Minireview

Action of thyroid hormones at the cellular level: the mitochondrial target

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Abstract Thyroid hormones exert profound effects on the energy metabolism. An inspection of the early and more recent literature shows that several targets at the cellular level have been identified. Since their effects on the nuclear signalling pathway have already been well-defined and extensively reviewed, this article focuses on the regulation of mitochondrial activity by thyroid hormones. Mitochondria, by virtue of their biochemical functions, are a natural candidate as a direct target for the calorigenic effects of thyroid hormones. To judge from results coming from various laboratories, it is quite conceivable that mitochondrial activities are regulated both directly and indirectly. Not only triiodo-L-thyronine, but also diiodothyronines are active in regulating the energy metabolism. They influence the resting metabolism in rats with 3,5-diiodo-L-thyronine seeming to show a clearer effect.

Key words: Thyroid hormone; Diiodothyronine; Mitochondrion; Receptor; Uncoupling protein; Energy metabolism

1. Introduction

The thyroid gland primarily produces two hormones, L-thyroxine (T4) and triiodo-L-thyronine (T3). These hormones are well known to exert profound effects on the energy metabolism. However, although the first evidence of an increase in metabolic rate in subjects given thyroid extract was reported at the end of the last century [1], the mechanism by which thyroid hormones influence the energy metabolism is still poorly understood. The roots of the currently favored hypotheses and of the debate concerning the cellular mechanism of action of thyroid hormones can be traced to early observations of their calorigenic effects. The key experiments were those performed by J.R. Tata and co-workers in the 1960s [2,3]. These authors showed that administration of thyroid hormones to hypothyroid rats induced an increase in their basal metabolic rate, while simultaneous injection of an inhibitor of protein synthesis, such as the actinomycin D, completely inhibited the stimulatory effect of T3. These results implicated the nucleus as the prime candidate for the location of the signalling pathway at the cellular level that is involved in mediating the effect of T3 on the energy metabolism. Mitochondria, on the other hand, by virtue of their biochemical functions, are natural candidates as the target for the calorigenic effects of thyroid hormones. This is also reasonable in view of the extensive changes that occur in the mitochondrial compartment in response either to thyroid hormones or the physiological states that involve changes in the activity of the thyroid gland (such as aging [4], exposure to cold [5,6], feeding [7] and thyroid status [8]).

During the last three decades, on the basis of results purporting to show either the mitochondrion or nucleus as the location of the major signalling pathway, several mechanisms have been proposed to explain the calorigenic effects of thyroid hormones. However, none has received universal acceptance, possibly because of the perceived problems arising from the wide variety of experimental methods and conditions used in the various investigations (see [9]). Actually, the peripheral availability of iodothyronines mostly depends on local deiodination of T4 and consequently on the activity of deiodinase enzymes. For that reason, T4 deiodination is crucial in the local homeostasis of thyroid hormones. The fact that deiodinase enzymes respond in different ways to different treatments (thyroidectomy, drug administration, etc., see [9]) could help to explain some of the conflicting evidence about the effects of thyroid hormones. Moreover, the use of some of these treatments could have led to the masking of some effects actually attributable to iodothyronines other than T4 and T3. Besides T4, it is known that T3, rT3 and other iodothyronines are substrates for deiodinase enzymes. If these enzymes are not inhibited (or are activated), it is quite conceivable that some effects seen following T4 or T3 administration might actually be due, at least in part, to some of their deiodinated products.

Studies of the effects of iodothyronines at the mitochondrial level have been mostly focused on T3, but recently diiodothyronines (T2s), in particular 3,5-diiodo-L-thyronine (3,5-T2), have been identified as possible peripheral mediators of the effect of thyroid hormones on cell respiration. This article will give an overview of the actions that thyroid hormones exert (both directly and indirectly) at the mitochondrial level. In particular, it will focus on the effects of diiodothyronines on the mitochondrial energy transduction apparatus.

