Respiration under Control of Uncoupling Proteins: Clinical Perspective

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Key Words

Uncoupling proteins · Energy metabolism · Thermogenesis · Body weight control · Mitochondria

Abstract

The term ‘uncoupling protein’ was originally used for the mitochondrial membrane protein UCP1, which is uniquely present in mitochondria of brown adipocytes, thermogenic cells that regulate body temperature in small rodents, hibernators and mammalian newborns. In these cells, UCP1 acts as a proton carrier activated by free fatty acids and creates a shunt between complexes of the respiratory chain and ATP-synthase resulting in a futile proton cycling and dissipation of oxidation energy as heat. Recent identification of new homologues to UCP1 expressed in brown and white adipose tissue, muscle, brain and other tissues together with the hypothesis that these novel uncoupling proteins (UCPs) may regulate thermogenesis and/or fatty acid metabolism and furthermore may protect against free radical oxygen species production have generated considerable optimism for rapid advances in the identification of new targets for pharmacological management of complex pathological syndromes such as obesity, type 2 diabetes or chronic inflammatory diseases. However, since the physiological and biochemical roles of the novel UCPs are not yet

clear, the main challenge today consists first of all in providing mechanistic explanation for their functions in cellular physiology. This lively awaited information may be the basis for potential pharmacological targeting of the UCPs in future.

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Introduction

In the last three decades mitochondria were recognized not only as simple energy generators harbouring oxidative metabolic reactions, but also as organelles also regulating various physiological phenomena such as calcium homeostasis or free radical generation as well as playing a key role in the signaling cascade of apoptosis. Thus, new roles for regulated respiration uncoupling mediated by mitochondrial carrier proteins as for example the adenine nucleotide translocase [1–3] but especially by members of the UCP family were found. Uncoupling proteins (UCPs) and respiration uncoupling seem to be implicated in numerous physiological and pathological processes of great importance, which becomes clear from this simple list of proposed UCP-functions: adaptive thermogenesis [4, 5], regulation of fatty acid oxidation [6, 7], participation in inflammation [8], prevention of reactive oxygen species (ROS) formation [9, 10], regulatory func-

Fig. 1. Coupling of energy at the inner membrane of mitochondria. The respiratory chain pumps protons (H^+) across the mitochondrial inner membrane (MIM) and generates a proton gradient. Energy used for this process is released from a redox-reaction whereby reduction equivalents ($R-H$) are oxidized by molecular oxygen (O_2) which itself in turn gets reduced to water. Incomplete oxygen reduction leads to production of ROS inducing cellular oxidative stress and damage. ATP synthase transforms ADP and organic phosphate (P_i) into ATP using the energy conserved in the H^+ gradient. Besides these tightly coupled processes protons leak back across the membrane using UCP or non-protein membrane pores (Leak). This 'uncoupling' dissipates the H^+ gradient as heat.

tions in type 2 diabetes [11], body weight regulation [12], prevention of atherosclerosis [13] and ageing [14]. A potentially protective role of at least one of the UCPs, i.e. UCP2, in cancer development is also discussed [15, 16].

Realizing that all these physiological and pathological features considered to be connected to respiration uncoupling are at the top of the list of the most challenging diseases of our time, the question arises as to the therapeutic potential of UCPs. The ultimate prerequisite for the development of pharmaceutical strategies using UCPs as target structures is a large knowledge of the mechanistic action and physiological function of these mitochondrial proteins.

Respiration Uncoupling and Energy Expenditure

Coenzymes NADH and $FADH_2$ generated in the citric acid cycle during fuel oxidation are reoxidized passing electrons to the respiratory electron transport chain. The electron transport in the respiratory chain pumps H^+ across the inner mitochondrial membrane from the matrix to the intermembrane space. This creates an electrochemical H^+ gradient, named *proton motive force*, which provides energy for adenosine triphosphate (ATP) synthesis by the ATP synthase. At least three protons are required to synthesize one ATP in the process of the so-called *oxidative phosphorylation* [17, 18]. The efficiency

