and environmental factors (3). It includes two apparently distinct defects: insulin resistance in skeletal muscle, fat, and liver, and an inadequate increase in insulin production by the pancreatic β-cells. These two defects ultimately result in fasting hyperglycemia (21, 387, 402). The development of T2DM is preceded by insulin resistance, but glucose homeostasis remains normal because of a marked increase in insulin secretion by pancreatic β-cells (85, 310, 380). This increase offsets the hepatic insulin resistance, suppresses the basal hepatic glucose production, and overcomes the defect in muscle glucose uptake (85, 86, 380, 387, 403). With the onset of impaired glucose tolerance, insulin resistance in muscle and liver increases (85, 86, 258, 380, 403) and there is also a further increase in the total insulin response to an oral glucose load (85, 86, 387). As the condition progresses from impaired glucose tolerance to overt T2DM, there is little or no further deterioration in insulin resistance, but rather a declined ability of the pancreas to maintain its high insulin secretory rate (85, 86). Initially, the defect in glucose homeostasis is evident by an excessive rise in glucose levels following feeding, followed by a rise in the fasting plasma glucose concentration.

The association between T2DM and obesity is well established. Cross-sectional (301) and prospective (196) studies have documented that the incidence of diabetes rises steeply with increasing body weight. The diabetogenic effect of obesity is related to three factors: attained body mass index, duration of obesity, and recent increase in body weight (400). Obesity is an insulin-resistant state, and both obesity and insulin resistance are risk factors for the development of T2DM (29, 65, 85). Like T2DM, the insulin resistance of obesity involves muscle, liver, and adipocytes. In addition to total fat content, the pattern of fat distribution is also an important predictor of the body's sensitivity to insulin. Individuals with preferential upper body fat accumulation (android) are more insulin resistant, hyperinsulinemic, and dyslipidemic than people with a preponderance of lower body fat (gynecoid) (189). This association has been attributed to the enhanced lipolytic activity of visceral fat cells, with increased delivery of FFA into the portal (causing hepatic insulin resistance) and systemic (causing muscle insulin resistance) circulations (9).

Both lean and especially obese type 2 diabetics are characterized by day-long elevations in the plasma free fatty acid (FFA) concentration which fail to suppress normally following ingestion of a mixed meal or oral glucose load (309) or in response to insulin (32, 147). FFAs are stored as triglycerides in adipocytes and serve as a source of energy during fasting conditions. Insulin is a potent inhibitor of lipolysis (147) and restrains the release of FFA from the adipocyte by inhibiting the enzyme hormone-sensitive lipase, leading to daylong elevated plasma FFA levels (147). Chronically increased plasma FFA also stimulates gluconeogenesis, and impairs insulin secretion from the pancreatic β-cells (178, 249, 348).

Enlarged fat cells are insulin resistant and have diminished capacity to store fat. When adipocyte storage capacity is exceeded, lipid "overflows" into muscle (145, 146), liver (344), and perhaps pancreatic ß-cells. The triglycerides in liver and muscle are in a state of constant turnover, and the metabolites [i.e., fatty acyl coenzymes A (CoAs), ceramides, diacylglycerol] of intracellular triglyceride lipolysis impair insulin action in both liver and muscle (31, 32, 140, 303). On a more speculative note, there are experimental data in rodent models of diabetes to support the "overflow hypothesis" of β-cell dysfunction. In the genetically obese Zucker fatty rat, diabetes develops at about 10–12 wk of life, and this is associated with a marked increase in islet triglyceride content and FA-CoA levels, which eventually lead to β-cell dysfunction and apoptosis. At present, it remains unknown whether similar changes occur in human β-cells (21). This sequence of events has been referred to as lipotoxicity (249, 384), and describes the deleterious effect of chronic FFA elevation on insulin secretion by the pancreatic β-cells.

d. Free fatty acids and insulin secretion After the ingestion of a mixed meal or infusion of lipid, the plasma FFA concentration rises, and FFA are transported into the islet β-cell via fatty acid-binding protein 2, subsequently leading to an increased insulin secretion by a variety of mechanisms (242, 249, 273, 348). The resulting increase in cytosolic fatty acyl CoAs works in tandem with hyperglycemia to enhance insulin secretion. Consistent with these *in vitro* observations, short-term (2 to 6 hours) elevation of the plasma FFA concentration in rodents and man has been shown to augment insulin secretion (249, 250, 358, 01), whereas an acute decrease in the plasma FFA concentration inhibits glucose-stimulated insulin secretion (249, 250). In contrast to the acute effect of elevated plasma FFA to enhance insulin secretion, longer-term (48 hours) exposure results in an impaired β-cell response to glucose both *in vitro* and *in vivo* in animals (231, 240, 330, 428, 429) and humans (55, 56, 178, 294). The inhibitory effect of chronically elevated plasma FFA appears to be more prominent in individuals with a genetic predisposition to develop T2DM (178). Conversely, a reduction in the plasma FFA concentration in type 2 diabetics improves insulin secretion (178, 295, 304).