2. Thyroid hormones and mitochondria

Numerous reports concerning the effects of T3 on mito-

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Abbreviations: T3, triiodo-L-thyronine; T4, thyroxine; 3,5-T2, 3,5-diiodo-L-thyronine; 3,3′-T2, 3,3′-diiodo-L-thyronine; RCR, respiratory control ratio; State 3, respiratory state in which ATP synthesis is at the maximal rate; State 4, respiratory state in which there is no ATP synthesis; UCP, uncoupling protein; TR, nuclear T3 receptor; TRE, thyroid hormone response element; mtTFA, mitochondrial transcription factor A; NRF-1, nuclear respiratory factor; mtDNA, mitochondrial DNA; p43, mitochondrial matrix T3-binding protein; PTU, 6-*n*-propylthiouracil; IOP, iopanoic acid; COX, cytochrome *c*-oxidase; RM, resting metabolic rate

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chondrial activity can be found in the literature (for review see [10]). Some of these reported effects are summarized in Table 1 and, in one way or another, they are all related to the effects that thyroid hormones exert on the cellular respiration (calorigenic effect). The oldest hypothesis put forward to explain the calorigenic effect of T3 was that of Martius and Hess [11]. These authors obtained the first evidence that the in vitro addition of T4 to mitochondria caused uncoupling, as measured by a decrease in the respiratory control ratio (RCR = respiratory state in which ATP synthesis is at the maximal rate (state 3)/respiratory state in which there is no ATP synthesis (state 4)) [11]. This hypothesis was subsequently discarded and regarded as not physiologically relevant since (i) large concentrations of T3 or T4 were required and (ii) the effects seen with thyroid hormone in vitro were not observed in vivo. Actually, mitochondria from animals in different thyroid states do not show any differences in RCR. This absence of a change in RCR, however, does not exclude the possibility that changes in state 3 and/or state 4 may occur. In fact, a concomitant increases in (i) the proton leak kinetic (enhanced proton conductance of the inner membrane), which is mostly responsible for state 4 of respiration, and (ii) phosphorylating machinery kinetic (ATP synthase, adenine nucleotide translocator, phosphate transporter), which is responsible for state 3 of respiration, can lead to an unchanged set-point for the coupling ratio [12]. Indeed, when compared to those from euthyroid animals, mitochondria isolated from hypothyroid animals display lower values of state 4 and state 3 respiratory rates, while mitochondria from hyperthyroid rats show higher values. The mitochondrial proton motive force, on the other hand, decreased in the order hypothyroid > euthyroid > hyperthyroid [13]. An uncoupling effect of T3 seems therefore to be a real effect and not an artifact. The molecular mechanism underlying these effects, however, is not completely defined. Harper and co-workers [14] related the increase in the mitochondrial proton leak elicited by T3 to an elevated permeability of the phospholipid bilayer due to a change in the lipid composition of the inner mitochondrial membrane. Recently, however, the discovery that uncoupling proteins (UCPs) are not present exclusively in brown adipose tissue (BAT), but in almost all tissues, shed new light on the mechanism of mitochondrial uncoupling and on the role played by thyroid hormones.

UCPs produce heat by generating a pathway that allows dissipation of the proton electrochemical gradient across the inner mitochondrial membrane without a coupling to any other energy-consuming process. Besides the UCP present in BAT (termed UCP1), other UCPs (termed UCP2, UCP3 and UCP4) are expressed in several tissues in humans and rats (for review see [15], see also [16]). It has recently been shown that T3 stimulates the expression of the mRNAs for both UCP2 [17,18] and UCP3 [19] and that a good correlation exists between UCP3 mRNA expression, mitochondrial coupling and the thyroid state [20]. This is particularly intriguing in view of (i) the evidence that a significant proportion of an organism's resting oxygen consumption is dedicated to opposing the proton leak and (ii) the old hypothesis of 'thyroid-induced uncoupling'. Uncoupling effects may also be elicited through the ADP/ATP carrier (AAC) involved in the mechanism of the uncoupling effect produced by long chain fatty acids [21]. Thyroid hormones, in fact, also regulate the expression of the mRNA for AAC [22].

These effects on the expression of genes involved in the function of the mitochondrial energy transduction apparatus belong to the nuclear-mediated effects of thyroid hormones. However, they are relevant to the present review because one of the questions that intrigues most of the investigators studying 'thyroid hormones and energy metabolism' is: 'are the mitochondria a direct or an indirect target of the effect exerted by T3 on the energy metabolism at the cellular level?'. This question has generated several possible answers. The most widely accepted idea is that held by those scientists who believe that the effects exerted by T3 on mitochondrial activities are indirect, all of them being mediated by an early effect at the nuclear level. These investigators are convinced of this for two main reasons. The first is that some results apparently showing a direct effect of thyroid hormones at the mitochondrial level seem not to be reproducible or they are ambiguous or they were obtained at very high doses. The second is that the nuclear pathway, by contrast, is well-established and much progress has been made in the last three decades in our understanding of this pathway.

