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Minireview

Thyroid hormone and uncoupling proteins

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Abstract Thyroid hormone (TH/T3) exerts many of its effects on energy metabolism by affecting gene transcription. However, although this is an important target for T3, only a limited number of T3-responsive genes have been identified and studied. Among these, the genes for uncoupling proteins (UCPs) have attracted the interest of scientists. Although the role of UCP1 seems quite well established, uncertainty surrounds the physiological function of the recently discovered UCP1 analogs, UCP2 and UCP3. The literature suggests that T3 affects both the expression and the activity of each of these UCPs but further studies are needed to establish whether the mechanisms activated by the hormone are the same. Recently, because of their larger range of expression, much attention has been devoted to UCP2 and UCP3. Most detailed studies on the involvement of these proteins as mediators of the effects of T3 on metabolism have focused on UCP3 because of its expression in skeletal muscle. T3 seems to be unique in having the ability to stimulate the expression and activity of UCP3 and this may be related to the capacity of T3 to activate the integrated biochemical processes linked to UCP activity, such as those related to fatty acids, coenzyme Q and free radicals.

Key words: Thyroid hormone; Uncoupling protein; Mitochondrion; Mitochondrial efficiency; Energy metabolism

1. Introduction

For the maintenance of a stable body weight, energy intake needs to match energy expenditure. A multitude of factors contribute to this stability, including genetic, physiological and behavioral factors. From a thermodynamic point of view, and considering the animal as a thermodynamic system, we can state that when the 'energy potential' of a system does not change all the energy expended in a transformation of the system appears, ultimately, as heat. This is certainly true in adult animals. In this state, the animal consumes oxygen to perform 'internal and external work' and the energy it expends may be measured either as oxygen consumption (indirect calorimetry) or as the heat produced (direct calorimetry), hence the terms 'calorigenesis' and 'thermogenesis'. Direct calorimetry, which may represent the best way to measure energy expenditure, was often used in the past. However, in the knowledge that there is a good correspondence among energy expenditure, heat production and oxygen consumption, the evaluation of energy expenditure is now most often achieved by measuring oxygen consumption.

Energy expenditure basically consists of three components: (a) obligatory energy expenditure (or basal thermogenesis), (b) facultative energy expenditure (or adaptative thermogenesis) and (c) voluntary energy expenditure.

Basal thermogenesis is represented by the heat produced (or oxygen consumed) as a by-product of the sum of all the anabolic and catabolic biochemical processes involved in the maintenance of the functions essential for life (respiration, circulation etc.). In poikilotherms (or ectotherms), this use of energy is minimal, but in homeotherms (or endotherms) basal thermogenesis is important to maintain a steady level of body temperature. About 20 years ago, Danforth and Burger [1] further divided this component into essential heat and obligatory heat.

Essential heat represents the energy lost (as heat) as a by-product of all the 'life-essential' metabolic processes. Under most circumstances in homeotherms, this heat is insufficient to maintain the body temperature and is supplemented by additional heat production termed obligatory heat.

It is by now an historical notion that thyroid hormones (THs) are unique in their ability to stimulate basal thermogenesis. In fact, among the most pronounced physiological effects attributed to THs is their influence over metabolism in adult endotherms. However, although this effect was deduced more than 100 years ago [2] and the demonstration that THs increase energy expenditure by lowering metabolic efficiency dates from the 1950s, it is surprising how little is known even now about the cellular–molecular mechanisms underlying these effects.

Over the years, several biochemical processes have been put forward to explain the mechanism by which THs stimulate thermogenesis [3]. Now, the control of specific genes at the transcriptional level is thought to be the major molecular mechanism. However, both the number and the identity of the TH-controlled genes remain unknown, as do their relative contributions.

