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Uncoupling Protein 3: Its Possible Biological Role and Mode of Regulation in Rodents and Humans

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The recently discovered uncoupling protein 3 (UCP3) is highly homologous to the mitochondrial inner membrane protein UCP1, which generates heat by uncoupling the respiratory chain from oxidative phosphorylation. The thermogenic function of UCP1 protects against cold and regulates the energy balance in rodents. We review *in vitro* studies investigating the uncoupling activity of UCP3 and *in vivo* studies, which address UCP3 gene expression in brown adipose tissue and skeletal muscle under various metabolic conditions. The data presented are, for the most, consistent with an uncoupling role for UCP3 in regulatory thermogenesis. We also discuss mediators of UCP3 regulation and propose a potential role for intracellular fatty acids in the mechanism of UCP3 modulation. Finally, we hypothesize a role for UCP3 in the metabolic adaptation of the mitochondria to the degradation of fatty acids.

KEY WORDS: Uncoupling proteins; fatty acids; skeletal muscle; brown adipose tissue; obesity; thermogenesis; gene expression.

DISCOVERY OF UNCOUPLING PROTEIN 3 (UCP3)

The first uncoupling protein (called now uncoupling protein 1: UCP1) was discovered by Ricquier and Kader (1976). It was found to be an inner mitochondrial membrane protein which, by dissipating the mitochondrial proton gradient driven by the respiratory chain, uncouples oxidation from phosphorylation and, therefore, produces heat instead of ATP. UCP1 was found to be exclusively expressed in brown adipose tissued (BAT). In rodents, BAT plays an important role in the exposure to a cold environment (non-shivering cold-induced thermogenesis) and to excess food intake (facultative diet-induced thermogenesis) (for review see Cannon and Nedergaard, 1985). In humans, BAT is present only in the newborn and involutes rapidly later in life (Cunningham *et al.*, 1985; Lean *et al.*, 1986). It was, therefore, generally admitted that, because of the lack of BAT, there was, at least under normal conditions, practically no UCP in human adults.

In 1997, i.e., 21 years after the first report on UCP1, two novel uncoupling proteins, UCP2 and UCP3 were discovered (Fleury *et al.*, 1997; Boss *et al.*, 1997). These proteins were found to belong to the mitochondrial carrier protein family. In this family, they had the highest amino acid sequence identity to UCP1, i.e., 55 and 56%, respectively (Fleury *et al.*, 1997; Boss *et al.*, 1997). The most closely related mitochondrial carrier protein, 2-oxoglutarate/malate carrier had only 32% amino acid sequence identity with UCP2 and UCP3. The novel uncoupling proteins were highly expressed, not only in rodents, but also in humans: UCP2 in most tissues studied (Fleury *et al.*, 1997) and UCP3 mainly in skeletal muscle in humans and in BAT and skeletal muscle in rodents (Boss *et al.*, 1997). Soon after the first description of UCP2 and UCP3, similar tissue distribution for these two proteins were reported (Gimeno *et al.*, 1997; Vidal-Puig *et al.*, 1997; Gong *et al.*, 1997). These discoveries reboosted the interest in UCPs as thermogenic proteins

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in humans and raised hopes for new targets in anti-obesity therapy.

IS UCP3 AN UNCOUPLING PROTEIN?

In vitro Studies

The function of UCP1 as an uncoupler of oxidative phosphorylation and the specific inhibition of its activity by GDP are well documented (Cannon and Nedergaard, 1985). The sequence homologies between UCP3 and UCP1 suggested, but did not prove, that these two proteins had similar uncoupling activities.

The UCP3 function was studied by Gong *et al.* (1997), who showed a decrease in the mitochondrial membrane potential in yeast transformed with UCP3, as compared to yeast transformed with an empty vector. These results were confirmed later, using the same technique, by Liu *et al.* (1998). We used C_2C_{12} myoblasts, which do not express UCP3 (Shimokawa *et al.*, 1998) and could also demonstrate, in a model closer to isolated muscle cells, that the expression of UCP3 significantly decreased cell mitochondrial membrane potential (Boss *et al.*, 1998) (Table I).