e. UCP2 and 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, it was surprising to find that UCP2 -/- knockout mice showed increased insulin sensitivity and appeared to be protected against high-fat diet–induced insulin resistance (176, 425). In addition, UCP2 has also been shown to have a direct role in the secretion of insulin, since UCP2 expression inversely correlates with β-cell ATP in both overexpression (62, 161, 398) and null expression models (176, 425), suggesting a role for UCP2 in energy metabolism by function-

ing as a negative regulator of insulin secretion (314). This "yin-yang"-effect of UCP2 in diabetes has been reviewed (210, 286) and UCP2 has also been suggested as a potential "diabetes gene" because of its negative effects in β-cells (238). In line with this, a recent report has described a functional polymorphism in the human UCP2 promoter that increases the risk of obesity but decreases the risk of type 2 diabetes (205, 420).

Until recently, regulatory proteins that participate specifically in down-regulation of insulin secretion have received little attention. The discovery that UCP2 is present in pancreatic islets and β-cell lines (430) 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) (63). An increased expression or activity of UCP2 in pancreatic β-cells may contribute to impairing insulin secretion by reducing the ATP/ADP ratio (341). The secretion of insulin depends on ATP at multiple steps in metabolism-secretion coupling (Fig. 14), and an estimated 98% of β-cell ATP production depends on mitochondrial oxidative processes (108). ATP is decreased in UCP2-overexpressing β-cells (62, 161), and increased in the corresponding tissues from UCP2 -/- knockout animals (176, 425), presumably due to an effect on the ability of glucose to hyperpolarize the mitochondrial inner membrane (62). State 4 respiration (i.e., when ADP is absent and ATP synthesis is inhibited with oligomycin) is increased in UCP2-overexpressing insulinoma cells (161), consistent with an uncoupling effect. Moreover, incubating islets with chemical uncouplers has classically been shown to inhibit insulin secretion (12). Because ATP participates at multiple rate-limiting or rate-potentiating steps of the insulin secretion pathway, one might predict that agents that cause a decrease in β-cell ATP would affect numerous downstream events. An obvious target is the ATP-dependent K-channel (K_{ATP}) in β-cells. When ATP (or, specifically, ATP/ADP) rises in response to substrate metabolism, the K_{ATP} channels close and the cells depolarize, leading to Ca^{2+}-dependent exocytosis. An induction of UCP2 followed by a reduction of cellular ATP content by 50%, led to a failure of K_{ATP} channels to close in response to elevated glucose (62). Likewise, an increase in K_{ATP} channel activity of UCP1-overexpressing β-cells led to a reduction in voltage-dependent Ca^{2+} influx (269). The reduction in insulin secretion secondary to the decrease in Ca^{2+} entry was only partially overcome by use of calcium ionophore A23187 (63), suggesting that ATP depletion affects regulatory sites other than K_{ATP} channels. Other sites modulated by ATP or ATP/ ADP have been less well characterized after UCP2 overexpression.

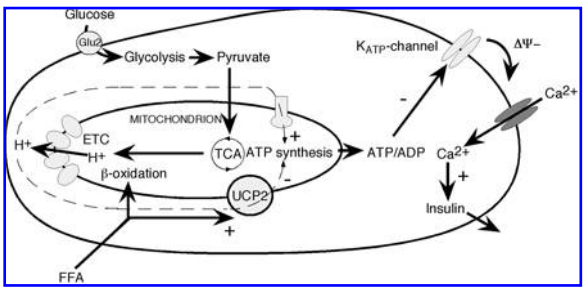

FIG. 14. Influence of UCP2 on insulin secretion of pancreatic β-cells. Glucose is taken up by a glucose transporter (Glu2), and oxidized in glycolysis and the TCA cycle. Substrates are fed into the electron transport chain (ETC) that pumps protons out of the mitochondrial matrix, forming a proton motive force. The proton motive force is used to synthesize ATP, leading to an increase in the ATP/ADP ratio, which leads to a closure of the K_{ATP} channels, with subsequent depolarization of the plasma membrane potential ($\Delta\Psi$). Ca^{2+} flows into the cell, triggering release of insulin. UCP2 inhibits insulin release by dissipating the proton motive force, thereby decreasing the ATP/ADP ratio.

