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Review Letter

Thyroid hormone uptake into the cell and its subsequent localisation to the mitochondria

Roderick P. Hafner

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, England

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The nature of thyroid hormone uptake into the cell and the possible involvement of the serum carrier proteins and receptor-mediated endocytosis in this process are reviewed. The evidence that there is a specific thyroid hormone-binding receptor in the inner mitochondrial membrane and the relation of this to the adenine nucleotide translocator is discussed. Direct effects of thyroid hormone on mitochondrial function that might be mediated by such a receptor are also considered.

Thyroid hormone; Active transport; Mitochondria; Receptor; Adenine nucleotide translocator

1. INTRODUCTION

The transport of the thyroid hormones 3,5,3′-triiodo-L-thyronine and L-thyroxine in the blood bound to albumin and thyroid binding globulin (human) or serum prealbumin (rat) is well known. Recently it has become clear that the uptake of thyroid hormone into cells is carrier mediated and that cells are able to maintain a free T_3 concentration greater than plasma. This has important implications for the control of whole body metabolism. It has been assumed that basal metabolism is largely controlled via a classical feedback inhibition between the thyroid and the hypothalamus/pituitary to control the free T_3 concentration, that in turn determined the basal metabolic rate of the organism. If the cell can control its own level of T_3 this view becomes too simplistic to be of much value. The mitochondria have also been suggested to be a site of direct T_3 action. The ability of the cell to regulate its own free T_3 concentration could provide a means for the cell to control the efficiency of mitochondrial respiration. It is therefore important to characterise the mechanism of T_3 uptake into the cell and the possible direct effects of this process on mitochondrial function, as this will place constraints on future models of the control of basal metabolism.

Correspondence address: R.P. Hafner, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, England

Abbreviations: T_3, 3,5,3′-triiodo-L-thyronine; T_4, L-thyroxine

2. UPTAKE OF THYROID HORMONE INTO THE CELL

The inability of T_3 to cross the plasma membrane because of the functional pore size of the membrane as well as the charged nature of T_3 at physiological pH led Rao et al. [1] to investigate the translocation of thyroid hormone into isolated rat liver cells. They presented the first evidence that this process was carrier mediated and that the free T_3 concentration in the cell is greater than that

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in the plasma [1]. Since then evidence has also been presented for a carrier-mediated uptake of thyroid hormone into isolated rat adipocytes [2], cultured mouse fibroblasts [3] and human red cell ghosts [4]. The finding that 30 out of 32 tissues in the rat exhibit a tissue-to-plasma gradient of T_3 [5] suggests that an even larger number of tissues may have active T_3 uptake, although the possibility that the tissue-to-plasma gradients in at least some of these sites are a consequence of intracellular binding cannot be excluded.

The uptake system in the rat hepatocyte is probably the best characterised. There appear to be two uptake systems, one high-affinity/low-capacity, and one low-affinity/high-capacity [1] for both T_3 and T_4 [6]. The low-affinity system has been suggested to represent binding to the cell surface as it can be eliminated by washing the cells with a medium containing thyroid binding globulin [6]. Analysis of the mutual inhibition of T_3 and T_4 uptake indicated that the high-affinity transport systems involved for T_3 and T_4 uptake are not the same [6]. These carriers exhibit saturable uptake [1,6] with defined V_{max} and K_m values. The process is energy dependent, and can be inhibited by oligomycin, cyanide and ouabain [6,7]. The ouabain sensitivity indicates that an Na^+ gradient may be important in the uptake of T_3. Further characterisation has indicated that the uptake is likely to be mediated by a glycoprotein. This is based on the sensitivity of the uptake to β-glucosidase, pronase and neuraminidase [7] as well as the inhibition of T_3 uptake by a monoclonal antibody that did not inhibit the Na^+,K^+-ATPase [8]. The uptake into cells has also been shown to be sensitive to 6-*n*-propyl-2-thiouracil and the microtubule-disrupting agents vinblastine and colchicine [9].