It is still possible that the observed decreases in mitochondrial membrane potential induced by UCP3 (Gong *et al.*, 1997; Boss *et al.*, 1998; Liu *et al.*, 1998) are artefacts produced by the insertion of the transfected protein into mitochondria. To really demonstrate that UCP3 is an uncoupling protein, it would be necessary to determine oxygen consumption and respiratory control in a well-characterized system (permeabilized cells or mitochondria) and the effect on these parameters of a well-known inhibitor of UCP1 activity like GDP. One should mention that, up to now, there is no evidence of an inhibitory effect of GDP on UCP3 activity. A complementary approach would be the study of oxidative phosphorylation in muscle mitochondria of UCP3 knockout animals as compared to wild type.

One interesting observation further supports the notion that UCP3 has an uncoupling activity. Lanni *et al.* (1999), in collaboration with our group, using hypohyroid, euthyroid, and triiodothyronine (T_3)-treated rats, observed, in the skeletal muscle, a positive correlation between UCP3 content and mitochondria state-4 respiration, as well as membrane proton conductance. The model of Lanni *et al.* (1999) supports the study on transfected cells and allows to go one step further toward the demonstration of an uncoupling activity of UCP3 (Table I).

In vivo Studies

Short and Long Isoforms of UCP3

In our report of the cloning of human UCP3, we described two different UCP3 clones: UCP3 long ($UCP3_L$) and short ($UCP3_S$), respectively. $UCP3_S$ lacks the VIth potential transmembrane domain and a large part of the putative purine nucleotide binding domain of UCP3 (Boss *et al.*, 1997). The two isoforms might be splice variants of the same gene as proposed by Solanes *et al.* (1997). The $UCP3_S$ protein can be postulated to be either inactive, because of the absence of the VIth transmembrane domain or constitutively active, due to the lack of inhibition by GDP, if the latter has any inhibitory effect on UCP3 activity. A few studies have addressed the problem of the possible biological function of $UCP3_S$ in humans. We (O. Boss *et al.*, 1997) and others (Millet *et al.*, 1998) have found that the two $UCP3_S$ and $UCP3_L$ transcripts are expressed at the same level in the muscle. It was also found that muscle $UCP3_S$ and $UCP3_L$ are coordinately regulated by fasting (Millet *et al.*, 1998) and NIDDM (Bao *et al.*, 1998). A new insight on a possible specific function of $UCP3_S$, as compared to $UCP3_L$, was suggested by a recent genetic study by Argyropoulos *et al.* (1998). Subjects heterozygous for an exon 6 splice junction polymorphism, which should preclude the

Table I. Is UCP3 an Uncoupling Protein?

System	Observed change	References
Transformed yeast	Mitochondrial membrane potential ↘	Gong *et al.*, 1997; Liu *et al.*, 1998
Transfected C_2C_{12} myoblasts	Mitochondrial membrane potential ↘	Boss *et al.*, 1998
Isolated muscle mitochondria (hyper- vs. hypothyroid state)	State-4 respiration ↗ Membrane proton conductance ↗	Lanni *et al.*, 1999

synthesis of $UCP3_L$ allowing only that of $UCP3_S$, had markedly reduced fat oxidation and a higher respiratory quotient, suggesting that a change in the $UCP3_L$/$UCP3_S$ ratio may have an effect on lipid metabolism.

Adaptation to Metabolic Changes

Although the study of the responses of UCP3 expression to metabolic changes should allow interpretations as to whether or not this protein behaves as a thermogenic protein, modulation data are only circumstantial and cannot be used to prove or disprove the hypothesis that UCP3 is an uncoupling protein. They are nevertheless very illuminating for understanding of the biological activity of the novel UCPs.

