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Review

Thyroid hormone action on mitochondrial energy transfer

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I. Introduction

Thyroid hormones influence numerous physiological and biochemical events in cells, ranging from regulation of growth and differentiation to the regulation of the metabolism of every class of foodstuff. Throughout the body, they are the main regulators of the basal metabolic rates. Thyroid hormones are especially active in liver, heart, kidney and brain but less in testis and not in spleen. Due to their stimulatory action on basal metabolism, thyroid hormones increase respiration. However, each tissue responds in a characteristic fashion: not all thyroid hormone effects are observed in all responsive tissues. This is reasonable in view of their complex mode of action; they influence cellular

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Abbreviations: ANT, adenine nucleotide translocase; PTU, propylthiouracil; T_4, L-thyroxine; T_3, L-triiodothyronine; D-T_3, D-triiodothyronine; T_2, L-3,5-diiodothyronine.

function in the long term range via nuclear receptors and in the short term range via extranuclear binding proteins. Plasma membrane binding sites not only mediate hormone transport but also may play a role in calcium, amino acid and sugar uptake, whereas mitochondrial binding sites could be responsible for a direct influence of thyroid hormones on mitochondrial function. Recently cytosolic enzymes, e.g., pyruvate kinase, have been identified as a target of thyroid hormone action.

This article focuses on regulation of mitochondrial energy transfer by thyroid hormones. Since little is known about the signalling pathways, except for the nuclear pathway, the article will also give an overview on short-term signalling pathways used by thyroid hormones.

II. Thyroid hormone signalling pathways

II-A. Binding sites

The first step in a signalling pathway is the binding of the hormone to a cellular receptor. Thyroid hormones can bind to: (i) the plasma membrane; (ii) cytoplasmic proteins; (iii) the mitochondria; and (iv) to the nucleus. Subcellular analysis reveals that 15% of liver cell L-triiodothyronine (T_3) is found in the nucleus, another 10–15% is associated with the mitochondria, and more than 50% is bound to other cell constituents, mainly cytosolic proteins [26]. The function of nuclear binding sites as nuclear receptors is well-established [109], whereas the roles of the other binding sites are less clear. Since some recent reviews deal with cellular binding and uptake of thyroid hormones [26,46,75], this chapter will mainly present findings relevant to mitochondrial effects.

II-A.1. Plasma membrane binding sites

Binding proteins have been characterised for rat liver plasma membranes with molecular masses ranging from 30 to 70 kDa [28,119], in rat kidney [36], in rat thymocytes [132], erythrocytes [4,13], in beef liver [65] and mouse fibroblasts [19]. High- and low-affinity types were identified with reported K_d values ranging from 0.3 to 3 nM and 25 to 220 nM, respectively [28].

In addition, evidence has been presented for a saturable, carrier-mediated uptake of thyroid hormone into hepatocytes [29,80], mouse fibroblasts [19], rat adipocytes [82], rat erythrocytes [111] and human red cell ghosts [63]. The transport system exhibits high and low affinity sites with K_m values in the range of 21–61 nM and 646–2800 nM, respectively. The uptake of T_3 in hepatocytes has been shown to be an energy-dependent process, sensitive to temperature, respiratory inhibitors and dinitrophenol (for reviews, see Refs. 26, 46, 75). It is sodium-dependent, sensitive to ouabain, (see, however, Ref. 12) and is likely to be mediated by a glycoprotein (sensitive to β-glucosidase, pronase and neuraminidase [29]). 30 out of 32 rat tissues exhibit a tissue to plasma gradient for T_3 [164], so that one might suggest that carrier-mediated uptake of thyroid hormones is general. T_3 and thyroxine appear to be taken up by different systems [80]. However, uptake of both T_3 and thyroxine was inhibited by a monoclonal antibody which bound to a 52 kDa plasma membrane protein [96], suggesting that both transport systems are at least similar.

Due to their similarity with amino acids, it has often been speculated that thyroid hormones are taken up by one of the amino-acid carriers in the plasma membrane. In rat erythrocytes T_3 binding and uptake appears to be mediated by the tryptophan transport system T [127]. However, for liver evidence is presented for at least one specific transport system for thyroid hormones [29,80].

Interestingly, in rat skeletal myoblasts [120], mouse fibroblasts [19] as well as hepatocytes [121] T_3 uptake is decreased in the presence of inhibitors of cell integrity and endocytosis, such as vinblastine, cytochalasine and colchicine, suggesting endocytosis (as T_3-protein complex) as a possible uptake mechanism for thyroid hormones. In line with this is the finding of thyroid binding globulin in human breast adipose tissue [122] and serum prealbumin within rat hepatocytes [7] and the observation that thyroid hormones are cotransported with serum protein [116]. However, the exact knowledge of the structure and function of a receptor is lacking and has to be established.

II-A.2. Cytosolic binding sites

The binding of T_3 and thyroxine to cytosolic proteins (CTBP) has been described for several tissues: liver, heart, brain, lung and kidney [20]. In rat liver, cytosolic proteins ranging from 26 to 70 kDa have been found to bind photoactivated T_3 [28]. Two CTBPs were characterised in rat kidney [54]. One is activated by NADPH and binds strongly to mitochondria as T_3-protein complex. It has a molecular mass of 58 kDa (K_a 2.4 nM^{-1}) with very high affinity for L- and D-T_3 [55]. The other is NADPH-independent, a dimer with a molecular mass of 76 kDa and has a different analog specifity. The amount of CTBPs was found to correlate with the developmental stages and was high in tissues that exhibit a high response to thyroid hormones [158].

The physiological function of CTBPs is not clear. Ichikawa and Hashizume [75] suggest that, beside their role as reservoir for thyroid hormones, they are necessary for intracellular transport of hormones to mitochondria and nuclei. Thus, CTBPs with a prealbumin-like structure were found in cytosol, nuclei and mitochondria [1] and the CTBP of human adipose tissue appears to be homologous to an endocytotic product of

a plasma membrane thyroid hormone binding protein (Ref. 122, see above). From uptake and intracellular binding kinetics, Oppenheimer et al. [108] concluded that CTBPs function as transport proteins for T_3 between the plasma membrane and the nucleus.

Others showed that plasma membrane thyroid hormone binding proteins which can enter the cell, have special enzymatic activities: a 55 kDa protein located in beef liver membrane with T_3-binding activity had protein disulfide isomerase activity [65]; it is also a subunit of prolyl-4-hydroxylase, glycosylation-site-binding protein of oligosaccharyltransferase and iodothyronine 5'-monodeiodinase. By binding to these enzymes thyroid hormones might stimulate their uptake and regulate their function within the cell.

Recent findings support the idea that thyroid hormones might also directly influence enzyme activity. It was reported that T_3 binds to a monomer of pyruvate kinase with high affinity (K_a 0.15 μM^{-1} [117]) and inhibits kinase activity [6,79,117]. The monomer has a molecular mass of 58 kDa which is identical to the NADP-dependent CTBP described in Ref. 54. Likewise, thyroxine binds to myosin light chain kinase from platelets [92] and rabbit skeletal muscle and inhibits Ca^{2+}-calmodulin stimulated activity (K_i 2.5 μM [50]). Others [143], using a gel overlay technique, demonstrated binding to isolated sarcoplasmic reticulum Ca^{2+}-ATPase, calsequestrin and a 55-kDa protein which was suggested to represent an anion-transporting protein of sarcoplasmic reticulum. Until the biological functions are shown, however, this suggestion remains pure speculation.