2.1. *The role of serum carrier proteins*

Some workers have been unable to identify active T_3 transport across the plasma membrane in isolated hepatocytes [10] while they did observe evidence for a free T_3 concentration difference between plasma and cytosol of kidney, liver and brain in situ [11]. This suggests the possibility of some direct involvement of a plasma component in facilitating active transport into the cell. Serum albumin appears to increase the V_{max} of the high- and low-affinity uptake systems in isolated hepatocytes without altering the K_m of either process [12]. This has been proposed to be due to enhanced diffusion through the unstirred layer at the cell surface [12]. A passive role for albumin in establishing a cytosol-to-plasma free T_3 gradient in vivo has been proposed [13] as a result of the inability of the workers in [10] to observe active transport into isolated hepatocytes while they did observe a tissue-to-plasma gradient in situ [11]. The model suggests that in the (human) hepatic microcirculation, dissociation of T_3 from albumin and thyroid binding globulin is enhanced so that the large pools of T_3 bound to albumin and thyroid binding globulin are available for passive transport into the cell. The free T_3 cytosol-to-plasma bulk phase concentration gradient is proposed to be a consequence of a high local free T_3 concentration in the plasma in the hepatic microcirculation. Presumably this model could also be applied to the reported free T_3 tissue-to-plasma concentration gradients reported in situ for kidney and brain [11]. However, the evidence for active T_3 transport into cells presented by several groups for several tissues [1–9,14] is sufficiently convincing that there is no need to invoke such enhanced diffusional models.

Is there any evidence for a more direct role of albumin in the transport of T_3 into the cell? As far as I am aware there has been no evidence presented that suggests albumin may enter the cell. There have, however, been reports that serum prealbumin (in the rat) or thyroid binding globulin (in man) may be transported into the cell. Evidence has been presented that prealbumin is able to pass through the plasma membrane into the cell [15]. This evidence was based upon monospecific antiserum assays and electron-microscopic radioautography of liver, brain and spleen after the injection of ^{125}I-labelled prealbumin or T_3. These authors have suggested that prealbumin complexed to T_3 can directly penetrate the plasma membrane. In another study [16] it was demonstrated that the T_3-binding protein in human breast adipose tissue is similar to serum thyroid binding globulin with respect to thermal inactivation profiles of binding and sensitivity to thiol group blocking agents. The cytosolic T_3-binding protein was also found to cross-react with a monospecific antiserum to human thyroid binding globulin. This evidence indicates that

thyroid binding globulin (which is synthesised only in liver) may be present in adipose tissue cytosol. Further experiments are required to clarify this situation.

If the uptake of thyroid hormone into the cell is mediated by thyroid binding globulin (or prealbumin in the rat), what is the role of the T_3-binding proteins present on the cell membrane [14]? It is possible that the T_3-binding proteins at the cell surface have no functional role in the uptake of T_3 into the cell. For example, it has been shown that in the red blood cell the activity of the plasma membrane Ca^{2+}-ATPase is controlled directly by T_3 [17,18]. This has also been shown to be true in the myocardium [19]. This enzyme would appear as a T_3-binding protein in T_3-binding assays, but it is not expected to be involved in the transport of T_3 into the cell. Recently the sequence of the membrane-associated thyroid hormone-binding protein from bovine liver has been determined [20]. This protein was shown to be 93% homologous with rat protein disulphide isomerase. Is this a functional relationship? In the absence of assays for uptake the presence of T_3-binding proteins on the plasma membrane does not exclude simultaneous serum carrier protein uptake.

2.2. *The possibility of receptor-mediated endocytosis*

If the carrier proteins are taken up into the cell as suggested by the work cited above, could receptor-mediated endocytosis be involved? Receptor-mediated endocytosis has been indicated in a previous study [21], where a rhodamine derivative of T_3 bound to specific binding sites on the cell surface, that clustered over clathrin coated pits prior to endocytosis. If T_3 entered the cell by receptor-mediated endocytosis it could do so bound to its transport system in a manner analogous to the transferrin iron-uptake system. This might explain the apparent localisation of thyroid binding globulin within (human) breast adipose tissue [16] and serum prealbumin in rat hepatocytes [15]. A receptor-mediated endocytosis of T_3 would also be consistent with the sensitivity of T_3 uptake into cells to the microtubule-disrupting agents vinblastine and colchicine [9]. If receptor-mediated endocytosis is involved then the suggestion that the membrane protein that binds T_3 is protein disulphide isomerase [20] acquires greater significance. This protein is characteristically associated with protein folding. If as has been suggested thyroid binding globulin [16] and prealbumin [15] are able to enter the cytosol, the involvement of this protein might provide a mechanism that allowed entry of the carrier protein into the cell, in a manner analogous to the reverse of secretory protein transport into the endoplasmic reticulum during protein synthesis. However, a receptor-mediated event cannot explain the uptake of T_3 into plasma membrane vesicles, which is not sensitive to microtubule-disrupting agents [9].