Interestingly, UCP3 overexpression produces similar effects, but of a lower magnitude than UCP2 on ATP, and these effects are not accompanied by inhibition of insulin secretion (161). On the other hand, UCP1 overexpression in β-cells totally suppressed the glucose-stimulated increment in ATP content and insulin secretion (269). Altogether, these studies support the idea that UCP2 catalyzes a proton translocation in β-cells, as suggested by studies in isolated yeast (120) or mammalian (118, 244) mitochondria and intact thymocytes (204), leading to depression of mitochondrially generated ATP.

f. Cell death in islets: lipotoxicity In the Zucker diabetic fatty rat, chronically increased plasma FFA levels initially lead to a physiological impairment in insulin secretion, and eventually to β-cell apoptosis (lipotoxicity). Also, incubation of human islets with FFA causes β-cell apoptosis (231). The concept of lipotoxicity in pancreatic islets is further supported by evidence that high FFA levels induce a variety of genes that influence fat metabolism (48, 399, 413). One key modulator that undergoes up-regulation after FFA exposure (48) or high-fat diet (176), and which has been associated with impaired insulin secretion (303), is carnitine palmitoyl transferase (CPT-1). CPT-1 is 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 within the β-cell generate oxygen radicals (417) that are potentially damaging to β-cells. Further, elevated fatty acyl CoAs increase the formation of ceramide, which in turn augments nitric oxide formation, which is detrimental to the β-cell (348). Since FFA treatment of pancreatic islets has been shown to induce UCP2 expression, the up-regulation of UCP2 has been suggested to be a protective mechanism against excessive lipid exposure and the associated increase in ROS production, lending further support to the role of UCP2 as an antioxidative agent and anti-apoptotic protein.

Acute FFA exposure induces ROS production in β-cells, primarily from complex I of the electron transport chain, and increased UCP2 expression and activity may be a protective response to lower the mitochondrial membrane potential and thus the production of ROS. Because β-oxidation depends on a highly polarized membrane, these conditions serve as negative feedback on further superoxide anion production to limit ROS-mediated cell damage during the lipotoxic insult (202), and will also directly lead to a reduced production of superoxide anion (201, 228) (see Figs. 3, 5, 6, and 15). Alternatively, FFAs appear to exert independent and distinct effects on the respiratory chain to inhibit electron transport (351),

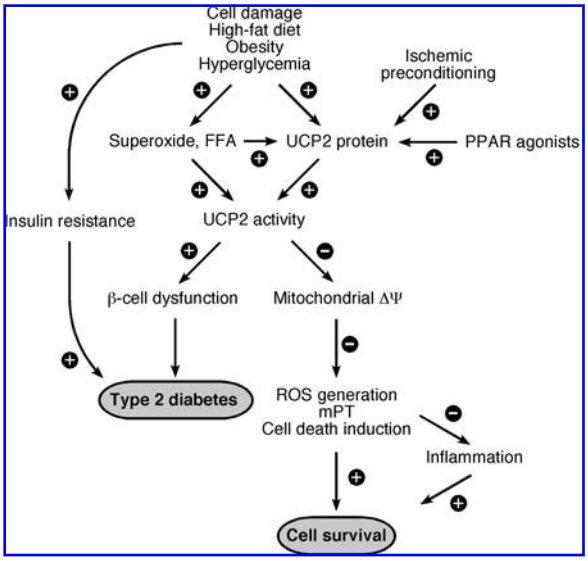

FIG. 15. Schematic drawing of the role of UCP2 in cell protection and development of diabetes. Cell damage, high fat diet, obesity, and hyperglycemia all lead to increased levels of superoxide and FFA, which stimulates UCP2 expression and activity. High levels of FFA also lead to ectopic fat accumulation in liver and muscle, which promotes insulin resistance and development of type 2 diabetes. UCP2 protein levels can also be increased by sublethal stress (ischemic preconditioning) and PPAR agonists. An increased UCP2 activity leads to a slight mitochondrial depolarization, with decreases in ATP and ROS generation, as well as a decreased risk of mPT induction and activation of mitochondria-mediated cell death. In the pancreatic β-cell, the reduction in ATP levels leads to a decreased insulin secretion, which promotes the development of type 2 diabetes. In cells subject to stress (e.g., following an ischemic episode), the reduction in ROS generation and mitochondria-mediated cell death inductions seems to promote survival and decreased inflammatory response.

leading to increased ROS production that could explain the increase in ROS following exposure to FFA (202).