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Abbreviations: UCP, uncoupling protein; TH/T3, thyroid hormone; FA^-, fatty acid anion; ROS, reactive oxygen species; FFA, free fatty acid; CoQ, coenzyme Q; GDP, guanosine diphosphate; BAT, brown adipose tissue; WAT, white adipose tissue

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Be that as it may, our knowledge of the mechanisms underlying thermogenesis and its control implies the involvement, directly or indirectly, of the mitochondrial energy-transduction apparatus [4]. About 50 years ago, it was suggested that THs increase metabolic rate by uncoupling electron transport from ATP synthesis [5]. This hypothesis was subsequently discarded because it was thought not to be physiologically relevant: the effects seen in vitro were not observed in vivo. More recently, however, the uncoupling hypothesis has gained new support because:

- in mitochondria, oxygen consumption is not perfectly coupled to ATP synthesis, part of the proton electrochemical gradient being dissipated as heat. Two main mechanisms may explain this imperfect coupling: passive re-entry of protons inside the matrix (proton-leak) and a failure of the proton pumps consisting in a reduced extrusion of protons in spite of the same electron transport rate (redox-slip). It now seems quite well established that proton-leakage is a general property of the inner mitochondrial membrane;
- it has been discovered that uncoupling proteins (UCPs) are present in almost all tissues and, due to their putative involvement in the mechanisms underlying cellular thermogenesis, they are obvious candidates as mediators of thyroid thermogenesis.

2. UCPs

The proton-leak across the inner mitochondrial membrane accounts for a significant part of an animal's resting metabolic rate and it represents a potential mechanism for energy dissipation or heat production. At the mitochondrial level, two types of proton-leak have been described: basal and inducible. The former is present in mitochondria in every tissue and, while the mechanism regulating it is not clear, it may be related to the lipidic environment of the membrane [6]. The inducible proton-leak, on the other hand, occurs through specific UCPs and is tightly regulated [7].

UCPs are homologous proteins constituting a subfamily of mitochondrial anion carriers [8] that are evolutionarily related and possibly derived from an ancestral protein that acted as a proton/anion carrier. UCP1, cloned in 1985 and called UCP until 1997, is located on human chromosome 4 and is exclusively expressed in brown adipose tissue (BAT) (for review, see [9]). Located in the inner mitochondrial membrane of BAT cells, UCP1, by uncoupling BAT mitochondria, leads to a physiologically important, hormonally regulated, heat production in response to cold or an inadequate diet, although some doubts have emerged recently concerning the latter mechanism [10].

Since 1997, several genes have been discovered that encode proteins closely related to the UCP1. In mammals, four such genes have been described: UCP2, UCP3, UCP4 and BMCP1 (brain mitochondrial carrier protein 1, also termed UCP5). This article will focus in particular on UCP1, UCP2 and UCP3.

UCP2 and UCP3 are adjacent genes located on human chromosome 11 at loci genetically linked to obesity and diabetes [9]. In contrast to UCP1, both UCP2 and UCP3 are expressed to a substantial degree in adult humans. UCP2 mRNA is present in a large number of tissues and is at high levels in white adipose tissue (WAT), spleen and pancreatic β-cells. Despite the ubiquitous presence of UCP2 mRNA, UCP2 protein has been detected in only few tissues [11]. UCP3 mRNA is strongly expressed in skeletal muscle and to a lesser extent in heart, BAT and WAT, while UCP4 mRNA is present in brain and the mRNAs for BMCP1/UCP5 are present in brain and liver (for review, see [9,12]). The homology of both UCP2 and UCP3 (55% and 57%, respectively) with UCP1 has led to the assumption that they also have a specific proton/anion carrier function. Indeed, the idea that UCP analogs might be expected to function as uncouplers was perhaps responsible for orienting the early investigations on the novel UCPs. However, the functions of UCP2 and UCP3 remain uncertain. They have been demonstrated to uncouple mitochondrial oxidative phosphorylation in a number of experimental models such as proteoliposomes [13,14], yeast heterologous expression systems [15,16] and transgenic mice [17,18], and it is now quite clear that heterologous or transgenic expression of these proteins leads to an increase in the proton conductance of the inner membrane. However, it is less obvious whether these uncouplings are due to the activity of the UCP1 homologs or instead represent a more general perturbation of mitochondrial function [19].