II-A.3. Mitochondrial binding sites

Mitochondrial binding sites for thyroid hormones have been demonstrated as early as 1975 [151]. They were found to be high-affinity binding sites, apparent in electron-microscopic autoradiographs 30 min after administration of [^{125}I]T_3 to hepatocytes [153] and located in the inner mitochondrial membrane [154]. The purified protein with a molecular mass of 28 kDa [154] had the same affinity for T_3 as the high affinity binding sites [153]. The primary sequence was also determined but the identity and function of the protein were not given.

The existence of mitochondrial binding sites was confirmed by [38,52]. The affinity constant was determined to be 0.1 nM^{-1} [38] with the highest affinity for L-3.3'-T_2, but with very high affinity (10 nM^{-1}) also for T_3 and T_4. Others [42] questioned the specificity of the mitochondrial thyroid hormone binding sites since they were not able to find specific binding sites at the mitochondrial inner membrane 2 min after i.p. injection of [^{125}I]T_3 into rats (binding to mitochondria was not replacable by excess unlabelled T_3). This was at a time, when specific binding sites in the nucleus were already apparent. In another study, the binding kinetics with mitochondria were found to be the same at 0°C as at 37°C, whereas this was not the case for nuclei [128]. Since the other studies [38,154] were done with isolated mitochondrial fractions which were incubated with labeled T_3 for 30 to 60 min, conditions that favour nonspecific binding, specific binding of thyroid hormones to mitochondria appears questionable. Another approach [153] used electron microscopy autoradiographs from intact hepatocytes incubated with [^{125}I]T_3. The electron micrographs were, however, of poor quality such that the silver grains were not easily identifiable and the assignment to mitochondria is by no means clear-cut, especially in view of the fact that no grains were found to be associated with the nuclei.

Since adenine nucleotide translocation, as well as distribution of adenine nucleotides between mitochondria and cytosol were shown to be affected in the hyperthyroid state (see subsection III-C), it was suggested that the translocase might be a reasonable candidate for mitochondrial binding of thyroid hormones [155]. Sterling examined [^{125}I]T_3 binding to isolated adenine nucleotide translocase. Preparations from beef heart mitochondria were shown to exhibit high-affinity, low-capacity binding. However, the sequence published for a T_3-binding 28-kDa protein in the inner mitochondrial membrane [155] had no similarity with the sequence of the 30-kDa adenine nucleotide translocase as given by Ref. 5. Rasmussen et al. [124] used intact rat heart mitochondria for affinity labeling with BrAc[^{125}I]T_3. Strong labeling, that was displaced by T_3 and analogues was shown for two proteins with a molecular mass of 48 and 49 kDa but not for the adenine nucleotide translocase, at 0°C, room temperature or after preincubation with the substrates or specific inhibitors.

Specific binding sites for T_3 were also identified in the outer mitochondrial membrane [53] with a K_a of 0.5 nM^{-1}. Binding was inhibited in vitro by calcium (K_i 75 μM) which released the receptor from the outer membrane. The authors ascribe the inability to identify T_3-receptors in the outer mitochondrial membrane by others to the presence of calcium in the incubation medium. However, the physiological significance of the receptor is left open.

II-A.4. Nuclear binding sites

Nuclear receptors are the longest known and best-characterised cellular thyroid hormone receptors. An early review is given by Oppenheimer [109], who in 1972 discovered high-affinity thyroid hormone binding proteins in the cell nucleus of rat liver and kidney [108]. The more recent reviews are those written by Cheng, and Ichikawa and Hashizume [20,75]. The latter group has cloned and purified the labile receptor protein and a summary of their recent overview is

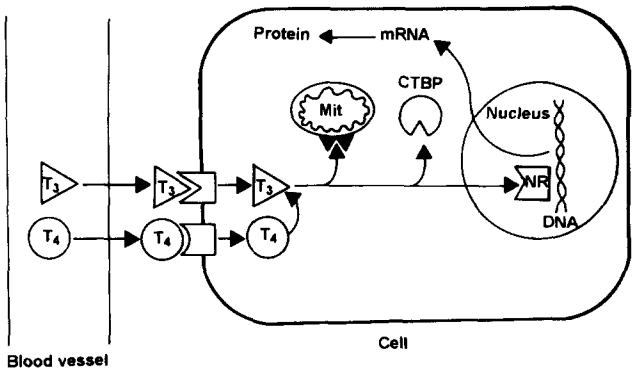

Fig. 1. Cellular binding sites for thyroid hormones. mit, mitochondria; NR, nuclear receptor; CTBP, cytosolic thyroid hormone binding site.

presented here: the nuclear thyroid hormone receptor is an acidic nonhistone protein with a molecular mass of 47–57 kDa. It binds tightly to chromatin, DNA and histones and distributes preferably in transcriptionally-active chromatin. The structure of the receptor is widely conserved among species. Heterogeneity is demonstrated at the genetic level and with the isolated protein but not yet realised in situ. It is most abundant in the pituitary gland, followed by liver, kidney, heart, brain, spleen and testis. Molecular biology techniques revealed that the nuclear thyroid hormone receptor belongs to the c-erb A superfamily.

The following findings qualify the nuclear binding protein as a true thyroid hormone receptor: (i) relative binding affinities of thyroid hormones and analogues correspond to their biological potency; (ii) nuclear occupancy by T_3 correlates with biological response; and (iii) abundance of receptors is correlated with biological responsiveness of tissues.

II-A.5. Summary

Fig. 1 attempts to summarize the findings accumulated in the previous paragraphs: T_3 and thyroxine bind specifically to plasma membrane proteins and are taken up into the cell. No clear evidence is given whether carriers and receptors are identical and the receptor function is not defined. In the cytosol, T_3 can interact directly with enzymes or reaches nuclei and mitochondria probably bound to specific transport proteins. Nuclear receptors are responsible for long-term actions of thyroid hormones within the cell, i.e., enzyme induction, whereas the role of mitochondrial binding sites remains to be clarified (see also subsection III-C.2).

II-B. Nuclear signalling pathway

According to earlier and more recent reports in the literature, the long-term action of thyroid hormones is mediated in higher organisms via regulation of nuclear transcription [109]. Regulation of transcription involves the the following events [26]: (i) Binding of T_3 to the nuclear receptor followed by a direct and indirect effect on transcription; (ii) a change in polymerase II activity followed by a change in cytosolic mRNA; (iii) a change in translation products; and (iv) metabolic and clinical effects. Regulation at the transcriptional level can influence every cellular event. Initiation of transcription starts as early as within 5 min following thyroid hormone application, as demonstrated for rat liver S14 mRNA in vitro [77], within 30–40 min for the mRNA of amino-acid transport systems in the plasma membrane in neurons [118], 2 h for mRNA coding for sarcoplasmic reticulum Ca^{2+}-ATPase in heart [125] and several hours to days for other mRNAs, e.g., cytochrome *c* [130].