Thus, the increased expression of 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 (64, 175).

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.

C. Cardiovascular events

The metabolic syndrome and diabetes are large risk factors for the development of cardiovascular disease, including atherosclerotic plaque build up, myocardial infarction, heart failure and cerebral stroke. Below, the role of UCP2 in these settings is reviewed.

a. Atherosclerosis Increased oxidative stress in vascular cells plays a key role in the development of endothelial dysfunction and atherosclerosis. UCP2 has previously been shown to protect against atherosclerosis in animal models (30) through inhibition of ROS generation in endothelial cells (216), or by inhibition of monocyte accumulation in the arterial wall (329). Two groups have investigated the role of the common -866G/A polymorphism in the UCP2 promoter, which may affect UCP2 gene expression in cells of the arterial wall. This polymorphism has previously been associated with obesity and beta-cell function (205). The results suggest a role of UCP2 in atherogenesis in humans as originally proposed from studies in animal and cell culture models (89, 287). Thus, measures to increase UCP2 expression in vascular endothelial cells may aid in preventing the development and progression of atherosclerosis in patients with the metabolic syndrome.

b. The heart The heart uses FFA as a main substrate for ATP production, and expresses UCP2 constitutively. Infusion of fat (389) led to an increased expression if UCP2, while a high-fat (cafeteria) diet led to a decreased expression of UCP2 in rats (237). Similarly to what we observed in the brain (244), a sublethal myocardial ischemia induces protection against a subsequent, longer ischemia (ischemic preconditioning). The preconditioning involves changes in gene expression, and one of the up-regulated genes is UCP2 (252). During myocardial infarction, cell death pathways similar to those following cerebral ischemia are activated, including oxidative stress and mitochondria-mediated cell death. When UCP2 was overexpressed in cardiomyocytes using an adenoviral vector, the cells were protected against oxidative stress (377).

Cardiac hypertrophy occurs as a response to an increased cardiac workload, both following exercise and as a result of hypertonia and increased peripheral resistance. Exercise-induced hypertrophy is generally beneficial, whereas hypertonia-induced hypertrophy is not. Strom *et al.* investigated changes in gene expression in the different forms of cardiac hypertrophy, and found that expression changes of genes involved in beta-oxidation of fatty acids and glucose metabolism, including UCP2, differentiated exercise-induced from maladaptive hypertrophy (361). Razeghi and co-workers investigated changes in gene expression in human failing and nonfailing hearts, and found that UCP2 was down-regulated in the failing hearts (308). These findings support a protective role of UCP2 in the treatment of ischemic cell death in the heart.

D. UCP2 in other settings

a. Development After birth, the plasma levels of FFA increase as a result of intake of milk with a high fat content, and this increase in FFA has been shown to induce expression of UCP2 in the lung (412) and in the brain (367). FFA-induced expression of UCP2 in the brain is likely to participate in antioxidant defense and may have a neuroprotective role in the case of brain injury (363). Epidemiological studies suggest that

infants of low birth weight show poor neonatal growth and increased susceptibility to adult diseases such as diabetes and lung disease. UCP2 and 3 have been implicated in the development of such diseases, and Mostyn and co-workers examined whether birth weight influenced the expression of UCP2 and UCP3 in adipose tissue, skeletal muscle, and lung. UCP2 and UCP3 expression in adipose tissue was lower in animals with a low birth weight compared with those with a high birth weight. Lung UCP2 and skeletal muscle UCP3 mRNA expression were unaffected by size at birth. The authors conclude that low birth weight is associated with tissue-specific effects on UCP expression, but it remains to be established whether these subsequently contribute to pathological conditions such as diabetes (262).

During neonatal cardiac development, the heart changes its substrate preference from glucose to FFA, and this is reflected as an increase in the expression of genes involved in control of cardiac FFA metabolism, including UCP2.

b. Aging The expression of UCPs has been found to decrease (198) or increase (19) in skeletal muscle as a result of aging. In the rat CNS, UCP2 expression increased in the spinal cord and brain in aging rats (259). UCP2 expression increased in the liver, but remain unchanged in heart muscle of aging rats (19, 385). It was suggested that the expression of UCPs are part of tissue aging, but the exact role remains to be elucidated. Given the inducible antioxidative role of UCP2, it is not surprising that an increased expression is found in tissues with a high metabolic activity. If an induced increase in expression can slow the aging process in different tissues remains to be determined.