An increase in the levels of the mRNAs for UCP2 and UCP3 has been shown not only in situations in which energy expenditure is significantly increased (fever [20], hyperthyroidism [21–23], high levels of leptin [24], cold exposure [25]) but also in one in which it would be expected to be depressed (starvation [26]). As yet, an increased expression of UCP analogs has not always been shown to be associated with increased mitochondrial uncoupling [27,28]. These results prompted the hypothesis that uncoupling is not the primary function of the newly discovered UCPs but rather a consequence of their real function. In particular, it has been hypothesized that UCPs may be involved in the handling of lipids, although whether such a physiological role of UCP2/UCP3 might be related to the control of lipid oxidation or to the prevention of lipotoxicity is unclear [29]. The observations that in skeletal muscle various physiological conditions associated with increased fat metabolism are correlated with a raised UCP3 mRNA expression and that UCP3 mRNA levels are 'lipid-sensitive' constitutes evidence that the association between UCP3 expression and fat metabolism is strong. Agonists of peroxisome proliferator-activated receptors (PPARs) (α and γ) affect the expressions of UCP3, indicating that these transcription factors, which are regulated by lipid metabolites, are involved in this association. Response elements for both TRs and PPARs have been shown to reside within the UCP3 promoter, although to our knowledge they are lacking in the UCP2 gene. Medvedev et al. have shown that the regulation of UCP2 expression is mediated indirectly by PPARγ through two E-box motifs in the UCP2 promoter, without PPAR binding being involved [30].

An involvement of fatty acids is inherent in some hypotheses put forward to explain the mechanism by which UCP1 elicits an uncoupling effect [31]. According to the proton-buffering model (see point A in Fig. 1), fatty acid-carboxyl groups act as H^+ buffers in conjunction with resident H^+-buffering amino acids in the translocation channel of UCP1, while in the 'fatty acid acting as a cycling protonophore' model, UCP1 is an anion carrier able to transport fatty acid anions outward [32]. In the intermembrane space, anions are protonated and return by a flip-flop mechanism into the matrix, where H^+ ions are released (see point C in Fig. 1). These hypotheses