Practically, all the changes in UCP3 expression levels reported up to now refer to UCP3 messenger. No quantification of the protein has been yet reported, because of the lack of a specific anti-UCP3 antibody. Assuming that UCP3, like UCP1, binds GDP, one might use, as an alternative way to quantify UCP3 protein [^{3}H]GDP binding assays, but no data on such measurement has been reported up to now.

The activity of a thermogenic protein functionally similar to BAT UCP1 should vary in parallel with whole body thermogenic needs. It should be increased upon cold exposure and during cold adaptation and decreased upon food restriction and fasting (Cannon and Nedergaard, 1985).

What is, in fact, observed in BAT? A 48-h cold exposure increased UCP3 mRNA expression in this tissue 1.5-fold (Boss *et al.*, 1998a). This effect was less pronounced than that on UCP1 (about fivefold). Therefore, in rat BAT UCP3, which is expressed under basal conditions at about the same level as UCP1 (Boss *et al.*, 1997), should contribute, but less than UCP1, to the response to cold. A fasting period of 48 h, on the other hand, decreased UCP3 mRNA expression in BAT by 74% (Boss *et al.*, 1998a). Therefore, in BAT, UCP3 behaves in response to cold and fasting as a thermogenic protein.

What about skeletal muscle? A 48-h cold exposure did not change UCP3 mRNA expression in rat tibialis anterior. Similar results were obtained by Larkin *et al.* (1997). However, a time course study, performed by Lin *et al.* (1998), showed that muscle UCP3 mRNA is increased between 6 and 24 h of cold exposure and is decreased after 6 days of cold acclimation. UCP3 might, therefore, contribute to non-shivering thermogenesis only in the acute response to cold exposure. Boyer *et al.* (1998) have shown a strong increase in muscle UCP3 in ground squirrels during hibernation. In muscle, food restriction, consisting of 40% reduction in food intake for 1 week, decreased UCP3 mRNA expression by 81% and more than 90% in mouse tibialis anterior or rat red quadriceps femoris, respectively (Boss *et al.*, 1998a; Cusin *et al.*, 1998). Therefore, in skeletal muscle, UCP3 behaves in response to acute cold exposure, hibernation, and food restriction as a true thermogenic protein.

The surprise came from the effects of fasting. A fasting period of 48 h, indeed, increased UCP3 mRNA expression in rat soleus and tibialis anterior, 2.2 and 6-fold, respectively (Boss *et al.*, 1998a). Similar results were obtained by other groups in rodents (Gong *et al.*, 1997; Weigle *et al.*, 1998). In humans, the same type of observation was reported. Indeed, in lean and obese subjects maintained on a hypocaloric diet (1045 kJ/d) a large increase in vastus lateralis muscle UCP3 mRNA expression was observed (Millet *et al.*, 1997, 1998). The reason for the increase in muscle UCP3 expression upon fasting is difficult to explain. It has been hypothesized that during fasting, BAT activity being blunted, muscle UCP3 activity increases to prevent a dangerous drop in body temperature (Boss *et al.*, 1998a).

MECHANISM OF UCP3 MODULATION: POSSIBLE ROLE OF INTRACELLULAR FATTY ACIDS

Weigle *et al.* (1998) could show that an elevation in circulating FFA by Intralipid infusion in fed rats mimicked the effect of fasting on muscle UCP3 mRNA expression. In humans, we found a correlation in a group of obese patients between circulating FFA levels and UCP3 expression in the vastus lateralis (Boss *et al.*, 1998b).

In BAT, cold exposure and fasting are known to, respectively, increase and decrease the sympathetic nervous system activity and therefore, should increase and decrease lipolysis and intracellular fatty acid level, respectively (Cannon and Nedergaard, 1985). In this tissue, all the above described modulations of UCP3 mRNA expression could, in theory, be explained only by changes in intracellular fatty acids (Table II).