3. LOCALISATION OF THYROID HORMONE TO THE MITOCHONDRIA

The mitochondria have received much attention as possible sites of thyroid hormone action, although a convincing demonstration of a direct effect of T_3 on mitochondrial function is lacking. There are many reports in the literature of T_3 rapidly associating with the mitochondria after administration to hypothyroid or euthyroid rats. However, a major discrepancy exists as to whether this association is specific or nonspecific.

Using electron-microscopic radioautography following administration of ^{125}I-T_3 to isolated hepatocytes [22] the mitochondria were identified as the only obvious site of T_3 accumulation during the first 30 min after administration. In another study injection of labelled T_3 into thyroidectomised rats led to a rapid localisation of T_3 to the mitochondria that then decreased over the next 3 h [23]. The interpretation of this study is made more difficult by apparent changes in the so-called 'cytosolic free protein concentration' during the first hour after T_3 administration. The finding that there was no change in the nuclear T_3 content during the first 4 h after T_3 administration in this study is also contrary to currently accepted schemes [10].

3.1. *A thyroid hormone receptor in the inner mitochondrial membrane?*

The question as to whether the distribution of thyroid hormone to the mitochondria is specific or nonspecific has been investigated by several groups. Sterling and co-workers [24,25] have reported a specific T_3-binding activity in the inner

mitochondrial membrane, but no binding to the outer mitochondrial membrane. This binding activity was identified in mitochondria from liver, kidney, myocardium and skeletal muscle, but not in brain, spleen and testis which do not respond to thyroid hormone with increased heat production. The association constants of thyroid hormone analogues to this inner mitochondrial binding activity correlated well with their physiological potency. In another study using gel filtration four T_3-binding activities in the outer mitochondrial membrane and two major T_3-binding activities in the inner mitochondrial membrane have been identified [26]. However, other studies have been interpreted in favour of nonspecific association of thyroid hormone with the mitochondria. For example, in the initial characterisation of the nuclear thyroid hormone receptor [27] under conditions specifically devised to identify thyroid hormone receptors, a rapid binding of ^{125}I-T_3 to mitochondria at 37°C was observed. Because the mitochondrial binding showed similar kinetics at 0°C to those at 37°C while the nuclear binding was completely inhibited at 0°C, it was concluded that the mitochondrial binding was nonspecific. In another study following the localisation of a small dose of labelled T_3 2 min after injection into thyroidectomised rats [28] it was concluded, on the basis of the inability to excess cold T_3 to displace the labelled T_3, that no specific binding site existed for T_3 in the mitochondria. The reason for these apparent discrepancies is not known. In the absence of a good functional assay (see below) for these putative receptors it is difficult to conclude that they have a physiological action.

3.2. *The adenine nucleotide translocator as a thyroid hormone receptor*

Subsequent to the initial reports from Sterling's laboratory of a T_3-binding protein in the inner mitochondrial membrane [24,25], Sterling [29] has suggested that the adenine nucleotide translocator may be the T_3 receptor. This suggestion was based on the co-purification of label with the adenine nucleotide translocator after photoaffinity labelling with ^{125}I-T_3. The lack of demonstration in this stsudy that mitochondrial proteins in general were not labelled makes the result less certain. The long incubation times used (24 h) make it possible that this binding was merely a consequence of attachment of the label to all mitochondrial proteins. In the earlier reports from Sterling's laboratory the T_3-binding activity was partially purified as a proteolipid with a molecular mass of 150 kDa [25]. The molecular mass of the adenine nucleotide translocator (31 kDa) makes it difficult to suggest that this is the same fraction as earlier identified. If the adenine nucleotide translocator is the mitochondrial T_3 receptor in liver, then the absence of the mitochondrial inner membrane T_3 receptor in brain, spleen and testis previously reported by Sterling's group [24,25] makes it necessary to postulate a different adenine nucleotide translocator in these tissues.