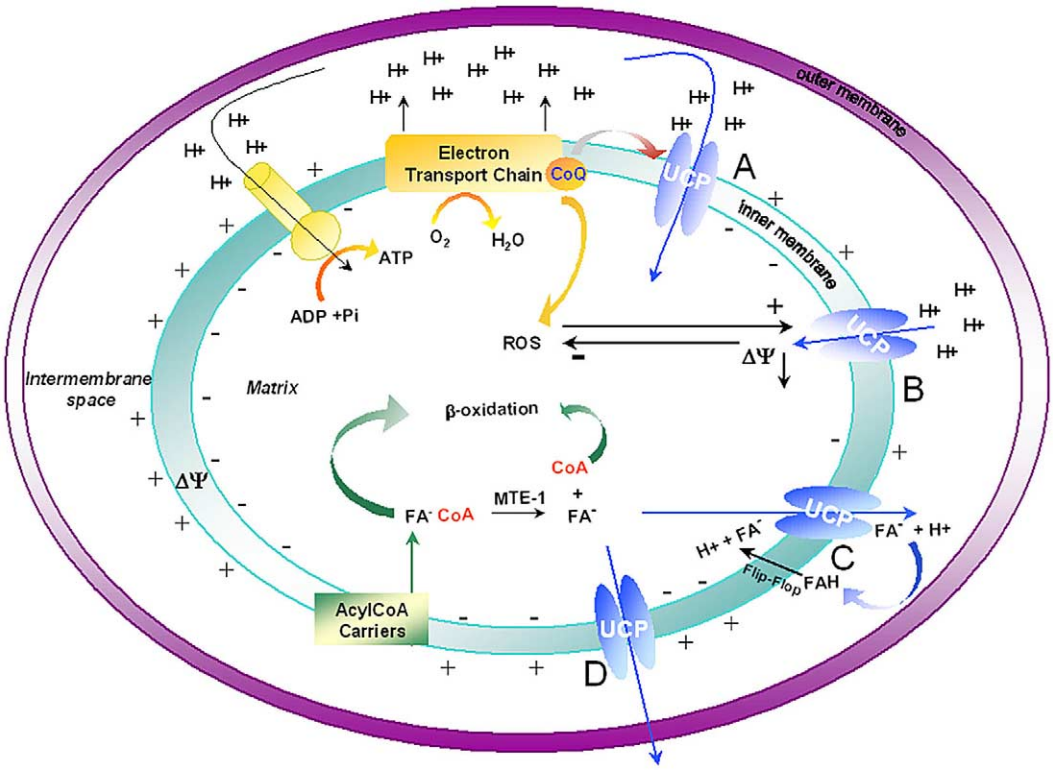

Fig. 1. The scheme illustrates the various hypotheses on UCP analog functions. (A) UCPs as uncouplers; (B) UCPs as ROS-activated mild uncouplers; (C) UCPs as fatty acid anions (FA^-) carrier implied in the FA cycle across the inner membrane; (D) UCPs (in particular UCP3) as FA^- exporters. For further details see the text.

are still under investigation and actually there are arguments for and against both models [33]. It has also been suggested that the activators of UCP1 are most likely not free fatty acids (FFAs) themselves but instead a fatty acid metabolite [34].

As an alternative, in the model proposed by Himms-Hagen and Harper [35], UCP3 is postulated to function as a transporter protein. However, the energy cost of operating of this cycle would result from the ATP utilization needed for cyclic fatty acid activation rather than from uncoupling via proton entry [35]. According to their model, UCP3 would function in concert with mitochondrial thioesterase I (a matrix enzyme that cleaves activated long-chain fatty acids to their corresponding FFAs and CoA-SH) to remove FFA anions from the matrix by transporting them across the inner membrane, thus allowing their return to the pool of fatty acid within the cytosol (see point D in Fig. 1). However, in this model UCP3 cannot maintain a steady transmembrane gradient of free non-oxidized fatty acids. Indeed, it is quite conceivable that it would be impossible to avoid protonation of fatty acid anions on the outer surface of the inner membrane and a subsequent downhill flip-flop of protonated fatty acids from the outer to the inner membrane leaflet. In addition, the hypothesis of Himms-Hagen and Harper does not explain the antioxidant effects exhibited by UCP2 and UCP3.

In fact, it has been proposed that the UCP1 homologs could be proteins that participate in the modulation of mitochondrial reactive oxygen species (ROS) production and therefore in the control of the redox state and oxidative stress of the cell (for review, see [29]). In fact, according to the earlier hypothesis of Skulachev [36], even if the uncoupling activity of a given UCP1 homolog is weak and difficult to detect under physiological conditions, it may have an important role in the mediation of 'mild uncoupling' and hence in the control of ROS production within the cell (see point B in Fig. 1). Evidence supporting such a role for UCP3 can be derived from studies revealing that skeletal muscle mitochondria from UCP3-knockout mice show a reduced proton-leak and increased ROS production [37]. A role for UCP2 in limiting ROS production and in the prevention of excess oxidative damage has been proposed by Nègre-Salvayre et al. [38] who showed an increased mitochondrial membrane potential and H_2O_2 generation in mitochondria not expressing UCP2. Arsenijevic et al. [39] demonstrated that macrophages from mice with disruption within the UCP2 gene generated more ROS and that these mice had greater resistance to parasitic infection than wild-type mice.