In muscles, which avidly take up fatty acids, the level of intracellular fatty acids should vary in parallel with that of circulating FFA. It is tempting to speculate that the increase in UCP3 expression induced by fasting is the consequence of the increase in circulating

Table II. Intracellular Fatty Acids and UCP3 mRNA Expression in BAT

Conditions	Theoretical changes in intracellular fatty acids	Observed changes in UCP3 expression	References
Cold exposure	Increase	Increase	Boss *et al.*, 1998a
β_3-Adrenoceptor agonist[a]	Increase	Increase	Savontaus *et al.*, 1998; Yoshitomi *et al.*, 1998
Leptin	Increase	Increase	Cusin *et al.*, 1998; Liu *et al*, 1998; Scarpace *et al.*, 1998
Fasting	Decrease	Decrease	Boss *et al.*, 1998a
Streptozotocin diabetes	Decrease	Decrease	Kageyama *et al.*, 1998

[a] No effect or a decrease in UCP3 mRNA expression observed by Gong *et al.*, 1997 and Emilsson *et al.*, 1998, respectively.

FFA level, excluding known endocrine adaptations to starvation (Samec *et al.*, 1998a, b). This hypothesis is supported by results of Samec *et al.* (1998b), who showed that, in 46-h fasted rats, the administration of a dose of nicotinic acid, which completely prevented the fasting-induced increase in FFA, decreased UCP3 mRNA expression in soleus muscle (slow twitch, oxidative) by 70%. This same dose of nicotinic acid did not change UCP3 mRNA expression in the gastrocnemius and tibialis anterior (fast twitch glycolytic, fast twitch oxidative glycolytic, respectively) muscles. Oxidative muscles are more dependent on circulating lipids as food substrates than glycolytic muscles. In fact, the plasma membrane fatty acid-binding protein ($FABP_{pm}$) level was found to be higher in slow than in fast twitch muscles and to correlate with the oxidative capacity of the muscle fiber type (Turcotte *et al.*, 1997). The sensitivity of oxidative, but not glycolytic muscles, to nicotinic acid described by Samec *et al.* (1998b) support, therefore, the hypothesis that circulating FFA are the major determinants of the induction by fasting of UCP3 expression in oxidative muscles and that, in glycolytic muscles, another mechanism is involved in the control of this phenomenon. It is noteworthy that the sympathetic nervous system, which seems to play a central role in the control of BAT UCPs expression, is unlikely to play a role in the control of UCP3 expression in muscle during fasting (Dulloo *et al.*, 1988) (Table III).

An interesting hypothesis would be that, when BAT activity is blunted, like in the fasted state or in STZ diabetic animals, FFA acts as a signal for shifting the control of body temperature from BAT UCP1 to muscle UCP3 activity.

$PPAR_\gamma$, which was shown to bind directly mono- and polyunsaturated fatty acids (Kliewer *et al.*, 1997), could play a role in the effect of fatty acids on UCP3 expression. In the only studies performed up to now to test this hypothesis, the $PPAR_\gamma$ agonists like pioglitazone or thiazolidinedione, administered to rodents, were found to increase UCP3 mRNA expression in BAT (Matsuda *et al.*, 1998; Kelly *et al.*, 1998) and, not change (Matsuda *et al.*, 1998; Kelly *et al.*, 1998) or decrease (Shimokawa *et al.*, 1998b) UCP3 expression in skeletal muscle. It could also be proposed that the stimulatory effect of fatty acids on UCP3 expression is indirect, involving β-oxidation and still unidentified intermediary products of this degradation pathway.