c. The immune system UCP2 has a role in the regulation of ROS production in the immune system (272). In UCP2 -/- mice, ROS production in macrophages was increased, and these animals were completely resistant to infection with *Toxoplasma gondii*, suggesting that UCP2 may affect the ability to fight infection (11). Following administration of lipopolysaccharide, expression of UCP2 is increased in several tissues (50), and this increase has been suggested to be stimulated by increased ROS levels (4). Given the importance of inflammation in development of cardiovascular disease as well as acquired brain injury, it is likely that the anti-inflammatory properties of UCP2 can have beneficial effects on disease progression.

d. The lung UCP2 is highly expressed in the lungs, and may have a role in the development of lung disease. However, the role of UCP2 in the lung is presently not clear. UCP2 expression in the lung increases with age in the sheep fetus (139), and is up-regulated by umbilical cord compression (138). Maternal nutrient restriction in sheep has been shown to have no effect (139) as well as to increase UCP2 mRNA in the lung (263). Birth weight in pigs (262) did not affect the level of UCP2 mRNA in the lung. In the rat, UCP2 expression in the lung increased after birth, and was increased by treatment with triiodothyronine as well as calorie restriction and plasma FFA levels (412).

e. The liver The expression of UCP2 in normal, healthy hepatocytes is low, but may be increased as a result of oxidative stress (247), steatosis (79, 247), or systemic reaction to a bacterial infection (78, 328). The role of UCP2 in the liver is not clear, but as in other tissues, the UCP2 rather appears to have a role in antioxidant defense (76) and possibly control of metabolism than in energy wasting and thermogenesis. Both beneficial and detrimental effects of increased expression of UCP2 have been described. It has been proposed that UCP2 may be induced and activated following hepatothermic therapy, a strategy designed to decrease body fat by maximizing hepatic fatty acid oxidation. Under these conditions, high mitochondrial redox potential would be expected, and induction of the uncoupling activity of UCP2 would represent a homeostatically appropriate antioxidant response (247). Mori and co-workers investigated the effects of pharmacological treatment of hyperlipidemia on expression of UCPs in different tissues. They found that a pharmacologically induced lowering of plasma FFA led to an increased activation of PPARα and an increased expression of UCP2 in the liver (261), and the authors suggest that the increased levels of UCP2 mediated an improvement in liver insulin sensitivity. Increased expression of UCP2 in the liver mediated by PPARα has also been reported by other investigators (8, 267).

Some studies suggest that increased levels of liver UCP2 may have important effects on ATP levels and energy homeostasis in the liver, for example, by inhibiting ATP production, which increases the risk of necrosis following transient ischemia (68–70). Also, it has been suggested that UCP2 has role in the development of nonalcoholic fatty liver disease, which is part of the metabolic syndrome (17). A majority of patients with pancreatic cancer have obstructive jaundice and diabetes with skeletal muscle insulin resistance. Surgery for these patients is associated with significant morbidity. Isaksson and co-workers (170) found that in an experimental rat model, obstructive jaundice was associated with increased liver expression of UCP2 (five-fold), rapid weight loss, and intact insulin action on skeletal muscle glucose metabolism. The jaundiced rats were hypoglycemic and hypoinsulinemic but demonstrated intact or enhanced insulin action on skeletal muscle glucose transport and glycogen synthesis *in vitro*. The authors conclude that obstructive jaundice, by up-regulated liver UCP2, may contribute to the cachexia and high surgical morbidity observed in these patients, but not to skeletal muscle insulin resistance in pancreatic cancer patients. In a clinical study, it was demonstrated that neither UCP2 nor UCP3 were up-regulated in skeletal muscle following pancreatic cancer, and it was concluded that UCP2 and UCP3 were unlikely causes of cachexia (87).

f. Pancreatitis UCP2 appears to be involved in cellular oxidant defense and in the regulation of cell death, both of which are important features of acute pancreatitis. Segersvard *et al.* investigated the expression of UCP2 in two models of acute experimental pancreatitis, and found that UCP2 mRNA was unchanged at 12 hours but was nearly 12-fold greater than control levels after 24 hours. UCP2 gene expression correlated with acinar injury, with parenchymal necrosis, and with the severity of the disease. Up-regulation of UCP2 in the pancreas may be a protective response to oxidative stress, but this increase may also have a negative influence on cellular energy metabolism. Therefore, acinar UCP2 may be an important modifier of the severity of acute pancreatitis (343).

g. Intestinal ischemia/reperfusion Glucagon-like peptide 2 (GLP-2) is an intestinal epithelium-specific growth factor. Guan *et al.* (148) investigated the protective effect of GLP-2 and its functional relationship with UCP2 on the small intestine injured by ischemia-reperfusion. GLP-2 attenuated the intestinal histological and functional damage caused by ischemia-reperfusion, and UCP2 expression was increased in GLP-2-treated mice. The authors suggest that effects of GLP-2 are related to the up-regulation of UCP2, which antagonized ROS production. On a similar note, it was recently suggested that UCP2 in the GI-tract has a role as a free radical scavenger, regulated by vagal innervation (227).