As stated before, an increase in the UCP analogs level has not always been found to be associated with an increased proton-leak. In recent years, however, it has become evident that a lack of mitochondrial uncoupling despite an upregulation of UCPs protein levels does not necessarily mean that they are devoid of the capacity to uncouple. Instead, the above situation might be the result of lowered concentrations of one or more putative cofactors. In fact, it seems clear that UCP-mediated uncoupling activity depends not only on FFA but also on factors such as coenzyme Q (CoQ) and superoxide.

Echtay et al. [14], who observed that CoQ is an obligatory cofactor for the uncoupling activities of UCPs in liposomes, suggested a role for CoQ consisting in a co-operation with fatty acids based on the physical contact between these two components at the membrane–UCP1 interface [14]. Further

studies showed that CoQ increased proton conductance in rat kidney (where UCP2 mRNA was present) but not in liver mitochondria (where UCPs are absent), that this increase required fatty acids, that it was prevented by guanosine diphosphate (GDP) and that it was abolished by superoxide dismutase (SOD), indicating that CoQ might mediate uncoupling through the production of superoxide [40]. These results have been questioned by Couplan et al. [41]. Ecthay et al. [42] showed that superoxide increases mitochondrial proton conductance through effects on UCP1, UCP2 and UCP3, and that such superoxide-induced uncoupling requires fatty acids and is inhibited by purine nucleotides (see point B in Fig. 1). They suggested that an interaction of superoxide with UCPs could be a mechanism for decreasing the concentration of ROS inside mitochondria [42] (for a schematic representation of the hypothesized functions of UCP, see Fig. 1). However, a still unknown function of UCP analogs, but possibly related to functions A, B, C and D in Fig. 1, may exist.

2.1. THs and UCPs

2.1.1. THs. Many of the physiological effects of T3 within cells are exerted at the level of transcription via interactions with specific TH receptors (TRα and TRβ) belonging to the superfamily of nuclear hormone receptors (for reviews, see [43]).

Although transcription is considered an important target for THs, only a limited number of genes directly regulated by TH has been identified (for review, see [44]). Recently, the introduction of quantitative fluorescent cDNA microarrays has allowed the identification of several novel T3-responsive genes both in rat [45] and human [46] which are both positively and negatively regulated. Among the T3-responsive genes detected so far, there are several nuclear-encoded respiratory genes, although it is as yet unclear whether or not these genes are directly regulated via an interaction of the T3–TR complexes with TREs in their promoter regions (for review, see [47]). Indirect induction has been suggested to occur via activation of intermediate cofactors as the nuclear respiratory factors-1 and -2 (NRF-1 and NRF-2) [48] or the thermogenic PPARγ co-activator 1 (PGC1) [49] or the recently characterized PGC1-related co-activator [50].

T3 also regulates the transcription of mitochondrial DNA [51]. A two- to eight-fold increase in the steady-state concentration of all mitochondrial RNAs after 1–3 days of T3 treatment has been reported [52]. This action might be exerted either directly through the binding of T3 to specific mitochondrial T3 receptors or indirectly through an induction of the expression of mitochondrial transcription factor A (actually, a nuclear-encoded gene [53]). Among the genes responsive to T3, those for UCPs have recently attracted the attention of scientists investigating the effects of T3 on energy metabolism (see below).

2.1.2. THs and UCP1. TH, in synergism with the sympathetic nervous system, is involved in BAT cold-induced thermogenesis (for review, see [54]). Hypothyroid rats do not survive cold well and fail to increase BAT recruitment (measured as UCP1 concentration and activation) in response to noradrenaline (NA). Although administration of physiological doses of T4 restores cold survival and BAT recruitment, the observation that higher doses of T4 are associated with a blunted BAT response to cold led to the idea that TH played only a permissive role in BAT-mediated thermogenesis. However, it later emerged that this permissive role was apparent rather than real.