in use of the formed H^+ gradient by the ATP synthase is called *coupling*. Hence 100% coupling would exist, if the machinery of the oxidative phosphorylation would work at the expense of the whole proton gradient formed [17]. However, the H^+ gradient is not only consumed by the ATP synthase, but also by carrier structures in the inner mitochondrial membrane like adenosine diphosphate (ADP)/ATP carrier, calcium carrier or glutamate/aspartate carrier, due to charge imbalance of their substrates [19]. A further part of the proton motive force is consumed by the phosphate carrier and other carriers employing substrate/ H^+ symport [19]. These mechanisms allow H^+ backflow to the matrix bypassing ATP synthase and thereby provoke *protein-mediated respiration uncoupling*. Additionally, under normal conditions, a portion of the created H^+ gradient is consumed by proton backflow to the matrix via non-protein membrane pores or protein/lipid interfaces and this is called *H^+ leak* [20, 21]. Thus, uncoupling is an inherent part of mitochondrial physiology.

Physiological Regulators of Protein-Mediated Respiration Uncoupling: The Family of the UCPs

UCPs are mitochondrial transporters present in the inner membrane of mitochondria (fig. 1). They are found in all mammals and in plants and belong to the family of

Table 1. Demonstrated and proposed roles for mammal uncoupling proteins

	UCP1	UCP2	UCP3	UCP4	BMCP1	KMCP1
Main tissue distribution	BAT	Ubiquitous	Skeletal muscle	Central nervous system	Brain	Kidney
Physiological role	Thermogenesis Limitation of ROS?	Insulin secretion Thermogenesis? Limitation of ROS and/or ATP?	Thermogenesis? Limitation of ROS and/or ATP? Fatty acid metabolism?	Thermogenesis? Limitation of ROS and/or ATP?	Thermogenesis? Limitation of ROS and/or ATP?	Thermogenesis? Limitation of ROS and/or ATP? Transport of glutathione?
Pathological implication	Cold intolerance Obesity	Regulation of inflammatory events? Protection against atherosclerosis, ageing and diabetes? Progression of cancer? Neuroprotection?	Obesity? Proapoptotic effects? Progression of cancer?	Neuromodulation? Neuroprotection against apoptosis?	Nervous system signaling via ROS?	

ATP = Adenosine triphosphate; BAT = brown adipose tissue; ROS = reactive oxygen species; UCP = uncoupling protein; ? = proposed roles.

mitochondrial anion carriers including for example the adenine nucleotide translocase [22]. It is widely accepted that the function of the original UCP, namely UCP1, is uncoupling of oxidative respiration from ATP synthesis, and that its physiological purpose is adaptive thermogenesis. UCP1, which is uniquely present in mitochondria of brown adipocytes, acts as a proton carrier activated by free fatty acids and enhances respiration and cellular heat production after its activation [23]. However, in humans and other large mammals, brown adipose tissue (BAT) disappears after infancy and there is minimal or no detectable UCP1 expression in adults [22].

Physiological functions and mechanistic action of the novel UCPs, identified in mammals within the past 9 years, are as yet poorly understood (table 1). These proteins include UCP2, UCP3, UCP4, BMCP1 (UCP5) and KMCP1 [22, 24–28]. UCP2 is ubiquitous and highly expressed in the lymphoid system, macrophages, and pancreatic islets [26]. UCP3 is mainly expressed in skeletal muscles [27]. Amino acid sequence similarity of UCP2 and UCP3 to UCP1 is 55 and 57%, respectively. It is of significance to note, however, that UCP4, BMCP1 and KMCP1 have only 30% similarity to UCP1 in amino acid sequence [4, 24, 25, 28]. UCP4 and BMCP1 are predominantly expressed in the central nervous system [24, 28], whereas KMCP1 is the UCP representative in kidney [25]. These latter mentioned three UCPs seem to be the ancestral prototype and phylogenetic analyses indicate that there are other mitochondrial anion carrier proteins more closely structurally aligned to UCP1 [4, 29].