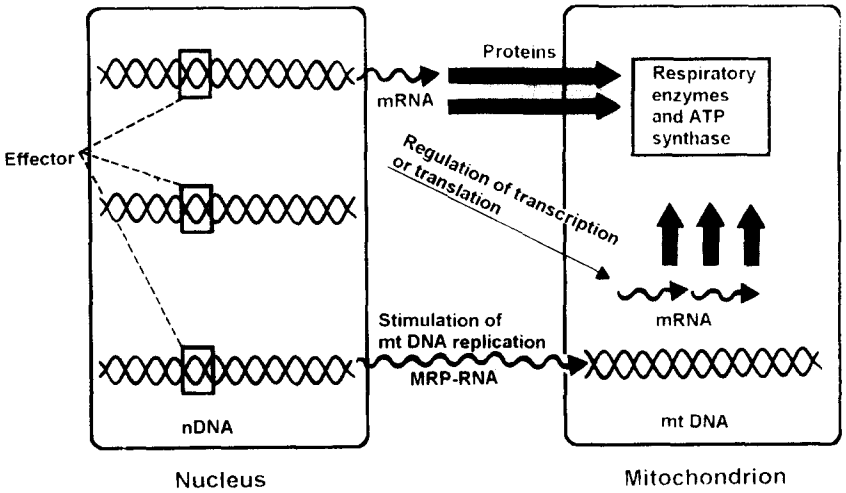

Fig. 2. Regulation of mitochondrial biogenesis by thyroid hormones. □ effector bound to the nuclear receptor; ⇝, upregulated mRNA; ⇉, newly synthesised proteins; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; MRP-RNA, RNA with site-specific endoribonuclease activity for mitochondrial RNA processing involved in primer RNA metabolism for replication of mtDNA. Modified from Ref. 105.

II-B.1. Regulation of the transcription of mitochondrial proteins

Thyroid hormone is the only physiological effector molecule known to exert global control over mammalian mitochondrial biogenesis. Studies from the early 1960s showed that the number, volume and membrane area of mitochondria are decreased in hypothyroidism [45]. Thyroid hormones increase the synthesis of nuclear encoded mitochondrial proteins and mitochondrially-encoded mitochondrial proteins [103,165]. There appears to be a general agreement, however, that influence on mitochondrial transcription is via nuclear action [75,103,165]. For the components of mitochondrial energy transfer there appears to exist a concerted activation at nucleotide sequence motifs, encoding proteins which regulate mitochondrial transcription [105] and translation [74,105,165]. A scheme is given in Fig. 2.

An interesting recent report showed that mitochondrial protein synthesis (measured by incorporation of labelled leucine) was regulated in liver (stimulatory) and kidney (inhibitory) by proteins isolated from the cytosol of young rats after thyroxine administration [74]. In aged animals, only inhibitory regulators were synthesised on thyroxine treatment.

For more details on the transcription of mitochondrial proteins, see subsection III-B.2.

II-B.2. Regulation of transcription of proteins involved in intracellular signalling

Thyroid hormones induce the transcription of peptide hormones such as growth hormone in rat pituitary tumor cells [102,129]. They also influence the hormone responsiveness by restoring plasma membrane receptors, e.g., for α-adrenergic agonists, vasopressin and angiotensin in hepatocytes of partially hepatectomized rats [72] or for β-adrenergic agonists in heart sarcolemma [32,163] and they increase the number of binding sites for vasopressin, angiotensin, α-adrenergic receptors and for glucagon in liver [2]. Thus, the permissive action of thyroid hormones on other hormone effects is understandable. Recently, it has been reported that thyroid hormones interact with other signalling pathways via regulation of the G-protein β-subunit mRNA expression [123], i.e., in hypothyroid rats mRNA-levels increased, and thus, the inhibitory control of adenylate cyclase in rat adipose tissue. Another group [133] reported that thyroid hormones increase the calmodulin content in thymocytes, liver, heart and brain, but not in skeletal muscle.

Other proteins relevant for intracellular signalling which are induced by thyroid hormones are sarcoplasmic reticulum Ca^{2+}-ATPase in rat heart [125], Ca^{2+}-ATPase in rat skeletal muscle [31] and Na^+/K^+-ATPase in liver and heart [149].

II-C. Extranuclear signalling pathways

Although it is widely accepted that thyroid hormones exert their main effect on the organism by regulating the transcription of proteins, evidence has accumulated during the past few years that thyroid hormones also do act through extranuclear pathways. These pathways have a faster time of onset (1 to 60 min) and duration (about 1 h). Despite the fast onset of the short-term effects, a nuclear pathway cannot be completely ruled out in this time range, since stimulation of transcription for S14 mRNA as early as 5 min after T_3 application has been demonstrated in vitro (see above). On the other hand, protein synthesis inhibitors like cycloheximide or actinomycin D had no effect on calcium and sugar uptake, as shown in Ref. 134. In addition, thyroid hormone actions on erythrocytes are self-evident examples of extranuclear effects. The so-called short-term actions comprise direct interactions with the plasma membrane, an increase in cytosolic calcium and cAMP, and direct interactions with cytosolic enzymes and with mitochondria.

II-C.1. Action at the plasma membrane

Recent reviews on the short-term actions of thyroid hormone at the plasma membrane level are Ref. 134 and, especially on Ca^{2+}-ATPase, Ref. 25. Thyroid hormones stimulate Ca^{2+}-ATPase, as well as calcium transport into the cell in erythrocytes of diverse origin [134]. T_3 increases intracellular calcium also in thymocytes in less than 1 min, reaching maximal values at 5 to 10 min; intracellular calcium starts to decrease at 8 min [135]. The effect is concentration-dependent, specific for T_3 and dependent on extracellular calcium. Sugar and amino-acid uptake is also enhanced [134,135]. Further, adenylate cyclase is stimulated and cAMP levels are increased.

In other tissues from rat, like diaphragm, liver and heart, the increase in Ca^{2+}-and sugar uptake could be reproduced [136,137]. Inorganic calcium channel blockers like Cd^{2+}, Mn^{2+} and La^{2+} inhibited basal and T_3-stimulated $^{45}Ca^{2+}$-uptake, whereas organic inhibitors like verapamil had no effect on the hormone stimulated uptake. It is concluded that thyroid hormone mediated calcium influx is not dependent on membrane depolarisation and that calcium is the first intracellular messenger of the direct, prompt and specific action of thyroid hormones at the plasma membrane.

Others [73] found in perfused liver a rapid uptake of calcium (within 1 to 20 min) following T_3-infusion, as determined from a decrease in the perfusate calcium with a calcium sensitive electrode, and likewise an increase in intracellular calcium, as measured with fura-2 in isolated hepatocytes. It had the same time-course as the stimulation of oxygen uptake and gluco-

neogenesis from lactate + pyruvate. Interestingly, not only T_4, but also T_2 but not D-T_3 had the same effect. The rise in cytosolic calcium induced by T_3 would also explain T_3-induced phosphorylation of pyruvate kinase and phosphorylase observed in isolated hepatocytes [146]. In single myocytes a rapid increase in intracellular calcium was measured following T_3-application, using fluorescent microinjected aequorin [86].

Taken together, there is plenty of evidence that thyroid hormones mediate a direct and rapid calcium influx into the cell in various tissues and that this increase in cellular calcium is paralleled by changes in intracellular metabolism. Therefore, calcium appears to be an intracellular messenger for the short-term effects of thyroid hormones.

On the other hand, the increase in cAMP, seen in thymocytes [135], does not appear to be general. In perfused liver no increase in cAMP was found following T_3-infusion within 60 min, a time-range where oxygen consumption, gluconeogenesis, amino-acid uptake and urea synthesis did increase, but not the transcription of enzymes typical for the nuclear action of T_3, e.g., phospho*enol*pyruvate carboxykinase or malic enzyme [98]. Although this long time-range does not appear to be supportive for a short-term action, no further evidence exists that indicates a short-term increase in cAMP mediated by thyroid hormones.

In hyperthyroid rats, however, cAMP is increased [100], probably by the induction of β-adrenergic and glucagon receptors (see above).

