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Plasma Membrane Transport of Thyroid Hormones and Its Role in Thyroid Hormone Metabolism and Bioavailability

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Although it was originally believed that thyroid hormones enter target cells by passive diffusion, it is now clear that cellular uptake is effected by carrier-mediated processes. Two stereospecific binding sites for each T_4 and T_3 have been detected in cell membranes and on intact cells from humans and other species. The apparent Michaelis-Menten values of the high-affinity, low-capacity binding sites for T_4 and T_3 are in the nanomolar range, whereas the apparent Michaelis-Menten values of the low-affinity, high-capacity binding sites are usually in the lower micromolar range. Cellular uptake of T_4 and T_3 by the high-affinity sites is energy, temperature, and often Na^+ dependent and represents the translocation of thyroid hormone over the plasma membrane. Uptake by the low-affinity sites is not dependent on energy, temperature, and Na^+ and represents binding of thyroid hormone to proteins associated with the plasma membrane. In rat erythrocytes and hepatocytes, T_3 plasma membrane carriers have been tentatively identified as proteins with apparent molecular masses of 52 and 55 kDa. In different cells, such as rat erythrocytes, pituitary cells, astrocytes, and mouse neuroblastoma cells, uptake of T_4 and T_3 appears to be mediated largely by system L or T amino acid transporters. Efflux of T_3 from different cell types is saturable, but saturable efflux of T_4 has not yet been demonstrated. Saturable uptake of T_4 and T_3 in the brain occurs both via the blood-brain barrier and the choroid plexus-cerebrospinal fluid barrier. Thyroid hormone uptake in the intact rat and human liver is ATP dependent and rate limiting for subsequent iodothyronine metabolism. In starvation and nonthyroidal illness in man, T_4 uptake in the liver is decreased, resulting in lowered plasma T_3 production. Inhibition of liver T_4 uptake in these conditions is explained by liver ATP depletion and increased concentrations of circulating inhibitors, such as 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, indoxyl sulfate, nonesterified fatty acids, and bilirubin. Recently, several organic anion transporters and L type amino acid transporters have been shown to facilitate plasma membrane transport of thyroid hormone. Future research should be directed to elucidate which of these and possible other transporters are of physiological significance, and how they are regulated at the molecular level. (*Endocrine Reviews* 22: 451–476, 2001)

Abbreviations: Arg, arginine; BBB, blood-brain barrier; BrAc[^{125}I]T_3, *N*-bromoacetyl-[^{125}I]T_3; BrAc[^{125}I]T_4, *N*-bromoacetyl-[^{125}I]T_4; CSF, cerebrospinal fluid; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; CP-CSFB, choroid plexus-CSF barrier; h, human; hc, heavy chain; HTC, hepatoma cell line; K_d, dissociation constant; K_m, Michaelis-Menten constant; Leu, leucine; NEFA, nonesterified fatty acid; NEM, *N*-ethylmaleimide; NTCP, Na^+/taurocholate-cotransporting polypeptide; NTI, nonthyroidal illness; OATP, organic anion transporting polypeptides; PDI, protein disulfide isomerase; PGT, PG transporter; Phe, phenylalanine; rBAT, related to basic amino acid transport; T_0, thyronine; TBG, T_4-binding globulin; T_3NS; T_3 sulfamate; T_4NS; T_4 sulfamate; Trp, tryptophan; T_3S, T_3 sulfate; T_4S, T_4 sulfate; TTR, transthyretin; Tyr, tyrosine

I. Historical Introduction

EARLY REPORTS ON uptake of thyroid hormones by cells and tissues of different species appeared in the early 1950s. For about two and a half decades it was assumed that the translocation of thyroid hormones over the plasma membrane of target cells was a process of simple diffusion. This assumption was based on the fact that thyroid hormones are lipophilic and, as the plasma membrane is constituted of a lipid bilayer, there seemed apparently no need to assume any other mechanism of translocation than that of diffusion. The belief in this concept was so strong that hardly any studies testing this assumption were performed in this period of time. The studies that were performed on thyroid hormone uptake by cells and tissues were predominantly directed at investigating the influence of temperature, pH, and extracellular thyroid hormone-binding proteins on the kinetics of this process. In the interpretation of the results of these studies, it was often taken for granted that thyroid hormones diffuse into the cells and that the driving force of this process is the concentration of the free hormone. This so-called "free hormone hypothesis" was formulated in 1960 by Robbins and Rall (1). They stated "that the free or diffusible thyroid hormone concentration in blood and extracellular tissues would determine the rate at which thyroid hormone is distributed to its loci of action and the rates at which it is degraded and excreted." As we will see in the following sections, this assumption is only partially correct. Plasma membrane translocation is a regulated process that is rate limiting for subsequent intracellular accumulation, action, and fate of the hormone. However, we will also see that, at least *in vitro*, the rate of uptake of thyroid hormones into the cell is determined not only by the efficacy of this plasma membrane translocation process but also by variations in the free hormone concentration in physiological and pathophysiological conditions. *In vivo* the situation is more complicated in that circulating inhibitors of thyroid hormone tissue uptake may be operative as well.