In comparison to the established uncoupling and thermogenic activities of UCP1, UCP2 and UCP3 appear to be involved in the limitation of free radical levels in cells rather than in physiological uncoupling and thermogenesis [4, 30, 14]. Moreover, UCP2 is discussed to be a putative regulator of insulin secretion and UCP3 could be involved in fatty acid metabolism [11, 31]. So far however, the physiological and biochemical roles of these inner mitochondrial membrane proteins are uncertain. Furthermore, there is very little information available about physiological functions of UCP4, BMCP1 and KMCP1.

Experimental Evidence for Physiological Roles and Implication of UCPs in Pathological Events

UCP-Mediated Adaptive Thermogenesis

One of the biggest energy expenditures in the mammalian body is thermal energy (heat). The production of this heat is called thermogenesis and can be divided in different types. Besides the work-induced type from exercise that heats up muscles the so-called thermo-regulatory form of thermogenesis is involved in keeping the temperature of the human body regulated. There are two types of thermo-regulatory or also called adaptive thermogenesis: the non-shivering and the muscle-mediated shivering one. Non-shivering thermogenesis also fits into a third and a fourth classification, which comprise diet-induced thermogenesis and cold-induced thermogenesis.

Table 2. Overview of the discussed UCP knock-out and knock-in models and their phenotypes

Strategy	Model system	Phenotype	References
Targeted disruption of the UCP1 gene	mouse	Cold sensitive	33
Targeted disruption of the UCP2 gene	mouse	Resistance to bacterial infection Higher ROS production levels Highly immuno-active MΦs Enhanced NF-κB activity in MΦs Enhanced development and progression of atherosclerosis Improved insulin secretion in beta-cells	8, 11, 13, 42, 43, 55
Transgenic or adenoviral UCP1 overexpression	mouse	Increased metabolic rates Lower levels of blood glucose, insulin and cholesterol Decreased ROS production Prevention of diet-induced insulin resistance, hypertension, hyperphagia, hypothalamic leptin resistance and obesity Activation of eNOS in VSMC	45, 47, 49, 50
Transgenic, adenoviral or retroviral UCP2 overexpression	in vivo: fly neurons in vitro: insulinoma cells, HeLa cells, VSMC	Decreased respiratory control ratio Diminished mitochondrial membrane potential Higher proton conductance of mitochondrial membrane Decreased ROS production levels and oxidative damage Reduced mitochondrial NADH and intracellular ATP levels Prevention of atherosclerotic processes Extended life span of flies	16, 37, 46, 65
Transgenic or adenoviral UCP3 overexpression	mouse	Decreased respiratory control ratio Diminished mitochondrial membrane potential Higher proton conductance of mitochondrial membrane Resistance to diet-induced obesity and hyperphagia Increased glucose clearance rate	12, 36, 37, 61

eNOS = Endothelial nitric oxide synthase; MΦs = macrophages; NF-κB = nuclear factor-kappaB; ROS = reactive oxygen species; UCP = uncoupling protein; VSMC = vascular smooth muscle cells.

Especially BAT, through the activity of UCP1, is responsible for non-shivering thermogenesis in newborn humans and in small mammals [32] (table 2). UCP1-mediated heat production causes a decrease in efficiency of oxidative phosphorylation leading to increased fat oxidation and to diminution in feed efficiency (ratio of weight gain to food intake) [32]. Mice that do not express UCP1 (UCP1 knock-outs) are markedly cold sensitive [33]. Recent identification of new homologues to UCP1 expressed in BAT, muscle, white adipose tissue, brain and other tissues together with the hypothesis that these novel UCPs may regulate thermogenesis and/or fatty acid metabolism have generated considerable optimism for rapid advances in molecular understanding of adaptive thermogenesis and for the identification of new targets for pharmacological management of obesity [34]. Intensive research

activity, however, led to significantly controversial results and a lively on-going debate about the physiological importance of the UCP1 homologues in thermogenesis and fuel/lipid oxidation. Whether e.g. UCP2 and UCP3 actually function as uncouplers is controversially discussed [4, 35]. Mitochondria from mice overexpressing human UCP3 in skeletal muscle have been shown to have a decreased respiratory control ratio and a diminished membrane potential, both suggestive for a decrease in coupling [12, 36]. Proton conductance was 4-fold greater in mice overexpressing UCP3 than in wild-type controls [36]. These and other studies support the contention that UCP2 and UCP3 are uncouplers of oxidative phosphorylation [26, 27, 37]. In relation to neuronal functions an exciting and provocative aspect of controlled mitochondrial uncoupling by UCPs – especially UCP2, UCP4 and BMCP1 – is