The mechanism of the interaction of thyroid hormones with the plasma membrane is a matter of speculation. Binding studies (see subsection II-A.1) have not been correlated with intracellular effects although the intracellular effects were shown to be dose-dependent [70,134,139]. As reported in Ref. 25, thyroid hormone stimulation of plasma membrane Ca^{2+}-ATPase or other calcium-transporting plasma membrane proteins, appears to involve a direct interaction with membrane lipids and proteins: (i) thyroid hormones in vitro were capable of stimulating or inhibiting red cell Ca^{2+}-ATPase depending upon dietary fat intake of the animals and, presumably, the lipid composition of the membrane [35]; (ii) other thyroid hormone analogues had greater or equal activity than T_3, e.g., thyroxine at the erythrocyte plasma membrane [25], T_4 or T_2 at the hepatocyte membrane [73]; (iii) whereas calcium influx was stimulated in a concentration range of thyroid hormone of 1 nM [135] to 1 μM [73], higher concentrations were ineffective or inhibitory, an observation typical for a molecule interacting with lipid bilayers; (iv) T_3 increases the plasma membrane potential in isolated perfused livers when it is administered at rather high (1 μM) concentrations, whereas plasma membrane potential is not increased in hyperthyroid rats [148] despite an induction of Na^+/K^+-ATPase [149]; (v) finally, when comparing the kinetics of calcium uptake into the liver cell induced by T_3, glucagon or vasopressin, the kinetics elicited by T_3 are distinctly different; it has a longer time of onset, is not biphasic and lasts longer [73]. It is suggested that T_3 does not mobilise intracellular calcium through a receptor-mediated process but induces only an influx, by directly interacting with a membrane protein. A direct interaction of thyroid hormones with plasma membrane proteins is also supported by the observed stimulation of sugar transport in rat thymocytes [138] and amino-acid uptake in liver [98].

An additional possibility of interaction of this hormone with the plasma membrane was suggested by the observation that stimulation of sugar uptake in cultured heart cells by picomolar concentrations of T_3 was insensitive to protein synthesis inhibitors, but sensitive to cytochalasin B [39], speculating that thyroid hormones like insulin, could initiate the translocation of glucose transporter molecules to the plasma membrane.

II-C.2. Action at the mitochondrial membrane

Thyroid hormones are taken up into the cell so that it is feasible that they interact directly with the mitochondrial inner membrane and influence mitochondrial energy transfer. High-affinity mitochondrial binding sites for thyroid hormones, especially L-3,3′-T_2 and T_3, have been found, but not related to a physiologic function of the mitochondria. Several authors have reported that T_2 is most effective at the mitochondrial site: a direct stimulation of respiration by T_2, but not T_3, in isolated mitochondria from hypothyroid rats was demonstrated [70]. Since a specific binding of thyroid hormones to mitochondria was not proven (see subsection II-A.3), the effects may be due to some non-specific interaction of diiodothyronines with mitochondrial membrane proteins and lipids.

Two papers [51,161] report ADP-ribosylation of a single inner membrane protein with a molecular mass of 11 kDa in the presence of 0.1 nM T_3. ADP-ribosylation is inhibited by nicotinamide [161]. The protein has not been identified and its function has not been characterised. Protein phosphorylation studies in isolated hepatocytes show phosphorylation of pyruvate kinase and phosphorylase with 1 μM T_3, but no phosphorylation of mitochondrial proteins [146].

Modification of mitochondrial membrane properties by fast-acting hormones, such as glucagon or α-adrenergic agonists, has been heavily debated, but rejected due to lack of identification of modified mitochondrial membrane proteins. Therefore, all mitochondrial effects elicited by hormones like glucagon and α-adrenergic agonists have been explained as a consequence of an increase in cytosolic calcium followed by a rise in mitochondrial free calcium [27]. As a result calcium

sensitive mitochondrial dehydrogenases are activated thus stimulating mitochondrial metabolism by an increase in substrate supply. Since T_3 also enhances cellular calcium uptake, the same mitochondrial signalling pathway may be true for this hormone.

III. Thyroid hormone action on mitochondrial energy transfer

Following the description of intracellular signalling pathways used by thyroid hormones, this Section will discuss the mode of thyroid hormone action on mitochondrial energy transfer. It might be influenced (i) by changing overall energy expenditure; (ii) by changing mitochondrial energy-generating capacity (protein synthesis); (iii) by enhancing the driving forces for energy consuming and generating pathways.

III-A. Long-term and short-term changes in extramitochondrial energy expenditure

Many effects of thyroid hormones are on energy requiring processes, e.g., growth, maturation, stimulation of carbohydrate and lipid metabolism and of ion transport, including renal regulation of mineral metabolism (for reviews, see Refs. 18, 101). A prominent energy requiring processes is the stimulation of gluconeogenesis in liver and kidney both in the long and short-term range [93,98,99]. Gluconeogenesis is enhanced either by increased substrate supply, e.g., transport of gluconeogenic amino acids [30,157], or induction of glucogenic enzymes [101]. It has also been debated as to whether a higher supply of ATP in the presence of T_3 drives gluconeogenesis. However, despite a possible increase in the rate of ATP-synthesis and transport (see below), the cytosolic phosphorylation potential of ATP was unchanged [78,139] or even decreased in livers from hyperthyroid rats [131]. On the other hand, if the rate of ATP supply is the limiting factor, oxidative phosphorylation could well exert control on biosyntheses in different thyroid states.

In addition, gluconeogenesis in the short-term range is probably increased by inhibition of pyruvate kinase via calcium-dependent phosphorylation of pyruvate kinase [101,146].

In hyperthyroidism, both catabolic and anabolic processes are accelerated, resulting in a loss of energy and generation of heat from 'futile cycles', especially in carbohydrate metabolism, but also in lipid and protein metabolism. The recycling of substrates can be responsible for as much as 30–40% of additional oxygen consumption in hyperthyroid mammals [140].

A stimulation of renal sodium transport via the Na^+/H^+-exchanger or Na^+/K^+-ATPase [18] and of Na^+/K^+-ATPase in liver, heart and skeletal muscle [144,149] are considered to be partly responsible for the higher oxygen uptake in the hyperthyroid state. The energy expenditure by these enzymes was reported to account for 50% in the transition from the hypo to the euthyroid state and for 80% from the euthyroid to the hyperthyroid state [144]. Renal sodium transport accounts for 10% of the overall oxygen consumption in the human tissue [18].

Reports differ between 5 and 90% of total cellular oxygen consumption to account for Na^+/K^+-ATPase stimulation in hyperthyroid tissues [101]. The contradictory data relating to the energy expenditure by the plasma membrane Na^+/K^+-ATPase may be explained by experimental difficulties when working with cells or with tissue slices from hyperthyroid animals. Hyperthyroidism leads to greater changes in membrane permeability in artificial systems, which are probably compensated by the Na^+/K^+ pump. Another important reason that estimates of the influence of Na^+/K^+-ATPase were too high was the use of ouabain for the assessment whithout taking into account that it caused a collapse of K^+ and Na^+-gradients in the cells and slices. Therefore, depending on the experimental model, artificial high or low rates of Na^+/K^+-ATPase can be measured [101]. Nevertheless, a considerable share of the extra oxygen consumption is at this step, although it cannot account for all the observed increase in oxygen consumption. The fact that isolated mitochondria from hyperthyroid rats show an at least two-fold stimulation of respiration compared to isolated mitochondria from euthyroid rats (see subsection III-B.3), indicates that, in addition to the increase in extramitochondrial energy turnover, there must be a direct influence on mitochondrial properties in higher thyroid states, leading to an increase in respiration.