2.1. Mitochondria as an indirect target

In the last few years, it has been demonstrated that T3 exerts profound effects on the expression of several genes. These effects of T3 at the genomic level are mediated by the nuclear T3 receptor (TR). This TR is intimately associated with chromatin and binds thyroid hormone with a high-affinity and specificity. The c-erbA proto-oncogene, the counterpart of the viral v-erbA oncogene, encodes the high-affinity TR. There are two genes, designated c-erbAα (TRα) and c-erbAβ (TRβ), encoding several thyroid receptor isoforms. (1) The TRα gene, by alternative splicing, gives rise to TRα1 and TRα2 and, by expression of an alternative strand, to Rev-ErbAα. TRα2 is also known as c-ErbAα2 or TRvariantα2, it does not bind T3 and, therefore, cannot be considered a true receptor. (2) The TRβ gene, through the use of alterna-

Table 1
Reported effects on mitochondrial parameters exerted by thyroid hormones (T4 of T3)

Parameters	Reported changes
H^+/O ratio	− or ↑
P/O ratio	− or ↓
Proton leak	↑
Proton slip	↑
Proton motive force (ΔpH and membrane potential)	− or ↑ or ↓
Activity of mitochondrial carriers (ADP/ATP carrier, UCPs)	↑
Activity of electron transport chain components	↑
Activity of mitochondrial enzymes (α-glycerophosphate dehydrogenase, succinic dehydrogenase, NADH dehydrogenase)	↑

Changes induced: − no change, ↑ increase, ↓ decrease. Note that conflicting results have been reported for some parameters.
For publication details of the papers reporting the effects shown in this table, see Table 1 on page 11 of [10].

tive promoters, gives rise to TRβ1 and TRβ2. Both are able to bind T3.

T3 receptors are transcription factors. They modulate transcription mainly by binding to specific DNA sites known as 'thyroid hormone response elements' (TREs). In the absence of T3, the TR has an intrinsic transcriptional repressor function. In most cases, the TRs act as heterodimers with the 9-*cis* retinoic acid receptor (RXR), but there are also multiple TR complexes that bind to TREs. In addition to RXR, in fact, many other molecules are directly or indirectly functionally associated to TRs (vitamin D3, peroxisome proliferator-activated receptor (PPAR), co-repressors, co-activators, etc.). However, these aspects of the molecular mechanisms involved in the nuclear pathway that mediates the action of thyroid hormone have been recently discussed in a number of excellent reviews [23–25], so they will not be discussed further in this article.

Activation or inhibition of nuclear gene transcription by T3, however, may be relevant to some effects that T3 exerts at the mitochondrial level. The regulation of the mitochondrial respiratory function by T3 may also be consequent upon a change in the expression of genes encoding components of the mitochondrial respiratory apparatus (respiratory genes) or components that activate or inhibit the mitochondrial genomic apparatus (for review see [26]). In mammals, about 100 gene products are necessary for the biogenesis of the mitochondrial respiratory apparatus and they are principally encoded in the nucleus. At present, it appears that only 13 mitochondrial components are encoded in the mitochondrial genome.