3.3. *Transport of thyroid hormone to the mitochondria*

The nature of the transport of T_3 to the mitochondria has not received much attention. It has been reported that the cytosolic T_3-binding protein increases the uptake of T_3 into isolated kidney mitochondria [30]. In an extension of the studies which indicated that T_3 was taken up into the cell in association with serum prealbumin in the rat [15] the same workers have indicated that serum prealbumin is targetted to the inner mitochondrial membrane [31], and have postulated that it binds to an 'acceptor' protein there to exert its effects. This involvement of a serum carrier protein in the subcellular distribution of thyroid hormone is not consistent with current views of intracellular transport and deserves further investigation.

3.4. *Direct effects of thyroid hormone on mitochondrial function*

The major problem in determining whether these putative T_3 receptors in the inner mitochondrial membrane are physiologically relevant or not is the lack of adequate experimental demonstration of any direct effect of T_3 on mitochondria. The main evidence that has been used by Sterling's group for a direct action of T_3 on mitochondria is a stimulation of the respiration rate of mitochondria isolated 30 min after the injection of T_3 into a rat in the presence of protein synthesis inhibitors [32]. The highly variable nature and size of this effect make its interpretation difficult. Other groups have reported no increase in mitochondrial respiration rate 15 min after the injection of T_3 into

thyroidectomised rats [23]. The possibility that the adenine nucleotide translocator is the mitochondrial T_3 receptor is not consistent with the action of T_3 on mitochondria being to increase respiration rate. The adenine nucleotide translocator has a lower flux control coefficient compared to control over respiration rate in mitochondria isolated from hypothyroid rats [33,34]. This makes it unlikely that stimulation of this step would increase the respiration rate.

In support of the adenine nucleotide carrier being a thyroid hormone responsive element of the inner mitochondrial membrane is the finding that the lowered V_{max} and raised K_m of this step, characteristic of the hypothyroid state, can be restored to normal values within 15 min of the injection of T_3 [35]. Although this supports the idea of a rapid effect of T_3 on mitochondrial function in hypothyroid rats it does not demonstrate that T_3 acts directly on this step or that it has any action at this step in the euthyroid animal. The workers in this study used a dose of T_3 designed to saturate the plasma binding sites and restore the free T_3 concentration to euthyroid levels. The finding that the extravascular T_3 binding is much larger than the intravascular binding [5] indicates that the free T_3 concentration actually achieved in this study is much lower than the euthyroid level. Because the kinetics of the carrier were completely restored to normal by what would appear to be a free T_3 concentration much lower than euthyroid it is hard to suggest that variation of the free T_3 concentration in the euthyroid animal would be able to regulate this step.

Bronk [36] has shown that the decreased respiration rate of mitochondria isolated from hypothyroid rats can be half restored 3 h after the administration of 30 μg of T_3. This effect was suggested to be the result of a direct action of T_3 on the mitochondria rather than being mediated by protein synthesis because the levels of the cytochromes did not change over this time course. However, the evidence that the cytochromes are important in the control of state 3 respiration rate in hypothyroid animals is slim (see [37] for a review). As the levels of some hepatic messenger RNAs have been shown to change within an hour of thyroid hormone administration [38] it seems premature to conclude that this increase in respiration was not mediated by protein synthesis.

A recent report [39] has indicated that T_3 added in vitro to mitochondria isolated from hypothyroid rats can control the ADP/O ratio in the presence of nanomolar concentrations of free Ca^{2+}. However, it has been suggested [40] that the apparent drop in ADP/O ratio previously reported by this group [23,34] is a result of net formation of phosphate acceptor leading to increased phosphorylation of ADP rather than a decreased efficiency of ATP synthesis [40]. The apparent Ca^{2+}-dependent drop in ADP/O ratio and its reversal by T_3 [39] may be a consequence of Ca^{2+}-dependent ATPase activity (or an enzymatic breakdown of ADP to AMP) that can be inhibited by T_3 in vitro. It cannot, therefore, be used as evidence for a receptor-mediated action of T_3 on the mitochondria.

4. CONCLUSIONS

In conclusion there is apparently sufficient evidence that the uptake of thyroid hormones into cells is an active mechanism and not passive diffusion. However, the exact nature of the translocation process remains obscure. Both receptor-mediated endocytosis and serum carrier proteins may be involved, and further experiments are required to characterise their roles in the uptake process. As far as the localisation of thyroid hormone to the mitochondria is concerned there is insufficient evidence for a receptor in the inner mitochondrial membrane or for a direct action of T_3 on the mitochondria. This localisation may not be a specific binding event, but rather a nonspecific distribution to a preferred lipid environment.

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