Finally, the thyroid-hormone-mediated stimulation of catabolism, such as β-oxidation and glycolysis [101,169] enhances substrate supply to the mitochondrial respiratory chain which could enhance mitochondrial energy production.

III-B. Long-term changes in mitochondrial properties

III-B.1. Lipid composition of the membrane

Thyroid hormones influence lipid metabolism [83,150] by induction of enzymes involved in lipid biosynthesis and degradation, and therefore alter the lipid composition of membranes. As early as 1968 it was suggested that the increase in sensitivity to uncouplers in hyperthyroidism may be due to the altered lipid composition of the mitochondrial membrane [59]. Hyperthyroidism increases microsomal fatty-acid synthesis and desaturation by a stimulation of acetyl-CoA carboxylase and fatty-acid synthase [83], a stimulation of carnitine palmitoyltransferase [126] and mitochondrial cardiolipin synthase [71]. In isolated hepatocytes stimulation of lipogenesis has been measured as early as 4 h after T_3-addition to monolayer cultures [37]. The increase in lipid unsaturation, membrane phase transi-

tion and the activation energy for succinate dehydrogenase was reversed in hypothyroid rats following thyroxine treatment [167]. More recent investigations show an increase in mitochondrial cholesterol and a decrease in phospholipids (increase in the ratio of cholesterol to phospholipids by about 40% and a 30% decrease in cardiolipin in hypothyroid rat liver [115]). Steady state fluorescence anisotropy of membrane probes revealed an increase in membrane order with increasing hormone level in mitochondrial membranes from rat liver with a concomitant increase in mitochondrial L-glycerol-3-phosphate dehydrogenase activity, and digitonin subfractionation showed an alteration in protein arrangement in the membrane [10].

The change in lipid composition of the mitochondrial membrane is also believed to alter the transport properties of mitochondrial carrier proteins. This was suggested already in the seventies for the adenine nucleotide translocase, where an increase in ADP uptake in isolated mitochondria from euthyroid compared to hypothyroid rat livers was observed. It was concluded that the activity change was not due to an increase in carrier molecules but the consequence of changes in the lipid composition of the inner mitochondrial membrane creating changes in the microenviroment of membrane proteins [59]. Later studies revealed that the K_m for external ADP is decreased and the V_{max} of the carrier is increased in hyperthyroid liver mitochondria and that both parameters are temperature-dependent [91]. Further, the sensitivity of the translocase to the classical inhibitors was enhanced and the fatty-acid content of the membrane was elevated.

On the other hand, in another study [9], the decrease in adenine nucleotide transport activity due to hypothyroidism was compared for liver, kidney, brain and testis: According to their report, adenine nucleotide transport activity decreased only in livers by 40%, whereas Na^+/K^+-ATPase activity decreased in all organs, except testis. However, the activities were referred to protein and, since organ mass changes differently from mitochondrial mass in different organs, this could mask true activity changes [46,94], also in the other organs.

The increase in carrier activity in hyperthyroid mitochondria is not only observed for the adenine nucleotide translocase, but also for other mitochondrial carriers. The group of Paradies showed an enhanced transport rate for the mitochondrial citrate [113], pyruvate [112] and phosphate carrier [115] and referred this to changes in the lipid composition of the inner mitochondrial membrane due to thyroid hormones.

III-B.2. Mitochondrial capacities and activities of electron carriers and enzymes

It has long been known that thyroid hormones regulate the biogenesis of mitochondria by increasing protein synthesis [17,168], including synthesis of mitochondrial respiratory chain proteins [33,106,160]. For the regulation of transcription and translation of mitochondrial proteins, see subsection II-B.1.

Biosynthesis of mitochondrial electron chain transport proteins. The induction of electron chain proteins appears to be a relatively late event in the long-term actions of thyroid hormones: cytochrome *c* mRNA levels in hypothyroid rat liver and kidney start to increase after 12 h and are highest between 24 and 48 h following administration of T_3 [130]. The increase is considerably higher in liver than in kidney. In a recent study [87] it was reported that different mitochondrial energy transfer proteins are expressed at different times after T_3-injection, e.g., cytochrome c_1 mRNA appears earlier whereas ATPase DCCD-binding protein mRNA later. Van Itallie [165] showed that the mRNA for subunit II of cytochrome oxidase, a mitochondrially-encoded protein, is increased in a hepatoma cell line cultured with thyroid hormone, but not the mRNA for nuclear encoded subunits. Further, an elevation in mitochondrial ATPase activity [24,76] and synthesis of β-subunit [76,104] appears to be induced by thyroid hormones.

In line with a role for thyroid hormone in the regulation of the efficiency of energy conversion is that thyroid hormone controls the transcription of uncoupling protein mRNA in brown adipocytes [11].

An overview of the effects of T_3 on the activities of all cytochromes in livers from thyroidectomised rats was given by Bronk [17], who showed that the increase was maximal after 48 h but already detectable after 3 h. However, no significant increase in the amount of cytochrome contents was observed, in contrast to other reports [3,130]. Furthermore, increases in the activities of cytochrome oxidase in rat liver [3,17] and in electron transport activity through the cytochrome bc_1 complex (due to an increase in ubichinon content), by thyroid hormones, have been reported [66,68].

Other effects on mitochondrial metabolism. The data relating to a regulation of the activity of ANT at the transcriptional level are controversial. Refs. 62 and 124 do not find an increase in the level of ANT mRNA in livers, kidney and heart from hyperthyroid rats. Contrary to these results evidence for an induction of mitochondrial ANT is presented in Refs. 87 and 88 when regarding the different subtypes of this protein: during development, multiple types of AdN-translocase genes (*T1–T3*) are differentially expressed [88]; in human muscle T_3-specific genes are expressed in the growing myoblast, T_2-specific genes in the intermediate phase, whereas T_1-specific genes are only expressed in the mature muscle. On the other hand, in mature rat livers only an increase in the transcription of the T_2-specific gene by thyroid hormones was reported [87]. It thus appears that for the mitochondrial adenine nu-

cleotide translocase, carrier activities might change in different thyroid states due to both changes in lipid composition of the membrane and in protein synthesis.

Likewise, a stimulation of mitochondrial glycerolphosphate dehydrogenase activity at the transcriptional level in the hyperthyroid state is debated. Clay and Ragan [21] found, by in-vitro translation, an increase of mitochondrial glycerolphosphate dehydrogenase mRNA in hypothyroid rat liver, in contrast to the finding that the activity of the enzyme was considerably higher in eu- and hyperthyroid rat livers (Ref. 85, see also subsection III-B.1). They suggested that it is the incorporation of the flavin moiety into the enzyme which is diminished in hypothyroid liver probably due to a restricted availability of riboflavin. In this context, a paper [90] is of interest, which shows that thyroxine regulates NADH-dehydrogenase and succinate dehydrogenase activities, both flavoenzymes.

The increase of mitochondrial capacities and activities, however, is not sufficient for a higher energy output as long as the substrate supply to the mitochondria is not enhanced. In hyperthyroid mitochondria, the calcium content is higher than in euthyroid mitochondria [43]. In line with this finding a higher thyroid state corresponded with higher mitochondrial contents of dicarboxylic acids and glutamate in rat liver [147], indicating a stimulation of citric acid cycle and mitochondrial substrate supply.