OTHER UCP3 MODULATORS

β_3-Adrenergic Agonists

We have found that a 30-h administration of a β_3-adrenoceptor agonist Ro16-8714 increased UCP3 mRNA expression in obese Zucker (*fa/fa*) rat BAT (Boss, Muzzin, and Giacobino, unpublished observations). Savontaus *et al.* (1998), in collaboration with our group, found that a chronic treatment with the

Table III. Circulating FFA and UCP3 mRNA Expression in Muscle

Conditions	Theoretical changes in circulating FFA	Observed changes in UCP3 expression	References
Intralipid infusion	Increase	Increase	Weigle *et al.*, 1998
Fasting	Increase	Increase	Boss *et al.*, 1998a; Gong *et al.*, 1997; Weigle *et al.*, 1998; Millet *et al.*, 1997, 1998; Samec *et al.*, 1998a,b
Streptozotocin diabetes	Increase	Increase	Kageyama *et al.*, 1998

β_3-agonist BRL 35135 also increased UCP3 mRNA expression in obese Zucker (*fa/fa*) rat BAT. Similar results were obtained with CL 316,243 by Yoshitomi *et al.* (1998) using hyperglycemic KK mice. It can be proposed that these effects of the β_3-adrenoceptor agonists, like those of cold exposure in BAT described above, are mediated by an increase in intracellular fatty acids. One should mention that Gong *et al.* (1997) did not observe any effect of an acute CL 316,243 treatment on BAT UCP3 mRNA expression and that Emilsson *et al.* (1998) observed a decrease in BAT UCP3 expression by an acute BRL 35135 treatment. The reasons for these discrepancies are not understood, but they might have to do with the different durations of the respective treatments.

The β_3-adrenoceptor is considered not to be expressed at a significant level in skeletal muscle. Nevertheless, the administration of β_3-adrenoceptor agonists *in vivo* has been shown to affect UCP3 mRNA expression in this tissue. The effects observed consisted of the induction by BRL 35135 of UCP3 mRNA expression in soleus muscle in lean Zucker rats (Emilsson *et al.*, 1998) and in no change in obese (*fa/fa*) rats (Savontaus *et al.*, 1998; Emilsson *et al.*, 1998). They might be mediated by the muscle atypical β-adrenoceptor reported to avidly bind BRL 35135 in this tissue (Liu *et al.*, 1996). This hypothesis was corroborated by the fact that CL 316,243, which does not seem to be an agonist for the muscle atypical β-adrenoceptor (Liu *et al.*, 1996), not only did not increase, but even decreased UCP3 mRNA expression in hyperglycemic KK mouse femoral muscle (Yoshitomi *et al.*, 1998). The fact that BRL 35135 does not stimulate muscle UCP3 expression in obese Zucker (*fa/fa*), whereas it does so in lean rats (Savontaus *et al.*, 1998; Emilsson *et al.*, 1998), might indicate that obesity induces a "resistance" of the muscle atypical β-adrenoceptor to its specific agonist.

The mechanism of action of β_3-adrenoceptor agonists on muscle UCP3 expression is unknown. It might be mediated by stimulation of muscle lipolysis and consequent increase in intracellular fatty acids and/or by the known second messenger of the β_3-adrenoceptor, cAMP.

Insulin

In STZ diabetic rats, Kageyama *et al.* (1998), in collaboration with our group, observed a decrease and an increase in UCP3 mRNA expression in BAT and gastrocnemius muscle, respectively. Both effects were totally corrected by insulin treatment. It is not quite clear whether or not insulin exerts a direct inhibitory effect on muscle UCP3 expression. In STZ diabetic rats, the effect of a lack of insulin might be indirect via the increase in circulating FFA (Kageyama *et al.*, 1998).

On the other hand, it might be proposed that the inhibitory effects of a chronic CL 316,243 treatment on hyperglycemic KK mouse muscle UCP3 expression reported by Yoshitomi *et al.* (1998) is, in fact, due to the restoration of insulin sensitivity by this drug. Again, this effect might be mediated by the decrease in circulating FFA induced by CL 316,243 treatment (Yoshitomi *et al.*, 1998).

In human patients, results that could help to understand the effects of insulin on muscle UCP3 expression are contradictory. In NIDDM, indeed, Krook *et al.* (1998) have reported a decrease and Bao *et al.* (1998) an increase in vastus lateralis muscle UCP3 expression.