In fact, it has been shown that the BAT content of T3 is strongly influenced by type II iodothyronine 5′-deiodinase (D2), which converts T4 into T3, and that D2 is activated by the sympathetic nervous system and inhibited powerfully by its substrate, T4. UCP1 levels vary with the T3 concentration in BAT, and D2 provides the T3 necessary for a maximal thermogenic response to adrenergic stimulation. The finding that T3 and the SNS interact in BAT-mediated thermogenesis is explained by mechanisms operating at the level of the UCP1 gene, which is under the tight control of NA and T3 (for review, see [54]).

The molecular basis of such synergism relies on the presence of two functional TREs and a cAMP-response element in the UCP1 gene promoter and on the proteins involved in cAMP generation [55].

2.1.3. THs, UCP2 and UCP3. Since the discovery of UCP2 and UCP3 in most tissues in mammals, including humans, and the proposal that they play putative roles as uncouplers, many studies have been performed to try to disclose an involvement of these proteins in the effect exerted by TH on energy expenditure. Administration of T3 to rodents leads to increases in the expressions of UCP2 and UCP3 in heart and skeletal muscle [21–23]. In association with the hypothyroidism–hyperthyroidism transition, increases occur in UCP3 mRNA expression in skeletal muscle and in mitochondrial uncoupling activity [23]. The latter result has been confirmed in vivo by a non-invasive method (nuclear magnetic resonance spectroscopy) both in rats and humans [56,57]. In rats, T3 administration led to an increased mitochondrial tricarboxylic acid (TCA) flux with a concomitant unchanged rate of ATP synthesis and, consequently, a lowered ATP/TCA flux ratio. In hypothyroid rats given a single injection of T3, a strict correlation in terms of time course has been shown among the induced increase in UCP3 protein levels in gastrocnemius muscle, the decrease in mitochondrial respiratory efficiency and the increase in the RMR of the whole animal [58]. In that study, the maximal increase in mitochondrial UCP3 density was reached at 65 h after the T3 injection, and the increase in RMR showed the same time course. At the same time point, mitochondria isolated from gastrocnemius muscle showed a significantly higher non-phosphorylating respiration rate, whereas the membrane potential showed a decrease, clearly indicating the occurrence of uncoupling [58]. These data provide in vivo evidence that UCP3 has the potential to act as a molecular determinant in the influence of T3 over RMR. In addition, Barbe et al. [59] have shown that T3 upregulates UCP2 and UCP3 mRNA expressions in human skeletal muscle and adipose tissue both in vitro and in vivo. In the above study [59], the increase in plasma free T3 levels that occurred following the T3 injection was associated with an increase in RMR and a decreased respiratory quotient, thus further pointing towards a role for UCP3 in the effect of T3 on resting metabolism. All these data are in favor of a T3-promoted increase in thermogenesis also via a mitochondrial uncoupling in skeletal muscle.

In contrast to these observations, it has been reported that UCP3 null mice given a 4 day course of T3 (at 100 μg/100 g body weight/day) show the same increase in RMR as wild-type controls [18], a finding that does not seem to support the involvement of UCP3 in the T3-induced increase in metabolic

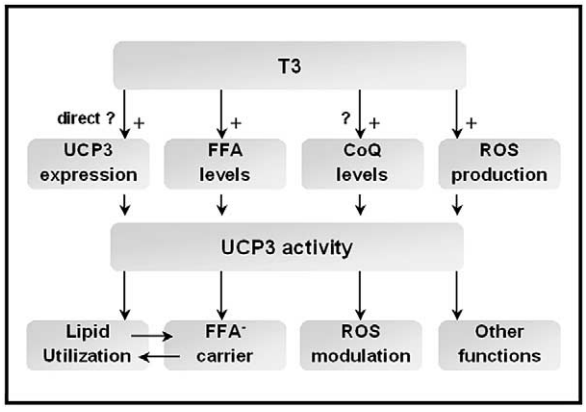

Fig. 2. Schematic representation of the biochemical pathways affected by T3 and related to UCP3 activity. (+) Activation; (?) not definitively established pathway.

rate. The discrepancy between this conclusion and the idea that UCP3 plays a role in TH-induced thermogenesis may be apparent rather than real. Among the possible reasons for the discrepancy being apparent are: (1) the very high doses of T3 used in UCP3-knockout studies (which could have overstimulated other thermogenetic mechanisms) and (2) in the same study, the possible conversion of T3 to other thermogenic iodothyronines, such as 3,5-diiodothyronine [60].