III. THERAPEUTIC APPROACHES USING UCP2

The physiological role and potential therapeutic applications of UCP2 are complex. In particular, the function of UCP2 appears to be tissue-specific, and may have beneficial effects in one cell type, while simultaneously having a negative effect on disease progression in a different cell type. The most striking example is type 2 diabetes mellitus, where increased levels of UCP2 in the pancreatic β-cell impairs insulin secretion, while increased expression of UCP2 in the endothelial cells help prevent atherosclerosis, and increased expression in the brain or heart appears to prevent ischemic cell death. Consequently, a therapeutic approach using UCP2 will have to include a strategy to modify expression of UCP2 in a tissue-selective manner. A review of the literature conveys the picture that the main function of UCP2 that has a therapeutic potential is that of limiting ROS production and inflammatory response, as well as inhibition of cell death. These properties make UCP2 an attractive potential therapeutic target in a number of major diseases, including neurodegenerative, cardiovascular, and potentially inflammatory disease. The suggested role of UCP2 in the regulation of metabolism and energy expenditure seem less clear, and UCP2 as a therapeutic target for the treatment of obesity and diabetes is still unproven.

A. *Pharmacologic inducers of UCP2 and possible applications (PPARs)*

The PPARs are ligand-regulated nuclear transcription factors that regulate gene expression by binding to specific peroxisome proliferators response elements within the promoter region (26, 356, 408). As mentioned in the introduction, PPAR agonists may be used to induce (among others) UCP2 in different tissues. The PPAR agonists are a family of compounds, directed at different subtypes of the PPAR nuclear receptors, including PPARγ, PPARα, and PPARδ. PPARγ exists as two isoforms, γ1 and γ2. PPARs associate with the retinoic acid receptor, and the PPAR/retinoic acid X receptor complex binds with cofactors to initiate gene transcription (26, 356, 408). The naturally occurring ligands for the PPARs are fatty acids and eicosanoids, which are active at micromolar concentrations (26, 408). The subtypes are encoded by different genes (26). Interestingly, the different PPAR subtypes upregulate UCP2 in different target tissues: PPARγ1 is primarily and highly expressed in adipose tissue (14, 318, 392, 418), whereas PPARγ2 is expressed in a wide variety of tissues (26, 356, 408). Of note is the low expression of both PPARγ1 and PPARγ2 in skeletal muscle (408), making this tissue an unlikely target for any direct action of the PPARγ agonists. PPARα is mainly expressed in the liver (8, 267), and PPAR∂ in skeletal muscle (265, 410). This ability to induce UCP2 in a tissue-selective manner may have important therapeutic implications. However, beside UCP2, the PPARs also induce expression of a number of other genes involved in fat metabolism, including other UCPs, which complicate the analysis of potential therapeutic roles of increased expression of UCP2.

B. *Therapeutic implications of UCP2 modulation in the CNS*

Several clinical studies are currently directed at minimizing mitochondria-related damage after acute brain injury. Free radical scavengers are used to counteract the oxidative stress caused by an increased production of ROS from mitochondria following injury, and inhibitors of mitochondrial permeability transition, such as cyclosporin A, are evaluated as a potential therapy following clinical TBI (73). Based on the initial reports on the role of UCP2 in neurodegenerative disease, it is reasonable to assume that UCP2 has a therapeutic potential in a number of neurodegenerative diseases by modulating and reducing mitochondrial ROS production and oxidative damage, as well as induction of mitochondria-mediated cell death (92, 244, 363). UCP expression and activity could be influenced by modulating dietary fat, and we have shown that a reduction in dietary fat in immature animals rapidly reduced neuronal UCP expression/activity and increased mitochondrial ROS production. These changes in mitochondrial UCP activity and ROS production decrease the resistance of the immature animals to excitotoxic insult, resulting in increased neuronal cell death following seizure activity, implicating a neuroprotective role for UCP2 and mitochondrial uncoupling in neuronal injury (367). The data also suggest that increasing dietary fat content would increase UCP activity and reduce ROS production, both of which we have recently demonstrated to occur *in vivo* (368). Importantly, preconditioning using sublethal insults has also been demonstrated to induce UCP expression, reduce ROS formation, and results in neuroprotection that is most likely mediated by the changes in UCP2 activity (244). These results implicate a potential role for UCP2-mediated neuroprotection in several neurodegenerative diseases including epilepsy, traumatic brain injury, and ischemia.