Fig. 2. Proposed physiological roles and possible implication of uncoupling proteins in pathological events. UCPs are described to play regulatory roles in cellular ageing, apoptosis and cancer development by limiting ROS generation, and in the case of inflammation and atherosclerosis they are even reported to exert supportive and protective functions. By promoting fatty acid oxidation and energy expenditure they influence either directly or indirectly regulation of body weight and of thermogenesis, furthermore they may increase fat catabolism in cancer cachexia. Limitation of ATP synthesis by uncoupling action of UCP is proposed to be a putative mechanism by which they could influence changes in cellular metabolism during tumour formation and ageing but also during loss of beta-cell function in diabetes. The ability of UCP to induce thermogenesis leads to their potential implication in regulation of body weight and cellular fat metabolism.

the potential to affect the temperature in the microenvironment of presynaptic terminals and hence to provide a basis for temperature as a neuromodulator [38, 39]. However, in summary satisfying answers to fundamental questions regarding the metabolic functions of the new UCPs are pending and more research is needed to elucidate their physiological functions in thermogenesis (fig. 2).

Involvement of UCPs in Inflammation

Inflammation is one of the most important cellular pathophysiological processes involving free radicals generated by mitochondria. According to the state of mitochondrial respiration, the respiratory chain generates superoxide anions, which are converted into hydrogen peroxide or hydroxyl-radicals; all summarized in the term ROS [40]. Altogether, till now published results strongly suggest that UCPs, particularly UCP2, are able to modulate mitochondrial ROS generation [41]. Expression of UCP2 is robust in spleen, lung and isolated macrophages, suggesting a role for UCP2 in immunity or inflammatory responsiveness [8]. Investigation of the response to infection with *Toxoplasma gondii* in mice with targeted disruption of the UCP2 gene (*Ucp2*^{-/-}) showed a complete resistance to infection, in contrast with lethality observed in wild-type littermates. Macrophages from *Ucp2*^{-/-} mice generated more ROS than that of wild-type mice in response to infection and had a 5-fold greater toxoplasma-

cidal activity in vitro compared with wild-type mice [8]. This supports a role for UCP2 in limitation of ROS and macrophage-mediated immunity. The molecular mechanisms of this elevated immune response of *Ucp2*^{-/-} mice were proposed to base on remarkably enhanced key steps in the activation cascade of nuclear factor-kappaB, including augmented I kappaB kinase activity and nuclear translocation of nuclear factor-kappaB subunits [42]. This in turn leads to higher expression of inducible nitric-oxide synthase and inflammatory cytokines [42]. From these results it was concluded that mitochondrial derived reactive oxygen from *Ucp2*^{-/-} cells constitutively activates nuclear factor-kappaB, resulting in a 'primed' state to both potentiate and amplify the inflammatory response upon subsequent stimulation [42, own unpublished data]. Furthermore, the reported down-regulation of UCP2 in immune cells during their activation in early stages of the response to bacterial lipopolysaccharides followed by up-regulation in UCP2 expression during later stages to protect all cells against oxidative stress [43] as well as a described decrease in UCP2 levels in ovarian cells during follicular growth but increased expression during the pre-ovulatory period, during which aspects of an inflammatory process are known to exist [44], represent further strong experimental evidences for a direct link of presence and action particularly of UCP2 and the regulation of inflammatory events.