2.2. Nuclear-encoded respiratory genes

While it is known that T3 influences (in the rat) the expression of nine nuclear-encoded respiratory genes [26], it is not clear at the moment whether or not these effects on the respiratory genes are mediated by a direct interaction of T3-TR complexes with TREs. Among these nine genes, only the promoter region of the liver cytochrome *c* has been characterized and, in transient transfection experiments, it did not support a T3-TRs-mediated induction of expression [27]. Work on human cytochrome c_1 and ATP synthase subunit genes further supports the idea that different mechanisms may underlie the effects of T3. Both contain putative TREs, but while the cytochrome c_1 promotor supports a TR-mediated induction of expression, the TRE of the F_1-ATP synthase subunit does not (for review see [26], see also [28,29]). In addition, a direct interaction of T3-liganded TRs with TREs should elicit a rapid induction, as seen for 'spot 14' (20 min), rat liver glucokinase and rat growth hormone (2 h) and rat phosphoenolpyruvate-carboxykinase (3 h). However, in the heart, direct T3-TRE induction of the α-myosin heavy chain gene and of calcium-ATPase takes 24–36 h to reach a maximal effect after T3 injection. As for the nuclear-encoded respiratory genes, the induction of mRNA levels by T3 usually reaches its maximum at 12–36 h, with the exception of glycero-3-phosphate dehydrogenase and the βF_1-ATPase subunit (for an account of all these aspects of the subject, see [26]). In summary, there is no conclusive evidence that a direct T3-TR interaction is always involved in the regulation of the transcription of respiratory genes. This raises the question as to how T3 might regulate respiratory gene expression if not via the thyroid receptor. A possible mechanism could be the stimulation of transacting factors which, in turn, activate the expression of the respiratory genes. These factors could include nuclear respiratory factor (NRF-1), which binds to the consensus sequences present in several nuclear-encoded respiratory genes [27]. Indeed, NRF-1 responds to T3 and represents a potential link between T3 stimulation and selective regulation of the expression of respiratory genes including mitochondrially encoded genes.

2.3. Mitochondrially encoded genes

In mitochondria, the genome is transcribed into 16 kb polycistronic RNA molecules, the transcription being initiated at a dual promoter sequence located in the D-loop region of the mitochondrial DNA (mtDNA) (for review see [30]). In early work, it was demonstrated (using non-specific substrates) that thyroid hormone regulates the rate of RNA synthesis [31]. More recently, it was shown that the steady-state concentration of all mitochondrial RNAs was increased 2–8-fold after 1–3 days of T3 treatment. Analysis of transcripts showed that part or all of the increase was accounted for by an elevated synthesis [32]. These results, which have been confirmed by other groups, support the idea that the mitochondrial protein synthesis is regulated by T3 at the transcriptional level. Two alternative mechanisms have been proposed to explain the regulation of mtDNA transcription by T3, through the activation of the expression of a mitochondrial transcription factor (mitochondrial transcription factor A (mtTFA)) [33,34] or directly through specific mitochondrial T3 receptors [35,36].

3. Non-nuclear action of iodothyronines: the direct mitochondrial pathway

As discussed before, the latency of the response to T3 is not an appropriate way to discriminate a nuclear-mediated effect of T3 from other, extranuclear, effects. This being so and in view of the presence of a mitochondrial genomic apparatus (which prevents us distinguishing between 'genomic' and 'non-genomic' effects), it would be preferable to divide the effects due to the iodothyronines into 'nuclear-mediated' (or nuclear) and 'non-nuclear-mediated' (or extranuclear) effects. The nuclear-mediated effects have been briefly described above and they have been extensively described by others [23–26]. The non-nuclear effects of thyroid hormones include all those effects or mechanisms for which a nuclear genomic dependence can be excluded.

In recent years, an increasing number of non-nuclear effects of iodothyronines have been described. Non-nuclear effects have been reported to occur in several compartments of the cell including most cell organelles, the plasma membrane, the cytoskeleton and the cytoplasm (for review see [37]). The latency to onset and other features differentiate the nuclear from the extranuclear actions of iodothyronines. Unlike the nuclear actions, the extranuclear effects are mostly evident within a short time (minutes or a few hours), show a better structure-activity relationship (they can be induced by iodothyronine analogues that have modest or no activity at the nuclear TR, such as T4, rT3, T2 etc.) and may be mediated by signal transducing pathways (cAMP, protein kinase, inositolphosphate). As the effects that iodothyronines may exert directly at the mitochondrial level are relevant to the present article, we shall focus our attention on them. There are some characteristics that need to be displayed by a given effect

before we can accept that it is mediated by a direct action on mitochondria. (a) It should be protein synthesis-independent, (b) it should be observable in vitro in a nuclei-free system and (c) it should be rapid in onset. In addition to all this, specific binding sites for iodothyronines should be present in mitochondria.

3.1. Mitochondrial binding sites

High-affinity binding sites for T3 were identified in the mitochondrial inner membrane in 1975 by Sterling et al. [38]. We confirmed the existence of such sites in 1981 [39]. However, in spite of this and several reports of rapid effects of T3 on mitochondria, the very existence and the physiological significance of these sites and of these effects have long been controversial. Further, a specific mitochondrial receptor for T3 has still not been identified. Quite recently, however, the existence of specific mitochondrial binding sites for T3 has received additional confirmation from the work of Morel et al. [40] and Wrutniak et al. [36].