Taken together, it is concluded that control of mitochondrial biogenesis by thyroid hormones is exerted at the transcriptional and translational level. It has broad time scales and organ specifity. This might explain the partially contradictory nature of the data. In addition, increase in activity does not necessarily correlate with an increased amount of enzyme but is partially due to membrane–protein interactions (see subsection III-B.1). Notwithstanding, the present evidence supports an increase in the overall capacity of mitochondrial electron transport chain proteins by thyroid hormones. Other indirect effects of thyroid hormone, e.g., increase in mitochondrial calcium and other mitochondrial metabolites are also responsible for the activation of mitochondrial metabolism.

III-B.3. Respiration and protonmotive force

A wealth of evidence has been presented that the thyroid state of an organ determines the activity of mitochondrial metabolite transport and citric-acid cycle and the capacity and activity of mitochondrial oxidative phosphorylation (see previous subsection). This is primarily reflected in a higher respiratory rate especially in liver [58,61,90,101], heart [107] and kidney [18]. The stimulation of oxygen uptake is observed not only in intact organs but also in isolated cells, e.g., hepatocytes [56], and in isolated mitochondria (e.g., Refs. 58, 142); it is not due to uncoupling of the respiratory chain despite the fact that high doses ($> 10^{-5}$ M) uncouple isolated mitochondria [95,159]. In perfused liver, it was shown that the increase in oxygen consumption elicited by 1 μM T_3 is sensitive to oligomycin [98]; in the same studies glucose, as well as urea production from alanine were stimulated, a strong argument against an inhibition of mitochondrial energy production by thyroid hormones.

Although a stimulation of respiration does not necessarily mean an increased protonmotive force (pmf) this was postulated already in 1979 by Shears and Bronk [142]. A higher pmf is indicated from findings in several recent papers. The membrane potential was monitored in liver submitochondrial particles from animals in different thyroid states using a fluorescent indicator [90]. Succinate or NADH addition yielded a higher fluorescence in livers from euthyroid animals than in livers from hypothyroid animals, indicating a higher mitochondrial membrane potential in the hyperthyroid state. Horrum et al. [69] measured pmf in isolated liver mitochondria at state 4 in different thyroid states and found an increase in pmf in the hyperthyroid group due to an increase in the proton gradient and the electrical potential across the mitochondrial membrane. These findings were confirmed for intact livers from rats in different thyroid states using fractionation of intact tissue in non-aqueous solvents [147]. Both groups used the distribution of labeled $TPMP^+$, a lipophilic cation, for the determination of the electrical potential and calculated from the distribution of labeled weak acids the proton gradient across the mitochondrial membrane. However, with the same technique, Paradies and Ruggiero [114] could not detect any change in the mitochondrial/cytosolic proton gradient in isolated mitochondria from hyperthyroid rat hearts; the membrane potential was not measured. Crespo-Armas and Mowbray [23] did not find an increased pmf in state-4 mitochondria from euthyroid compared to hyperthyroid rat measuring both membrane potential and proton gradient with marker distribution methods. Even a decrease in the membrane potential was found with higher thyroid state using isolated hepatocytes [40] and in isolated mitochondria (e.g., Ref. 49). It should be pointed out, however, that the differences in membrane potential and proton gradient measured between hypo- and euthyroid animals [69,147] are relatively small and become only significant in the hyperthyroid state, so that small experimental differences might overlay thyroid hormone effects. Further, it is perhaps difficult to compare data on this parameters in isolated mitochondria, hepatocytes and intact organs in different metabolic states using different substrates and inhbitors.

Thus, on the basis of the present evidence, it is not possible to decide whether there is an increase in protonmotive force in higher thyroid states in the in-

tact cell. Even a slight increase may, however, have considerable impact as driving force for mitochondrial substrate supply, since the mitochondrial/cytosolic metabolite distribution is exponentially correlated with the changes in the mitochondrial membrane potential and proton gradient [147]. However, a clear correlation between driving force and transport activity (e.g., mitochondrial/cytosolic proton gradient and pyruvate carrier activity [112]) or between driving force and subcellular metabolite distribution (e.g., the membrane potential and the mitochondrial/cytosolic distribution of aspartate, glutamate [147]) could not be demonstrated.

III-B.4. Long-term thyroid hormone control of mitochondrial energy conversion

Since the increase in respiration is not only observed in intact organs and cells, but also in isolated mitochondria, enhanced overall energy expenditure cannot account solely for the higher respiratory rate. As stated in subsections III-B.1–3, a direct change in mitochondrial properties has to be taken into consideration. It appears reasonable that the increase in mitochondrial capacities, especially in the respiratory chain components, leads to a higher respiration rate. On the other hand, several observations favour a more complex mode of action because (i) the induction of mitochondrial electron chain proteins does not occur at the same time but during a long period ranging from 3 h to 1 week; the levels of several proteins that go up in the earlier period go back to normal after 1 week, e.g., the β-subunit of mitochondrial ATPase [87]. The stimulation of respiration, however, is observed within 1 h and lasts during the life time of the thyroid hormone. (ii) In many cases it is not the amount of protein which is higher, but the measured activity, e.g., adenine nucleotide translocase T1 and other mitochondrial carriers, or the activity of the cytochrome oxidase. Thus, the mechanisms of the changes are, at least in part, of a more indirect nature.

Kinetic, as well as thermodynamic studies indicate that this suggestion is real: in principle all these studies examine the properties of electron transport proteins, mitochondrial ATPase and adenine nucleotide translocase, involved in oxidative phosphorylation, in different thyroid states. The kinetic studies comprise inhibitor titrations of respiratory and phosphorylating activities in isolated mitochondria and cells, whereas the thermodynamic studies measure proton permeabilities in state 4, state 3 and in uncoupled mitochondria, as well as P/O and H^+/O ratios, and control coefficients for the various steps in oxidative phosphorylation.

In state-4 mitochondria thyroid hormones increase the proton permeability across the inner membrane and, thus, state-4 respiration [14,67,142,166]. Also, the permeability to K^+ is enhanced [48,142]. The majority of the investigators in the field more or less ascribe the changes in membrane permeability to protons to an altered membrane lipid composition in higher thyroid states. Thus, the proton permeability of liposomes prepared from phospholipids extracted from inner membranes of hyperthyroid mitochondria was found to be higher than in liposomes prepared from hypothyroid mitochondria [14]. Further, isolated cytochrome oxidase from hypo-, eu- and hyperthyroid mitochondria, reconstituted into the liposomes, all showed identical proton current/voltage curves, indicating that the hormonal effects were not an inherent change in the properties of the enzyme.

Another interesting possiblity raises the finding that thyroid hormones in vivo increase the sensitivity of the mitochondria to cytoplasmic, low-molecular-mass, water-soluble proteins which mediate uncoupling of oxidative phosphorylation by inducing the transport of sodium and protons into the mitochondria [34].

Taken together, evidence for a change in mitochondrial inner membrane proton permeability is strong. Most authors ascribe this increase in proton leak to the change in the membrane lipid composition in higher thyroid states. However, this proton leak is more than compensated for by the higher capacity of oxidative phosphorylation.