Preventive Functions of UCPs in Atherosclerosis

In patients with diabetes mellitus increased oxidative stress especially in smooth muscle cells of vessels has been suggested to be implicated in the pathogenesis of accelerated atherosclerosis. A major factor in ROS-dependent pathogenesis of atherosclerosis under diabetic conditions and hypertension are high levels of blood glucose. Hyperglycaemia-induced mitochondrial superoxide overproduction inhibits among others eNOS activity [45], a main regulator of blood pressure acting in a vasodilatory manner. Changes in eNOS activity were found in aortas from diabetic animals in vivo [45]. Inhibition of eNOS by ROS is reversed by blocking mitochondrial superoxide production by overexpression of UCP1 under hyperglycaemic conditions [45]. Since UCP2 is also described as an important regulator of intracellular ROS production [8], the stimulating hypothesis arises that UCP2 could function as an inhibitor of the atherosclerotic process. Park et al. [46] demonstrated that UCP2 by adenovirus-mediated transfer of the UCP2 gene could modify atherosclerotic processes in smooth muscle cells of vessels in response to high glucose. The authors proposed agents increasing UCP2 expression in vascular cells as potential preventive tools in counteracting development and progression of atherosclerosis in patients with diabetes and hypertension [46]. Since the mechanisms linking hypertension and insulin resistance are poorly understood, another approach was performed by crossing transgenic mice expressing UCP1 in skeletal muscle with lethal yellow (A(y)/a) mice, genetically obese animals known to have elevated blood pressure [47]. Blood pressure and serum leptin were lower in uncoupled offspring mice than in parental (A(y)/a) mice, what indicates that respiratory uncoupling under certain circumstances is able to reverse insulin resistance and to lower blood pressure in genetic obesity. Thus, experimental in vivo evidences demonstrate that a controlled respiratory uncoupling could decrease the risk of atherosclerosis in type 2 diabetes by preventing insulin resistance-related hypertension [47]. Interestingly, when non-diabetic low-density lipoprotein receptor-deficient mice (LDLR^{-/-}) were transplanted with bone marrow from either UCP2-deficient mice (Ucp2^{-/-}) or wild-type mice (Ucp2^{+/+}), a marked increase in atherosclerotic lesion size in the thoracic aorta as well as in the aortic sinus of Ucp2^{-/-} transplanted mice compared with control Ucp2^{+/+} transplanted mice was found and reported to correlate with enhanced oxidative stress in Ucp2^{-/-} transplanted mice. This result strongly suggests furthermore a protective role for UCP2 against atherosclerosis [13].

Regulatory Functions of UCPs in Type 2 Diabetes Mellitus

Reduction of the amount of ATP generated through oxidation of fuels by an UCP-stimulated uncoupling procedure suggests UCPs as candidate genes for human obesity or type 2 diabetes mellitus (T2D) [11, 12, 48–50]. Besides a large variety of proposed functions of UCP2, including control of ATP synthesis, regulation of fatty acid metabolism or control of ROS production [4, 8, 22], this UCP is discussed to regulate insulin secretion in pancreatic islets and thereby may play an important role in linking obesity to T2D [11]. Furthermore, mapping of the gene of UCP2 to a region linked to obesity and hyperinsulinaemia as well as the fact that the UCP2 gene was shown to be under control of fatty acids and thyroid hormones in vivo provide strong evidence for a possibly important role in the loss of beta-cell function in diabetes [26, 51]. High-fat feeding markedly impaired glucose-induced insulin secretion in islets from rats and this was accompanied by an increase in UCP2 levels in the islets. The authors of this observation concluded that hyperlipidaemia induced by high-fat feeding affects insulin secretion in islets by a mechanism which may involve, at least in part, modulation of UCP2 expression [52]. Further experimental evidences support this conclusion by revealing that genetically engineered mice overexpressing different UCP homologues were resistant to diet-induced obesity [12]. In addition, a -866G/A polymorphism in the UCP2 gene, which enhances its transcriptional activity, was associated with increased risk for T2D in obese subjects. Moreover, UCP2 mRNA expression was significantly correlated with insulin resistance [53, 54]. Accumulating evidence using UCP2 knock-out mice shows that beta-cell UCP2 expression is up-regulated by glucolipotoxic conditions and that increased activity of UCP2 decreases insulin secretion [11, 55]. The finding that lack of UCP2 dramatically improves insulin secretion constitutes, to date, the most pertinent path to investigations in a therapeutic perspective [55].