Morel et al. [40] studied the kinetics of the internalization and specific subcellular binding of T3 in mouse liver both in vivo and in vitro. These authors showed by quantitative electron microscopy autoradiography that, after the injection of radiolabelled T3, specific binding was displayed by five cell compartments (including mitochondria). Surprisingly, specific binding was not evident in the cytosol, in which T3-binding proteins are found. Cabello's group [36], using a photoaffinity labelling technique, identified two T3-binding proteins in rat liver mitochondrial extracts. One (molecular weight (MW) 43 kDa) was located in the matrix and the other (MW 28 kDa) in the inner membrane. These results are in partial agreement with the results obtained by Sterling et al. [38] and by us [39]. The same group [36], using antibodies against the two binding domains of c-erbA α1 identified, by Western blot, two proteins (mitochondrial matrix T3-binding protein (p43) and inner mitochondrial membrane T3-binding protein (p28)) whose location and molecular weight were identical to the mitochondrial T3-binding proteins previously described. Bigler et al. [41] had previously demonstrated that truncated c-erbAα1 proteins are synthesized from the c-erbA mRNA encoding the full-length TR (47 kDa) by using an internal AUG codon. Using an expression vector provided by these authors, Wrutniak et al. [36] overexpressed a truncated 43 kDa c-erbAα1 protein in CV1 cells and, by cyto-immunofluorescence experiments, demonstrated that this truncated TRα protein is specifically imported into mitochondria. Interestingly, the same authors have identified five sequences highly related to TRE within the rat mitochondrial genome and they showed that p43 binds to one of these sequences in the D-loop region, which contains the promoters of the mitochondrial genome. These results are very intriguing and suggest the hypothesis that p43 could function as a T3-dependent mitochondrial transcription factor.

All these data suggest that mitochondria could be an important direct target for T3, even though a possible involvement of p43 in the effect exerted by T3 on the energy metabolism has not yet been thoroughly investigated. However, this does not exclude the possibility that mitochondrial binding sites for T3 may play a very important physiological role in regulating the mitochondrial transcription apparatus. This is reasonable for two reasons, (a) T3 influences the mitochondrial biogenesis and turnover, (b) the mitochondrial biogenesis or turnover needs the coordinated participation of the nuclear and mitochondrial genetic apparatus. In fact, the early results obtained by us and by others showing that T3 regulates the mitochondrial population and the mitochondrial nucleic acid level [31,32,42,43] already suggested just such a possibility. Results seeming to confirm this possibility were obtained by Martino et al. [44], who showed a direct action of T3 on mitochondrial RNA-polymerase in isolated mitochondria, and very recently by Enriquez et al. [45]. The latter authors studied the effect of T3 (both in vivo and in vitro) on in organello mtDNA transcription and on the in organello footprinting patterns in the mtDNA regions involved in the regulation of transcription. They confirmed a direct influence of T3 on the mitochondrial transcription apparatus and, in particular, they showed that T3 selectively modulates the alternative H-strand transcription initiation sites without a previous activation of nuclear genes.

3.2. Diiodothyronines and mitochondria

In the last decade, a growing number of researchers have been focusing their attention on the possibility that iodothyronines other than T4 and T3 may be active in regulation of the energy metabolism. In particular, several studies have suggested that diiodothyronines such as 3,5-T2 and 3,3′-diiodo-L-thyronine (3,3′-T2) could be of biological relevance.

As mentioned above, in 1981, we confirmed the presence of T3-binding sites in a rat liver extract rich in inner mitochondrial membranes but, quite surprisingly, these sites also showed a great affinity for 3,3′-T2 [39]. In view of the fact that such a molecule was considered a biologically inactive product of T4 or T3 catabolism, these observations generated some doubts about the physiological relevance of these sites. More recently, however, Horst et al. [46] demonstrated that 3,5-T2 stimulates oxygen consumption in isolated perfused rat liver (other analogues had no effect). In the same study, T3 also showed a stimulatory effect but it was completely abolished by the addition to the perfusion medium of propylthiouracil (PTU), suggesting that T3 stimulates oxygen consumption only after its transformation to 3,5-T2 (even if the biochemical pathway leading to the formation of 3,5-T2 through T3 deiodination has still not been demonstrated).