UCP2 expression is induced in a number of tissues by PPARγ agonists (94, 255), and treatment with PPARγ-agonists improved survival of motorneurons in culture (283), as well as outcome following cerebral ischemia (349, 372). It is suggested that the protective effect was related to neurotrophic properties (283), to the anti-inflammatory effects of the drug (372), or mediated through up-regulation of antioxidant enzymes (349). The levels of UCP2 in the cells or brains were not measured in these studies, but it is possible that up-regulation of UCP2 was involved in all of the neuroprotective mechanisms suggested. In another study, the response of mice lacking PPARß receptors (PPARβ KO) following middle cerebral artery occlusion ischemia was evaluated. The PPARβ KO mice had a two-fold increase in infarct size compared with wild-

type (WT) mice. Brain oxidative stress was dramatically enhanced, and no induction of uncoupling protein 2 (UCP2) mRNA was observed (10), suggesting (but not proving) that a PPAR-mediated increase in UCP2 may be an important physiological reaction to limit ischemic damage.

C. PPAR-agonists in treatment of the metabolic syndrome and diabetes

The most well investigated clinical condition where UCPs are expressed using PPAR-agonists is type 2 diabetes mellitus (T2DM), where thiazolidinediones are used clinically today with a documented positive effect on disease progression. T2DM is associated with 1) too much body fat; 2) an abnormal distribution of fat with deposition in muscle, liver, and visceral adipocytes; and 3) large, insulin-resistant fat cells with a compromised capacity to store triglycerides. Further, the dysfunctional fat cells promote insulin resistance, inflammation, hypercoagulability, dyslipidemia, and possibly hypertension. Consequently, a disordered fat cell metabolism is very important to the development of glucose intolerance in T2DM (248).

Thiazolidinediones are synthetic ligands that are potent PPARγ agonists (26, 356, 408). PPARγ is a critical transcription factor in the differentiation of preadipocytes into adipocytes (312, 324, 325, 379). PPARγ agonists induce a number of genes in the adipose tissue, and the net effect of these changes is to enhance glucose and FFA transport into the adipocyte, to stimulate triglyceride synthesis, decrease intracellular concentrations of triglyceride metabolites in muscle, liver, and pancreatic β-cells, and to contribute to improvements in muscle/hepatic insulin sensitivity and pancreatic function in T2DM (21). Consistent with these molecular actions, all thiazolidinediones cause a marked reduction in plasma FFA concentration and inhibit lipolysis in T2DM patients (291). As the plasma FFA concentration declines, fat is mobilized out of the muscle and liver, with improved insulin sensitivity in these organs. In rodents, thiazolidinediones stimulate adipogenesis in subcutaneous fat depots, and induce apoptosis of large fat cells in both visceral and subcutaneous regions in rodents, leading to a shift of body fat from visceral to subcutaneous depots (1). These findings have been confirmed also in clinical studies (184). Surprisingly, these studies show an association between weight gain and improved glycemic control. Normally, weight gain brought about by overeating is associated with insulin resistance and deterioration in glycemic control. The glucose-lowering efficacy of the thiazolidinediones is related to their ability to bind to PPARγ (291, 356, 408), and similar beneficial effects can be expected from nonthiazolidinedione PPARγ agonists (26, 408).

Treatment with thiazolidinediones leads to an increased expression of UCP2 in several tissues, including β-cells of the pancreas (347). As can be expected, such an increase in UCP2 expression leads to a decreased ability of the β-cells to secrete insulin (171). However, the antidiabetic effect of thiazolidinedione treatment suggests that the positive effect of reduction in plasma FFA and peripheral insulin resistance outweighs the potentially negative effects of increased β-cell UCP2 levels.