UCPs and Body Weight Regulation

Body weight regulation is a complex phenotype also depending either directly or indirectly on the action of UCPs that mediate dissipation of energy as heat [48, 49]. When UCP1 was expressed in murine liver using adenoviral vectors in mice with high-fat diet-induced diabetes and obesity and in standard diet-fed lean mice, hepatic UCP1 expression reversed high-fat diet-induced hyperphagia and hypothalamic leptin resistance, as well as insulin resistance in muscle [50]. In transgenic mice ex-

pressing UCP1 in skeletal muscle resistance towards high-fat diet-induced obesity, lower levels of glucose, insulin and cholesterol as well as an increased metabolic rate have been observed [49]. Thus, enhanced UCP expression in liver and muscle should be considered a new potential therapeutic target for the metabolic syndrome.

Interestingly, when studying 261 subjects from the Quebec Family Study, Oppert et al. [56] found a higher frequency of a specific allele version in the UCP1 gene in individuals who gained more body fat over time. Furthermore, central obesity in whites as reflected by an increased waist-to-hip ratio is discussed to be associated with UCP1 64A/T and UCP3 -55C/T polymorphisms [57]. When assessing the association between the UCP3 -55C/T polymorphism and the risk of obesity, the conclusion was drawn that UCP3 -55C/T polymorphism carriers have apparently a lower risk of obesity [58]. In the case of UCP2 a common G/A polymorphism in the UCP2 promoter region is shown to be significantly associated with enhanced adipose tissue mRNA expression by comparison of 340 obese and 256 never-obese middle-aged subjects [48].

Moreover, sex differences have been shown in cold-, diet- and overweight-induced expression of BAT UCP1 and also in correlation of muscle UCP3 with overweight [59]. Male but not female rats showed tendency to have increased overweight-induced levels of abdominal muscle UCP3 mRNA relative to their age-matched controls [60]. Sex-dependent differences, as well as sex differences in body weight gain under a hypercaloric diet, could be related to the different respective biological functions of males and females, taking into account the fact that the gender effect in future studies on obesity could be of interest. Besides UCP1, especially UCP3 expression has been suggested as a potentially important determinant affecting obesity risk, keeping in mind that UCP3 seems to play a prominent role in the regulation of energy metabolism. Most notably, expression of UCP3 mainly in skeletal muscle mitochondria and the potency of skeletal muscle to act as a thermogenic organ make UCP3 an attractive target for studies towards manipulation of energy expenditure to fight disorders such as obesity and T2D. The observation that mice overexpressing human UCP3 in skeletal muscle are hyperphagic but weigh less than their wild-type littermates and show an increased glucose clearance rate provides evidence that skeletal muscle UCP3 has the potential to influence metabolic rate and glucose homeostasis in the whole animal [61]. Furthermore, comparison of the proton leak in diet-resistant and

diet-responsive overweight women and the expression and gene characteristics of UCP2 and UCP3 by Harper et al. [62] leads to the conclusion that proton leak and the expression of UCP3 but not of UCP2 correlates with weight loss success and may be candidates for pharmacological regulation of fat oxidation in obese diet-resistant subjects.