In state-3 mitochondria the share of proton leak to the overall respiratory increase is rather low. Here it appears to be only a minor factor responsible for increased respiration. During state-3 respiration, a high turnover of the respiratory chain proton pumps increases 'intrinsic uncoupling' ('slipping') of the proton pumps. In mitochondria from T_3-treated rats increases in the ratio of respiration/pmf and upwards shifts of flow-force relationships (respiration or ATPase vs. pmf) were found using inhibitor titrations of the various proton pumps. By comparing the experimental results with computer simulations on the basis of a chemiosmotic model of energy transduction, it was concluded that T_3 increases intrinsic uncoupling, especially at the cytochrome oxidase and ATPase step [89]. If this is true, H^+/O and P/O-ratios should be decreased in hyperthyroid mitochondria. However, contradictory results have been raised with regard to these values: H^+/O, as well as the P/O ratios were the same in all thyroid states [47] using rat liver mitochondria. In another study, using intact heart [141], the fluxes through ATP-synthase measured with ^{31}P-NMR, and oxygen uptake were used for the calculation of the P/O ratio. A lower ratio was determined in hypothyroid organs; however, the values were not corrected for the substrate level phosphorylation by glycolysis which has been shown to be higher in hyperthyroid rats [169] so that the difference in P/O ratios between hypo- and hyperthyroid state could be an artifact. In contrast, a higher P/O ratio was determined in liver mitochondria, isolated 15 min after treatment of rats with

T_3 [23]. This probably reflects a short-term, extranuclear effect. Thus, the contradictory results do not allow a decisive conclusion on P/O or H^+/O ratios. This might suggest that slipping is not very strong in hyperthyroid states probably because the increase in activity of the pumps is more due to an increase in capacity than due to indirect effects, so that the turnover of a single molecule is not increased.

In spite of the higher activity of cytochromes in the hyperthyroid state (see subsection III-B.2) titration with respiratory chain inhibitors had the same effect on respiration in hypo- and hyperthyroid state-3 mitochondria [49] and hepatocytes [41]. This may be because the respiratory chain components have insignificant flux control over state-3 respiration rate [49]. These experiments suggest a stimulation by thyroid hormones at the ATP-synthase and adenine nucleotide translocase step [41,49]: experimental data for a higher ADP-uptake in mitochondria from higher thyroid states [8,60,97] were confirmed in isolated state-3 mitochondria [49] and hepatocytes [41] where a higher activity of ANT was measured with inhibitor titration experiments. In addition, in mitochondria from T_3-treated animals the translocator was shown to operate at higher extramitochondrial ATP/ADP ratios from which it was concluded that the translocase is more active in the higher thyroid state [64,166]. This is in line with our findings in intact hyperthyroid rat livers [139] where the cytosolic ATP/ADP ratio was also found to be increased (and mitochondrial ratios to be decreased), indicating a higher translocation activity. Regarding oxidative phosphorylation, the flux control coefficient for ANT, however, was found to be lower in hypothyroidism [64,166] and control was restored by T_3-treatment in isolated mitochondria without increasing the respiration [166]. This shows that other steps in oxidative phosphorylation than the translocation of ATP are even more increased in the hyperthyroid state, which, accordingly, could well be ATP synthesis [49,67,89].

III-B.5. Summary

Table I summarises the changes of mitochondrial parameters in the hyperthyroid state. Taken together these findings lend support to the concept that the stimulation of respiration in isolated mitochondria in higher thyroid states is due to changes in mitochondrial ATP-synthesis and transport. The higher capacity of respiratory chain components in higher thyroid states has no control over the rate of respiration because these steps are normally not limiting in oxidative phosphorylation. Whereas thyroid hormones decrease the efficiency of mitochondrial ATP-synthesis due to intrinsic uncoupling [89], this slightly lower efficiency of phosphorylation is more than compensated by a higher capacity and activity of ATP synthase, so that overall ATP supply is enhanced by thyroid hormones. 'Intrinsic uncoupling' of the ATP-synthase reaction in hyperthyroid mitochondria is the consequence of the higher turnover of this enzyme which, however, might allow the observed increase in ATP-export, i.e., a higher rate of ATP supply for cytosolic biosyntheses (see above), in the presence of a reasonably high cytosolic phosphorylation potential [145]. The concerted action of the altered membrane properties, modulating the activity of membrane proteins, e.g., electron transport chain and metabolite carriers, together with induction of synthesis of new proteins of cellular catabolic pathways and the energy-transfer system, increasing the capacity, lead to a higher substrate supply to the mitochondria and an increase in energy production for the cell and cellular biosynthetic output. Due to the increased ATP-turnover, slipping of proton pumps and proton leak, hyperthyroidism is accompanied by thermogenesis, an effect relevant during cold adaptation.

TABLE I

Changes in mitochondrial parameters in the hyperthyroid state

References are in brackets. –, no change; ↑, increase; ↓, decrease.

Changes in the lipid composition of the inner membrane	[10,59,71,83,112–115,126,167]
Increase in electron transport chain activities	[3,17,24,66,68,76]
Increase in mitochondrial enzyme activities	
Glycerolphosphate dehydrogenase	[21,85]
Succinate dehydrogenase, NADH-dehydrogenase	[90]
Increase in mitochondrial carrier activities	
Adenine nucleotide translocase	[9,59,87,88,91]
Other carriers	[112–115]
Uncoupling protein in brown adipocytes	[11]
Increase in respiration	
Liver	[56,61,90,101]
Hepatocytes	[56]
Liver mitochondria	[58,142]
Heart	[107]
Kidney	[18]
Change in pmf	[69↑,142↑,147↑,23–]
Change in ΔpH_{mit}	[23–,114–,147↑]
Change in mitochondrial membrane potential	[69↑,90↑,147↑,40↓]
Increase in proton leak	[14,67,142,166]
Increase in K^+-permeability	[48,142]
Slip in H^+-ATPase	[89]
Change in H^+/O ratio	[47–]
Change in P/O ratio	[4–,141↓]

III-C. Short-term changes in mitochondrial ATP-generation

III-C.1. Oxidative phosphorylation

Since oxidative phosphorylation is uncoupled by nitrophenols and halophenols, the uncoupling effect of the physiological halophenol thyroxine, at high doses, has been investigated and demonstrated in isolated mitochondria already in the 1950s [57,95,159]. Later, it was postulated that thyroid hormones have also a physiologic action in stimulating oxidative phosphorylation directly within minutes (for a recent review, see Ref. 156). High-affinity binding sites for T_3 at the mitochondrial level were demonstrated; the binding protein was characterised and a direct stimulation of oxygen uptake in isolated liver mitochondria by doses of T_3 below the uncoupling range were reported [152]. However, the studies on the binding sites lack functional tests and for the stimulation of oxygen uptake, only one trace was presented. From that moment on, little evidence was presented in favour of a direct stimulation of oxygen uptake in isolated mitochondria. Bronk [16] could enhance oxidation rate and P/O ratio only in submitochondrial particles from rat liver by physiological doses (10-times below uncoupling concentrations) of thyroxine or T_3. A recent investigation [70] showed a stimulation of oxygen uptake with T_2 but not with T_3 in state-3 mitochondria, pointing to a possible importance of this analog in the fast mitochondrial pathway (see below). On the other hand, whereas convincing evidence for a direct action on isolated mitochondria is still lacking, several groups report a fast-term stimulation of oxygen uptake (within minutes to 1 h) in intact cells and organs. The stimulation of respiration has been observed in the presence of protein synthesis inhibitors in mitochondria isolated from livers of thyroidectomised rats, treated with T_3 for 20 min [15], in perfused euthyroid rat livers within 1 min after infusion of T_3 or T_2 [73], but not with T_3 + PTU [70], in isolated hepatocytes from hypothyroid or euthyroid rats after 3 min of incubation with T_3 [56], in mitochondria isolated from rats within minutes after T_2, but not T_3 + PTU treatment [110], and in mononuclear human blood cells within minutes of T_4, or T_2 application [81]. It is, thus, reasonable to conclude that increase in respiration is a true effect of the short-term action of thyroid hormones, probably with higher specifity for T_2. Since no data on serum or tissue T_2 are available, the physiological role of T_2 remains open.