2.1.4. Does T3 activate the integrated biochemical pathway linked to UCP3 activity?. From an analysis of the literature it seems evident that in various situations in which there is an upregulation of UCP2 and UCP3, the THs are unique in their capacity to regulate the expressions of UCPs while at the same time to inducing mitochondrial uncoupling. This could be a consequence of their capacity to synergistically stimulate, or interact with, the complex network of biochemical pathways underlying the activation of UCPs (such as those related to FFA, CoQ and ROS) (see Fig. 2). Actually, TH is well known to induce lipolysis and thus at least part of the effect of T3 on the expression of the UCPs could be due to newly released FFA.

The increase in FFA associated with hyperthyroidism can also affect the functionality of UCP3 within the mitochondria. The clear-cut changes in proton-leak kinetics in rat skeletal muscle mitochondria that accompany the hypothyroidism–hyperthyroidism transition disappear when bovine serum albumin is included in the incubation medium [23]. Moreover, this transition is accompanied by increases in the endogenous levels of mitochondrial FFA and in the sensitivity to FFA shown by both the mitochondrial respiration rate and the membrane potential, and all of these correlated with the level of UCP3 [61]. These data are in agreement with data published by Simonyan et al. [25] who, while studying the effect of cold exposure and T4, reported an increase in UCP3 concentration and a higher sensitivity of skeletal muscle mitochondria to added FFA.

As stated above, recent studies in vitro seem to point towards CoQ and superoxide as essential cofactors for the UCPs-inducible, GDP-sensitive proton-leak, but a physiological demonstration of this phenomenon in an in vivo or ex vivo system is of major importance before we can accept that it has a physiological meaning.

Some evidence supporting just such a physiological role came from a recent study [62] in which we showed (a) that mitochondria from fasting rats, provided they have a source of FFA, do not show uncoupling despite higher levels of UCP3, (b) that after T3 administration to these rats, a GDP-inhibitable, SOD-sensitive uncoupling is observed. In the same study, we noted that the total amount of CoQ is drastically reduced in fasting rats, while T3 administration prevents this reduction. Further, the addition of CoQ to mitochondria from fasting rats (in the presence of an FFA source) induced UCP3-mediated uncoupling (GDP- and SOD-sensitive). These results, by providing evidence that in vivo, as well as in vitro, FFA, CoQ and ROS may be important cofactors in the induction of UCP3-mediated uncoupling, support the idea that T3 is able to stimulate, at one and the same time, UCP3 expression and activity by virtue of its capacity to activate integrated biochemical pathways leading to the establishment of the conditions needed for UCP3 activity: ROS formation and adequate FFAs and CoQ levels (see Fig. 2).

3. Conclusions and perspectives

The effect of TH on energy metabolism may be explained by the activation of several biochemical processes integrated in a complex way. From an analysis of recent results, it seems clear that UCPs are important mediators of the effects of T3 at the cellular level and that T3 is capable of integrating the various biochemical pathways involved in UCP expression and activity, such as those on lipid metabolism, CoQ levels and ROS production. The objectives of future studies on THs and UCPs should lie in three basic areas: (1) clarification as to whether the effects of TH on UCP2/3 are direct or, at least in part, mediated by other factors such as lipids; (2) improvements in the methods by which mitochondrial functions, related to the presence of UCPs, can be investigated in vitro; (3) further clarification of the physiological role played by UCPs, to which end in vitro studies should be accompanied by in vivo and ex vivo investigations.

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