It is remarkable that, to the best of our knowledge, the first publication on thyroid hormone transport points to an energy-dependent uptake process (2). In this report, transport of T_3 into ascites carcinoma cells was inhibited by KCN, a metabolic blocker that suppresses ATP formation, indicating that energy is involved in the uptake mechanism. The authors of this study concluded that "this amino acid does not escape the cellular concentration process to which all other amino acids so far studied are subjected." This report apparently escaped attention and was "rediscovered" by Sorimachi and Robbins in 1978 (3).

In a review in 1957 (4), Robbins and Rall proposed that thyroid hormone action is a function of the free hormone in the blood. However, in view of the extremely low concentration of unbound T_4 in blood, they suggested that tissues are extraordinarily sensitive to thyroid hormone, or that T_4 has to be concentrated in target cells. This latter suggestion leaves open the possibility of an active transport process. On the basis of their studies using tissue slices at different incubation temperatures and metabolic activities, Freinkel *et al.* (5) concluded that the establishment of concentration differentials for T_4 between tissue slices and suspending media constitutes an equilibrium-binding phenomenon rather than an active transport. Hogness *et al.* (6) suggested that the higher concentration of T_4 and T_3 in rat diaphragm as compared with that in the incubation media was evidence for a true chemical binding. They did not consider the possibility of energy-dependent transport against a concentration gradient. Two groups of investigators, Beraud *et al.* (7) and Ingbar and Freinkel (8), were of the opinion that extra- and intracellular thyroid hormone binding-proteins govern transmembrane transfer of free diffusible hormone. In their studies of the uptake of T_4 and T_3 by rat diaphragm, Lein and Dowben (9) assumed that the kinetics of uptake they observed were based on diffusion into the tissue and subsequent binding of hormone to intracellular proteins. In his review on distribution and metabolism of thyroid hormone, Tata (10) suggested that the plasma membrane did not play an active role in the movement of free hormone from the vascular to the tissue compartments. Hillier (11) published a series of studies related to uptake and release of T_4 and T_3 in different organs. To our knowledge, he was the first to assess saturability of these processes. Studying the perfused rat heart, saturation of these processes could not be detected using free hormone concentrations ranging from 13 pM to 1.3 μM. As we will see below (*Sections II* and *III*), the highest concentration used is sufficient to saturate the high-affinity component of the uptake process detected in rat hepatocytes and many other cell types, although discrepancies have been described. One of the reasons why any saturation of the uptake mechanism might have escaped detection is that the conditions under which the studies were performed were not optimal to maintain intracellular ATP concentrations. This means that any energy-dependent, carrier-mediated process might have become undetectable. This possibility is in line with another observation from the same study (11), that thyroid hormone uptake was independent of changes in incubation temperature. In a follow-up study (12), Hillier concluded that extracellular thyroid hormone binding-proteins are an important factor determining the total amount of hormone taken up by the rat heart. Studying uptake and release of T_4 and T_3 in rat liver under similar "ATP-poor" conditions and using hormone concentrations up to 0.13 μM, he arrived at similar conclusions, in that uptake and release were temperature independent and that uptake was importantly influenced by extracellular hormone-binding sites (13). The assumption that thyroid hormones easily penetrate plasma membranes was strengthened by Hillier's next studies (14) using liposomes prepared from egg-yolk lecithin. He reported that these membranes were readily permeable to T_4 and that the binding of both T_4 and T_3 to liposomes and to rat heart tissue is similarly dependent on pH.

In summary, until 1970 it was generally believed that thyroid hormones enter target cells by simple diffusion. This assumption was based on the fact that thyroid hormones are lipophilic and could therefore easily traverse the lipid-rich bilayer of the cell membrane. Transport of thyroid hormones into cells was envisaged to be mainly regulated by binding forces of extra- and intracellular thyroid hormone-binding proteins, directing the free moiety of thyroid hormone passively through the plasma membrane.