These results encouraged us and others to start making greater efforts to uncover a putative physiological role for iodothyronines other than T4 and T3 in regulation of the energy metabolism. As part of this effort, we have shown that chronic administration (3 weeks) of either 3,5-T2 or 3,3′-T2 significantly enhances both the mitochondrial respiratory rate and cytochrome *c*-oxidase (COX) activity [47,48]. T3 also had a stimulatory effect but showed a different initial time course, the effect of the T2s was significant 1 h after the administration of a single dose, while the effects of T3 were significant only after 24 h. In the same studies, it was also observed that other iodothyronines (3′,5′-T2, 3-T1, T0) had no effect on the COX activity. These results induced us to focus our attention on two of the diiodothyronines, 3,3′-T2 and 3,5-T2.

The metabolic effects of 3,5-T2 have also been demonstrated by others in rats [49] and humans [50]. O'Reilly and Murphy [49] also showed that these effects are independent of protein synthesis. A rapid stimulation of rat liver cytochrome oxidase activity by T2s has also been demonstrated in vitro in a nuclei-free system [51].

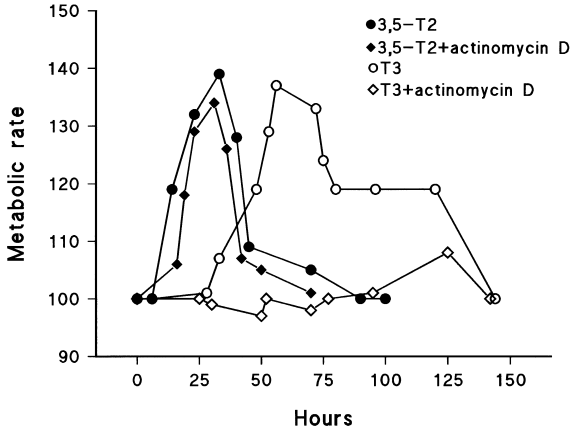

Fig. 1. Changes in the RM of hypothyroid rats following administration of a single dose of iodothyronines (25 μg/100 g BW) with or without actinomycin D (8 μg/100 g BW). Hypothyroidism was induced by combined treatment with PTU and IOP. The metabolic rate of each rat at time 0 (immediately before the injection) is given the value 100.

The direct effects of 3,5-T2 on the mitochondrial metabolism have been confirmed in mitochondria isolated from the liver and red muscle of the goldfish [52], thus indicating that the influence of T2s is not restricted to mammalian species.

All these date strongly suggest that a direct mitochondrial pathway, without nuclear mediation, underlies the effects exerted by T2s on mitochondrial activities. If this is so, specific binding sites for T2s should be present in mitochondria. In fact, saturable binding sites for both 3,5-T2 and 3,3′-T2 have been detected in rat liver mitochondria [53,54]. These sites exhibit a high-affinity and low-capacity, characteristics that support their possible role as mitochondrial specific sites that could mediate the effects of T2s. The exact biochemical nature of these sites, however, remained unidentified.

By top-down elasticity analysis, we have shown a stimulation of the activity of both cytochrome c-oxidizers and the cytochrome c-reducers components of the respiratory chain, 1 h after the injection of 3,5-T2 [55]. These data suggest that 3,5-T2 may interact with some components of the respiratory chain and they are in agreement with previous results showing a direct stimulation of COX activity in experiments using the isolated enzyme from bovine heart [56]. In a recent report, Arnold and Kadenbach [57] showed that (in addition to the mitochondrial membrane potential, the substrate pressure in the respiratory chain and the oxygen concentration) the respiration of animal cells is also controlled by the matrix ATP/ADP ratio, via an interaction of nucleotides with COX. In fact, ATP produces an allosteric inhibition of the COX activity. In a further investigation, Arnold et al. [58] showed that 3,5-T2 specifically binds to subunit Va of the COX complex and completely abolishes the allosteric inhibition of respiration induced by ATP. More recently, Arnold and Kadenbach (personal communication) found that 3,5-T2 induces a decrease in the RCR of COX, based on Δp. Subunit Va of the COX complex is a candidate to be the receptor mediating the effects that this iodothyronine exerts at the mitochondrial level. Overall, these effects should result in an influence of 3,5-T2 on the metabolic rate and thermogenesis in vivo.