UCPs and Mitochondrial ROS Formation, Cellular Ageing and Apoptosis

Oxidative stress and mitochondrial dysfunction are associated with disease and ageing. Oxidative stress results from overproduction of ROS generated by mitochondrial respiration, often leading to peroxidation of membrane phospholipids and production of reactive aldehydes [10]. Furthermore, mitochondrial ROS by shortening telomeres are an important determinant in cellular ageing (fig. 2). According to this free radical theory of ageing, oxidative damage from mitochondrial ROS is a major cause of cellular decline, i.e. apoptosis, during ageing. In aged cells, mitochondrial membrane potential ($\text{mt}\Delta\mu\text{H}(+)$) is reduced by a slower respiration and among others this change induces apoptosis [63]. UCPs are described to stimulate respiration by lowering $\text{mt}\Delta\mu\text{H}(+)$ which directly leads to diminished ROS formation and in turn to a reduced frequency of apoptotic events [10, 63, 64]. This protective process mediated by UCPs is also known as 'mild uncoupling' of oxidative phosphorylation [10]. In available literature, particularly UCP2 is described as a potent modulator of mitochondrial generation of ROS, including H_2O_2 , O^{2-} and OH-radical [41]. This supports a role especially for UCP2 in cellular (patho-)physiological processes involving free radicals generated by mitochondria, such as oxidative damage, inflammation or apoptosis [9, 10, 14]. In this context a recently published study is worth to be mentioned. Significant extension in life span of the fly *Drosophila melanogaster* without compromising fertility or physical activity was shown when human UCP2 (hUCP2) was targeted to the mitochondria of adult fly neurons. In this case, an observed decrease in ROS production and oxidative damage by action of hUCP2 is concluded to be sufficient to extend life span of flies [65]. All actually available data concerning the role of UCPs in apoptosis and ageing have in common that UCPs per se cannot cause apoptosis. There is furthermore a broad consensus in the fact that there are UCP-type specific differences in the role of UCP action leading either to protective or death-sensitizing effects. For example, neurons and their synaptic terminals are found to be protected by the neuron-specific UCP4 as

well as the neuronal expressed UCP2 against dysfunction and apoptotic death by a mechanism involving suppression of oxyradical production and stabilization of cellular calcium homeostasis [66, 67]. In contrast to this protective function of UCPs some publications describe UCPs as death-sensitizing components. In an UCP-transfected cell line a higher responsiveness to stimulation of mitochondria-mediated apoptosis as well as a higher mitochondrial Bax/Bcl-2 ratio, representing a prominent apoptotic marker, were described [68]. The authors of this study deduced that presence of UCPs – in this case UCP3 – sensitizes cells to apoptotic stimuli involving mitochondrial pathways [68].

In a study undertaken to determine whether age-related changes in UCP expression occur, tissue-specific changes of UCP2 and UCP3 gene expression were found to be associated with ageing [69]. An observed increased UCP2 expression in ageing liver was proposed to limit ATP production and was related to changes in mitochondrial gene expression in older animals [69]. Furthermore, an *in vivo* observed decrease in skeletal muscle aerobic capacity with advanced age in humans was interpreted as a consequence of the dramatic reduction of UCP3 content associated with decreased uncoupled respiration of skeletal muscle mitochondria [70]. Consistent with a tighter coupling, increased free radical production might contribute to the metabolic compromise in ageing. Taken together, the data suggest a potential role of UCPs as tissue-specific regulators of mitochondrial ROS formation and ATP production during apoptotic processes and cellular ageing.

UCPs in Neoplastic Events

Cancer cell survival depends on adaptive mechanisms including modulation of oxidative stress responses and acquiring drug resistance [15, 71]. Tumour cell drug resistance is the major problem in achieving successful cancer treatment. Moreover, drug-resistant cells express high levels of mitochondrial UCP2 [71]. UCP2 expression is increased for example in most human colon cancers and the level of expression appears to correlate with the degree of neoplastic changes [71]. These findings may foster the idea that UCP2 as a negative regulator of ROS production is part of a novel adaptive response by which oxidative stress is modulated in cancer cells. Furthermore, not only in colon cancer expression particularly of UCP2 was found to be elevated. In some hepatocarcinoma cell lines and in activated mouse myeloid leukemia cells UCP2 protein is expressed 10-fold higher compared with undifferentiated non-transformed cells [72, 73]. In addition,