II. Binding of Thyroid Hormones to Isolated Cell Membranes

A. Binding kinetics

The earliest studies analyzing specificity of binding of thyroid hormones to plasma membranes of target cells were reported in 1975 by Tata (15) and in 1976 by Singh *et al.* (16). Although detecting saturability of binding of thyroid hormones to different cellular constituents, including plasma membranes, Tata questioned the biological relevance of these binding sites (15). Singh and his group studied inhibition of binding of T_3 and T_4 to intact hemoglobin-free erythrocyte membranes by thyroid hormone analogs (16). Specificity of binding was demonstrated for both T_4 and T_3 by structure-dependent inhibition by the analogs. The major finding of this study was that the avidity of erythrocyte membranes was greater for T_3 analogs than for T_4 analogs but was similar for L-T_3 and L-T_4.

Several reports concerned binding of thyroid hormones to plasma membranes of rat hepatocytes (17–20). Pliam and Goldfine (17) reported on two binding sites for L-T_3, one with high affinity and low capacity and one with low affinity and high capacity. Mean apparent dissociation constant (K_d) values were 3.2 nM and 220 nM, respectively (Table 1). Similar values were found by others (18), who also reported on high- and low-affinity binding sites for L-T_4, with mean apparent K_d values of 0.57 nM and 23.8 nM, respectively, distinct from the T_3 binding sites (Table 1). Specific T_4 binding was inhibited by thiol-blocking agents and by proteases. L-T_4 was bound with high specificity regarding iodine substituents and alanine side chain modifications (20). Studies of L-rT_3 binding to rat hepatocyte membranes also revealed two binding sites, the high-affinity site being different from that of L-T_4 (21).

A number of studies have also reported on the binding of thyroid hormones to human and rat erythrocyte membranes (22–29). Both in human and rat erythrocyte membranes, two saturable binding sites for L-T_3 were identified; a high-affinity, low-capacity and a low-affinity, high-capacity binding site. Apparent K_d values for the high-affinity binding site in human erythrocytes varied between 0.2 nM and 140 nM and for the low-affinity binding site between 5 nM and 26 μM (22, 24, 25, 27). Specific binding was dependent on the presence of reduced protein-SH groups and showed high specificity for L-T_3, with L-T_4 being far less avidly bound (24). For rat erythrocyte membranes, apparent K_d values for T_3 varied between 9 pM and 21 nM for the high-affinity site and between 0.4 nM and 50 μM for the low-affinity site (23, 26–29) (Table 1). Also here, specific binding was dependent on the reduced state of protein–SH groups, and the high-affinity binding site appeared to be related to the amino acid transport system T (27, 28). Binding was (stereo)specific, in that D-T_3 and L-T_4 were less potent in competing for these sites than L-T_3, whereas rT_3 and triiodothyroacetic acid were inactive (23). The considerable variation in apparent K_d values reported in these studies is probably due to differences in test conditions and techniques, but may also be caused by involvement of multiple transporters (see *Section IX*).

Binding of thyroid hormones to plasma membranes of other cell types and species was also reported. High-affinity binding sites for T_3 and T_4 in plasma membranes of rat kidney and testis were characterized by apparent K_d values in the low nanomolar range, whereas those of the low-affinity binding sites were in the high nanomolar range (Table 1). Specific binding sites for L-T_3 and L-T_4 could not be detected in rat spleen (18). In plasma membranes of human placenta, two specific L-T_3 binding sites were found with apparent K_d values of 2.0 nM and 18.5 μM (30). D-T_3, L-rT_3, L-T_4, and D-T_4 were less effective in displacing L-T_3 from both binding sites. In plasma membranes of a mouse neuroblastoma cell line, L-T_3 binding sites showed apparent K_d values of 8.4 nM and 7.3 μM, with lower affinity of both sites for D-T_3 (31).