Little evidence is available on the short-term influence of thyroid hormones on the mitochondrial protonmotive force. A short paper [94] reports that the proton-pumping activity of submitochondrial particles from rat liver was increased by very low concentrations of T_4 and T_3 (10^{-11} and 10^{-13} M) from 50% (70%) to 130% (170%) of control activity, respectively. This fits with the findings reported in Ref. 148 in perfused rat liver, where an increase in pmf, resulting solely from an increase in the proton gradient, but not the mitochondrial membrane potential, was found after infusion of T_3. Both findings are in agreement with studies using liver mitochondria from thyroidectomised rats pretreated with T_3 15 min prior to isolation of mitochondria [23], where an increased efficiency of proton pumping resulting in a higher mitochondrial proton gradient and H^+/O ratio was found, but no change in the membrane potential.

T_3 infusion into isolated rat livers for 1 h decreased the mitochondrial ATP/ADP ratio considerably at a constant cytosolic ratio in the presence of a high gluconeogenic rate [139]. Therefore, it was suggested that mitochondrial adenine nucleotide transport is stimulated as in euthyroid and hyperthyroid rat livers. On the other hand, as in the hyperthyroid state, control strength for adenine nucleotide translocation is increased rather than decreased in liver mitochondria from euthyroid rats pretreated for 15 min with T_3 [64]. Further, binding of thyroid hormone to the adenine nucleotide translocase, postulated by Sterling [155] was not confirmed by Rasmussen et al. [124]. Therefore, a short-term, direct stimulation of mitochondrial adenine nucleotide translocation by thyroid hormone is not supported by the available evidence. An indirect effect, i.e., by the altered proton gradient, cannot be ruled out.

In Table II, the influence of thyroid hormones on mitochondrial parameters via an extranuclear action is

TABLE II

Immediate changes in mitochondrial parameters induced by thyroid hormones

Effect	Agonist	Reference
Increase in respiration		
Perfused rat liver	T_3, T_4, T_2, not T_3 + PTU	70, 73, 110
Hepatocytes	T_3	56
Rat adipocytes	T_3	134
Mononuclear blood cells	T_4, T_2	81
Liver mitochondria	T_3, T_2 not T_3 *	15, 152, 70 *
Increase in proton pumping activity		
Liver submitochondrial particles	T_4, T_3	16, 94
Increase in ΔpH_{mit}		
Perfused rat liver	T_3	148
Increase in P/O ratio		
Liver submitochondrial particles	T_4, T_3	16
Liver mitochondria	T_3	23
Increase in H^+/O ratio		
Liver mitochondria	T_3	23

summarised. On the basis of these findings a mechanism is suggested by which thyroid hormones directly could regulate mitochondrial energy transfer.

III-C.2. Short-term thyroid hormone control of mitochondrial energy conversion

For a long time, the short-term interactions of thyroid hormones with cellular energy metabolism have been a rather exotic field, investigated only by few research groups. In the last ten years, however, evidence has accumulated, that short-term stimulation of energy metabolism is real and of importance in the range of actions exerted by thyroid hormones. The first unequivocal evidence for an extranuclear action of thyroid hormones came from studies with erythrocytes that do not have a nucleus, showing a stimulation of Ca^{2+}-transporting systems. This has been confirmed also for other cells which contain a nucleus like liver, heart and kidney cells. With the exception of the effects of T_2 directly on mitochondria, practically all the available data can be explained on the basis of a lipophilic interaction of thyroid hormones ($T_2 > T_3 > T_4$, but not D-T_3) with the plasma membrane followed by a permeability change for calcium. Calcium itself or by an activation of adenylate cyclase (in the case of thymocytes) could then initiate all the changes observed in energy metabolism within the cell (see above). A subsequent rise in mitochondrial calcium would activate mitochondrial metabolism. Another possibility could be that the rise in cytosolic calcium induces ADP-ribosylation of an inner membrane protein as suggested in Refs. 51 and 161, and thereby change the permeability properties of the inner membrane.

The binding of thyroid hormones to (one or more) mitochondrial sites, however, is ambiguous; competition experiments did not provide clear-cut evidence, for the mitochondrial binding sites to be specific. A definite relationship between hormone binding and its effect is not established.

Of interest are investigations indicating that T_2 might be the one hormone analog which is most effective at the mitochondrial site: (i) Cytochrome oxidase activity is restored in hypothyroid rat liver faster with T_2 than with T_3 [84]. (ii) Stimulation of state-3 respiration in isolated mitochondria only with T_2, but not with T_3 was found [70]. In addition, several groups (see subsection III-C.1) observed that respiration is only stimulated by T_3, if it is applied to the intact cell so that the cellular deiodinase can convert T_3 to T_2. PTU, an inhibitor of the cellular deiodinase abolishes the stimulation of respiration. (iii) In human mononuclear blood cells T_2 stimulated respiration, but not glucose uptake, suggesting a specific role at the mitochondrial site for T_2 [81]. Thus, T_3 would be responsible for the nuclear actions of thyroxine, and L-T_2 for the mitochondrial short-term actions [84].

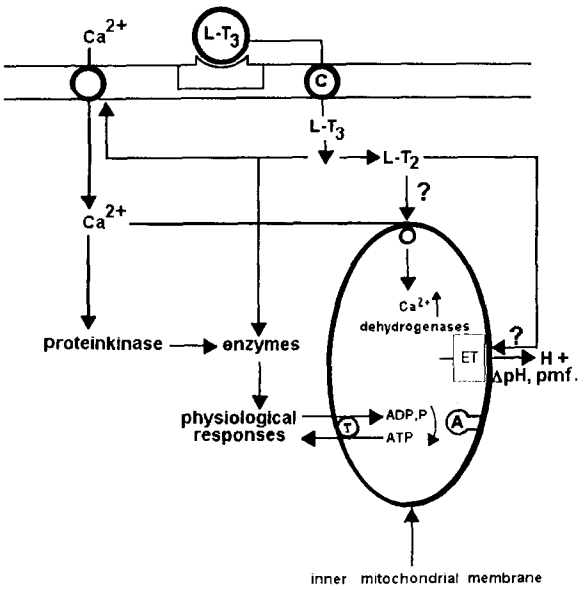

Fig. 3. Extranuclear action of thyroid hormones on mitochondrial energy transfer. C, plasma membrane transport system for T_3; A, mitochondrial ATPsynthase; T, adenine nucleotide translocase; ET, electron transport chain.

The mode of action of L-T_2 at the mitochondrial site is not investigated. Increase of proton leak is not probable since the proton gradient is increased [94,148]. An attractive hypothesis would be a direct interaction of T_2 with a mitochondrial calcium transporting system similar to an interaction of thyroid hormones with calcium transporting systems in the plasma membrane. Changes in the permeability of the inner membrane for calcium, followed by an activation of mitochondrial dehydrogenases and of respiration, could be responsible for increases not only in the mitochondrial proton gradient [44] and pmf but, as a consequence, mitochondrial metabolite transport and mitochondrial substrate supply.

Supporting data for this hypothesis were also reported by Thomas et al. [162], showing an influence of nanomolar calcium on mitochondrial P/O ratio, and from Corrigal and Mowbray [22], showing that the mitochondrial oxidation rate is increased and pyridine nucleotide fluorescene decreased in hypothyroid mitochondria from homogenates preincubated for 15 min with T_3.

Fig. 3 summarises the possible extranuclear mechanisms of thyroid hormone action on mitochondrial energy transfer based on the available evidence. Further studies, especially dealing with the role of T_2, are needed.

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