up-regulation of UCP2 mRNA has been demonstrated in thyroid oxyphilic oncocytoma tumours in comparison to the paired control tissues. This rare subgroup of thyroid tumours is characterized by a significantly lower ATP synthesis, suggesting that a coupling defect in oxidative phosphorylation may be a cause of mitochondrial hyperplasia [74, 75]. A similar and further confirming observation was made by analyzing the human tumour HeLa cell line, where increased UCP2 expression leads to rapid and dramatic fall in mitochondrial membrane potential and to reduction of mitochondrial NADH and intracellular ATP [16]. Another approach in research for a possible role of UCPs in cancer development was performed with the implantation of a fast-growing lung tumour into mice which resulted in a clear cachectic state characterized by a profound muscle wasting and was accompanied by a significant increase in both UCP2 and UCP3 gene expression in skeletal muscle and heart [76]. Interestingly, comparison of mRNA levels of UCP3 in skeletal muscle from gastrointestinal adenocarcinoma patients with the respective controls led to the conclusion that elevations in muscle UCP3 activity may enhance energy expenditure and this in turn could contribute to tissue catabolism during tumour burden [77]. Confirming results of another study, where a mouse model system shows significant elevations of UCP mRNA levels in different tissues in response to injection of an adenocarcinoma-secreted lipid-mobilizing factor, additionally suggest that UCPs may serve to increase fat catabolism in cancer cachexia [78]. These and other results [79] agree with the possible roles of UCPs in participation in a counter-regulatory cytoprotective mechanism to lower production of ROS and in parallel in the increase of energy expenditure associated with tumour growth. On the other hand, the latter mentioned putative contribution of UCPs to the well-characterized abnormalities of metabolism observed in cancer as for example weight loss attributable to enhanced energy expenditure could not be confirmed in the case of pancreatic cancer [80].

Clinical Perspectives and Therapeutic Potential of the UCP Family Members

There is much to be done to decipher the full array of actions of UCPs since mechanistic explanations for reported effects are still not available (table 3). For this reason, it is difficult to comment upon the therapeutic potential of UCPs and the benefits of their pharmacological targeting. One of the current challenges and obstacles of

UCP research is the lack of broad understanding not only of their exact function in cellular physiology but also of which endogenous substances activate UCPs and, perhaps more importantly, which pharmacological compounds could selectively activate different UCPs. The availability of this information will be quintessential to an in-depth analysis of the functionality of these proteins as pharmacologic target structures. However, from the early signs it becomes clear that elucidation of physiological functions of UCPs may lead to identification of novel drug targets for prevention and treatment of different diseases.

Thus, UCP1 and brown fat thermogenesis is and remains a target of continued interest for attenuating nutrient efficiency and counteracting obesity and insulin resistance since future strategies could involve the conversion of white fat cells into thermogenic brown-fat-like adipocytes [81]. However, most physiological studies have not enhanced the understanding of the roles of UCP2, UCP3 and other members of this family. Phenotypes of mice with inactivated UCP2 or UCP3 genes are not related to either defective body temperature or body weight regulation and it has been proposed that these transporters are involved in fatty acid metabolism and in down-regulation of mitochondrial ROS production linking them with epidemic diseases like cancer or chronic inflammation.

In summary, these are still 'early days' in UCP research and a meaningful understanding of the functionality of this mitochondrial transporter family is till now not present at all. Nevertheless, the promising recent findings summarized in this review provoke considerable opti-

Table 3. What the clinician should know about UCPs and respiration uncoupling

Respiration uncoupling and energy expenditure are inherent parts of mitochondrial physiology.

UCPs are mitochondrial transporters present in the mitochondrial inner membrane.

UCP1 acts as a proton carrier and uncouples respiratory chain action from ATP synthase by using oxidation energy for thermogenesis.

Physiological and biochemical roles of UCP1 homologous UCP2, UCP3, UCP4, BMCP1 and KMCP1 are uncertain.

UCP-mediated respiration uncoupling seems to be implicated in important pathological processes (diabetes, obesity, atherosclerosis, cancer).

UCPs = Uncoupling proteins.

mism that pharmacologic targeting of UCPs in future will offer an entirely unique approach to fight against today's most challenging diseases.

Acknowledgements

The work of our laboratory is supported by Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Association de Recherche sur le Cancer and the Institut de Recherche Servier. T.N. is supported by the grand ECFP6 'Diabetesity' contract No. LSHM-CT-20003-503041 in aid of the European Union.

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