Other studies are definitely needed to answer the question of a direct effect of insulin on muscle UCP3 expression.

Triiodothyronine (T3)

T3 treatment or hyperthyroidism are known to increase thermogenesis in rodents and in humans. T3 administration in euthryroid rodents has been reproducibly shown to increase UCP3 mRNA expression in BAT (Gong *et al.*, 1997; Larkin *et al.*, 1997) and in skeletal muscle (Gong *et al.*, 1997; Larkin *et al.*, 1997; Lanni *et al.*, 1999). These results show that UCP3 in BAT and muscle behaves in response to T3 as a thermogenic protein and might, at least in part, be the mediator of the *in vivo* thermogenic response to T3.

Leptin

We have administered leptin intracerebroventricularly in rats. Using pair-fed animals as controls, we found that leptin increased UCP3 mRNA level twofold in BAT and prevented the decrease in UCP3 mRNA level induced by pair feeding in muscle (Cusin *et al.*, 1998). Similar data were obtained by Scarpace *et al.* (1998), who showed an increase in BAT UCP3 mRNA expression after subcutaneous leptin infusion with a minipump, and by Liu *et al.* (1998), who showed an increase in muscle UCP3 mRNA expression after

administration of adenoviral vectors expressing leptin in obese (*ob/ob*) mice.

The effects of leptin on BAT have been shown to be mediated by stimulation of the sympathetic nervous system (Collins *et al.*, 1996). It can, therefore, be proposed that these effects, like those of cold exposure in BAT, are secondary to an increase in intracellular fatty acids. The effect of leptin on muscle is difficult to explain and the elucidation of its mechanism needs further study.

Altogether, the effects of leptin on UCP3 expression are in line with a general positive effect of leptin on energy dissipation.

UCP3 AND OBESITY

In obese (*fa/fa*), as compared to lean Zucker rats, we observed a 42% decrease in UCP3 mRNA level in BAT and 41% decrease in soleus muscle (Boss *et al.*, 1998a). Similar results have been reported by Emilsson *et al.* (1998), also using Zucker rats.

In obese Zucker (*fa/fa*) rats the leptin receptor OBRb is mutated and nonfunctional (Phillips *et al.*, 1996). Leptin has been shown to stimulate UCP3 expression in BAT and muscle of lean animals (Cusin *et al.*, 1998; Scarpace *et al.*, 1998; Liu *et al.*, 1998). It can therefore be hypothesized that the decrease in BAT and muscle UCP3 reported in obese Zucker (*fa/fa*) rats is due, at least in part, to the lack of leptin signaling.

In obesity, most of the factors mentioned in this review may participate in the control of muscle UCP3 expression, e.g., the increase in circulating FFA, the insulin resistance, and a decreased efficiency of leptin. The situation is, therefore, very complex.

In humans, no change in muscle UCP3 has been reported in obese as compared to lean patients (Millet *et al.*, 1997; Boss *et al.*, 1998 b; Nordfors *et al.*, 1998). However, in the obesity NIDDM-prone population of Pima Indians, Schrauwen *et al.* (1999) have shown that UCP3 mRNA expression in skeletal muscle is negatively correlated with BMI and positively with metabolic rate. Altogether, this report indicates that UCP3 might be involved in energy balance regulation, but that a change of its expression should not be a frequent cause or consequence of obesity.

IS UCP3 ACTIVITY RELATED TO FATTY ACID β-OXIDATION?

The fact that the level of intracellular FFA seems to be a positive modulator of UCP3 expression in BAT and, under some circumstances, in muscle may suggest that UCP3 is a fatty acid mitochondrial carrier, but it should not be considered an argument against an uncoupling activity of UCP3.

It might suggest, however, the interesting hypothesis that, when a cell expressing UCP3 is faced with the necessity of burning fatty acids instead of glucose, it shifts its mitochondrial oxidative phosphorylation to a less coupled state. Fatty acid degradation would, therefore, be associated with a greater energy dissipation than glucose degradation.

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