Evidence supporting these conclusions has in fact been obtained in our laboratory. We have shown that T2s enhance the resting metabolic rate (RM) when injected, at physiological doses, into hypothyroid rats [9]. This stimulatory effect of T2s on RM is independent of an effect on the protein synthesis and is more rapid than that exerted by T3, with 3,5-T2 showing a clearer effect (see Fig. 1 and [59]). In these experiments, we basically followed the experimental design employed by J.R. Tata [2,3], with the exception that in our case, the hypothyroidism was induced by simultaneous administration of PTU and iopanoic acid (IOP). This treatment induces a severe hypothyroidism and, at the same time, produces a strong inhibition of deiodinase enzymes, thus permitting us to exclude the possibility that a proportion of the effects consequent to the administration of iodothyronines might be due to some of their deiodinated products (see Section 1). In normal animals (i.e. those in which deiodinase activities are not inhibited), such a possibility cannot be excluded in experiments involving the injection of either T4 or T3. In fact, in the papers by J.R. Tata and co-workers, the authors noted that when T3 was injected into normal animals, rather than hypothyroid animals, a different steepness of the curve showing a change in the basal metabolic rate as a function of time was observed. In our laboratory, after injecting T3 into normal animals, we have observed the same phenomenon (unpublished results), that is that the time course of the increase in RM looks much like the effect elicited by 3,5-T2. Taken together, these recent findings furnish support for a direct mitochondrial pathway mediating the actions of thyroid hormone. They also indicate that the diiodothyronines (or at least 3,5-T2, because it shows the clearest effect on RM) should be considered an active iodothyronine and not a simple product of T4 or T3 metabolism.

4. Conclusion

It would be convenient, not to say desirable, to explain the various actions of thyroid hormones at the cellular level by a unitary molecular model. However, while this is an attractive idea, the bulk of available evidences indicates that the situation is far more complicated. In addition, the evidence coming from several laboratories showing that iodothyronines other than T4 and T3 possess relevant physiological properties have further complicated the picture. With regard to the effects they exert on the energy metabolism, however, it seems quite clear at the moment that there are two ways by which iodothyronines may regulate mitochondrial functions. The first way is 'nuclear-mediated' and involves a regulation of the synthesis of respiratory genes and of mtTFs, T3 is the principal mediator in this pathway. The second way is a direct action on mitochondria. In this pathway, T3 and T2 may both be involved. T3, by binding to a specific mitochondrial receptor and affecting the transcription apparatus, may thus act in a coordinate manner with the T3 nuclear pathway to regulate the mitochondrial biogenesis and turnover. T2s, or at least 3,5-T2, seem to act directly on the energy transduction apparatus by binding with some components of the respiratory chain (such as subunit Va of the COX complex), thus regulating the coupled and uncoupled respiration.

Concerning their action at the transcriptional level, it seems well-established that thyroid hormones regulate both nuclear and mitochondrial transcription, but some questions remain unanswered. For example, do the effects that the hormones exert on the nuclear and mitochondrial transcription function

(a) so as to produce organelles richer in respiratory components, (b) so as to produce organelles more or less efficient in the ATP synthesis or (c) so as to regulate the mitochondrial turnover? It seems unlikely, to us, that a rapid regulation of the mitochondrial respiration is achieved by synthesizing respiratory components to be inserted into pre-existing membranes. It is more likely that in the regulation of cellular respiration by iodothyronines, a double mechanism is operative. One would be a short-term mechanism, useful for a rapid response to sudden physiological variations in the energy requirement. Diiodothyronines, or at least 3,5-T2, may play an important role in this mechanism through a direct interaction with some mitochondrial components. The other would be a long-term mechanism, useful for responding to chronic stimuli (days or weeks) such as a long period of cold exposure or a change in the diet or developmental stage. T3, through the regulation of both the nuclear and the mitochondrial transcription apparatuses, would play an important role in this second mechanism. This long-term mechanism would ultimately produce a new mitochondrial population that is more or less active (depending on the presence of respiratory components or cristae) and/or more or less efficient (depending on the presence of UCPs, AAC etc.).

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