D. Cardiovascular disease

High plasma levels of FFA contribute to the development of cardiovascular disease, and induce expression of UCP2 in the cardiomyocytes. It has been suggested that an increased expression of UCP2 may protect the heart from ischemic events (252, 377), but also that high levels of UCP2 may lead to energy depletion and increased susceptibility to myocardial ischemia (266). However, it was never shown that the increased expression of UCP2 was responsible for the energy depletion, and it may well be that the increased levels of UCP2 was an effect of high levels of plasma FFA in the patients studied. Also, based on other reports of the cell protective effect of UCP2, it seems plausible that increased levels of UCP2 would mainly be beneficial during episodes of myocardial infarction and heart failure (252, 308, 361, 377). Further, UCP2 has been shown to inhibit the development of atherosclerosis (30, 216, 329), which is a predisposing factor for the development of myocardial infarction, cerebral stroke, and diabetes-related decrease in peripheral circulation. These findings suggest that an increased expression of UCP2 in the cardiovascular system may be an attractive therapeutic target to inhibit the development of cardiovascular disease.

Given the antidiabetic effects of PPARγ-agonists, and the close relationship between diabetes and cardiovascular disease, indirect beneficial effects of treatment with PPARγ-agonists on the development of cardiovascular disease can be expected. Experimental data indicate that treatment with PPAR-agonists is effective against hypertension by acting directly on endothelial cells (279), and that they have a positive effect on heart metabolism and work capacity (141), as well as a protective effect in myocarditis through anti-inflammatory properties (421). However, it is presently not clear if the protective effects observed are related to UCP2 expression and/or activity.

IV. CONCLUDING REMARKS

Although the role(s) of UCP2 in normal physiology are not completely understood, it is apparent that this mitochondrial protein is present and active in several tissues of the body. Recent evidence has pointed to a pivotal role for UCP2 in a number of major diseases, including the metabolic syndrome, diabetes, aging, obesity, and several acute and/or chronic neurodegenerative diseases. As yet it is not apparent what the best strategy for exploiting UCP2 to combat/control these diseases would be or if modulation of UCP2 would be a viable and productive tool in this battle. However it is quite clear that based on several decades of experimental data numerous consequences of increasing UCP2 activity (e.g., reduction of ROS, inhibition of mitochondria-mediated cell death, reductions in mitochondrial calcium loads) are beneficial in important diseases in man. It is also clear that UCP2 has a role in the development of type 2 diabetes, and that mitochondrial uncoupling *per se* is a viable target for the treatment of obesity, but probably not mediated through an increased UCP2 activity (see Fig. 15). From this experimental data, it can be concluded that tissue specific control of UCP2 expression will be instrumental to the success of any therapeutic ef-

fort. Given the growing interest within the field, the next decade of research on the role of UCP2 in health, disease and therapeutics holds great promise.

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ABBREVIATIONS

$\Delta\Psi$, mitochondrial membrane potential; AIF, apoptosis inducing factor; ADP, adenosine diphosphate; ANT, adenine nucleotide translocase; ATP, adenosine triphosphate; BAT, brown adipose tissue; BMCP1, brain mitochondrial carrier protein 1 (UCP5); BMI, body mass index; BSA, bovine serum albumin; CA1–3, cornu Ammon 1–3 (Fields 1–3 of Ammon´s horn); CCI, controlled cortical impact; CNS, central nervous system; CoQ, coenzyme Q; CPT-1, carnitine palmitoyl transferase 1; CytC, cytochrome C; DNP, 2,4-dinitrophenol; ETC, electron transport chain; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; FFA, free fatty acids; H_2O_2, hydrogen peroxide; $HO_2^{\bullet-}$, hydroperoxyl radical; IPC, ischemic preconditioning; K_{ATP}, ATP-dependent K^+-channel; MCAO, middle cerebral artery occlusion; MnSOD, manganese superoxide dismutase; mRNA, messenger ribonucleic acid; mPT, mitochondrial permeability transition; mPTP, mitochondrial permeability transition pore; NF-κB,nuclear factor kappa B; NMDA, *N*-methyl- D-aspartate; NO, nitric oxide; OGD, oxygen-glucose deprivation; $O_2^{\bullet}$, superoxide anion; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, peroxynitrite; PPARs, peroxisomal proliferator-activator receptors; RCR, respiratory control ratio; ROS, reactive oxygen species; SNS, sympathetic nervous system; SOD, superoxide dismutase; SREBP, sterol responsive element binding protein; T2DM, type 2 diabetes mellitus; TTC, 2,3,5-triphenyltetrazolium chloride; UCP1–5, uncoupling protein 1–5; UCP -/-, UCP2 knockout mice; UCP-2/3tg – UCP, 2/3 overexpressing animals; UCP-2/3wt, wild-type littermates of UCP-2/3tg; VDAC, voltage-dependent anion channel.

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