TABLE 1. Specific binding of L-T_3 and L-T_4 to isolated plasma membranes of different tissues from different species (mean values)

Tissue	T_3		T_4		Ref.
	Kd_1[a]	Kd_2[b]	Kd_1[a]	Kd_2[b]	
Rat hepatocytes	3.2 nM	220 nM			17
Rat hepatocytes	15 nM	270 nM	0.57 nM	23.8 nM	18
Rat hepatocytes	15.8 nM	237 nM	4.54 nM	127.0 nM	18
Rat kidney			10 nM		19
Human erythrocytes	140 nM	26 μM			22
Human erythrocytes	0.2 nM	5 nM			24
Human erythrocytes	34 nM	ND[c]			25
Human erythrocytes	0.2 nM	18 μM			27
Rat erythrocytes	19 pM	20 nM			23
Rat erythrocytes	9 pM	0.4 nM			26
Rat erythrocytes	20 pM	ND[c]			27
Rat erythrocytes	21 nM	50 μM			28
Rat erythrocytes	4.5 nM	ND[c]			29
Rat testis	266.0 nM	ND[c]	27.77 nM	285.7 nM	18
Rat spleen	ND[c]	ND[c]	ND[c]	ND[c]	18
Human placenta	2.0 nM	18.5 μM			30
Mouse neuroblasts	8.4 nM	7.3 μM			31

[a] High-affinity binding site.
[b] Low-affinity binding site.
[c] Not detected.

B. Analysis of binding protein(s)

A series of publications by Cheng and co-workers (30, 32–35) concerned the identification of T_3 and/or T_4-binding membrane proteins in different cell types by affinity-labeling techniques. In their experiments using human placenta (30), GH3 cells (32, 33), mouse Swiss 3T3 fibroblasts (33), and human A431 epitheloid carcinoma cells (33), the proteins were envisaged to be associated with the plasma membrane and to have a molecular mass between 55 (32, 33) and 65 kDa (30). Peptide mapping of the proteins labeled with *N*-bromoacetyl-[^{125}I]T_3 (BrAc[^{125}I]T_3) or BrAc[^{125}I]T_4 showed very similar patterns (33), indicating that the same protein was probably involved. Later immunocytochemical studies, using four different monoclonal antibodies against the 55-kDa thyroid hormone-binding protein, showed that this protein was loosely associated with the endoplasmic reticulum and nuclear envelope, although some association with the plasma membrane could not be excluded (34). In a later study by Kato *et al.* (35), this protein was shown to be identical to protein disulfide isomerase (PDI). This finding was confirmed by Horiouchi *et al.* (36), who detected both T_3-binding

and PDI activity in a 55-kDa protein isolated from a plasma membrane-enriched beef liver fraction. Although some PDI may indeed be associated with the plasma membranes, most of this enzyme is located in the lumen of the endoplasmic reticulum (37). In contrast to the high reactivity of PDI toward $BrAcT_3$ and $BrAcT_4$, it shows only low affinity for underivatized T_3 and T_4 (38). Since, moreover, PDI is not an integral membrane protein (37, 38), it seems unlikely to be involved directly in plasma membrane transport of thyroid hormone.

Photoaffinity labeling of erythrocyte membranes with L-T_3 has identified a protein with an apparent molecular mass of 55 kDa (39). T_3 binding to this protein was critically dependent on the presence of phospholipids. Tryptophan but not leucine or D-T_3 competed with the L-T_3 binding site, indicating stereospecificity and a possible relationship with the amino acid transport system T (39). Using a monoclonal antibody that specifically inhibited uptake of T_3 in rat hepatocytes, a putative carrier protein was detected with an apparent molecular mass of 52 kDa (40). Affinity labeling of mouse neuroblastoma plasma membranes with $BrAc[^{125}I]T_3$ has detected a 27-kDa protein (31). Since the size of this protein is identical to that of the type I iodothyronine deiodinase, which is also readily labeled with $BrAcT_3$ (38), it is unlikely to be related to a thyroid hormone transporter.

In summary, the first studies showing specific binding of thyroid hormones to isolated cell membranes appeared in the mid-1970s. Most extensively studied were cell membranes from human and rat erythrocytes and rat hepatocytes. For each T_3 and T_4, two stereospecific binding sites were detected in these membranes; one with apparent K_d values in the lower nanomolar range, and the other in the (sub)micromolar range. Specific binding for both hormones was dependent on the reduced state of protein-SH groups. T_3-binding proteins have been identified in rat erythrocyte and hepatocyte membranes with apparent molecular masses of 55 and 52 kDa.

III. Transport of Thyroid Hormones into Isolated Cells

The first evidence, to our knowledge, that transport of thyroid hormones into intact cells is not a passive, but an energy-dependent, process was reported by Christensen *et al.* in 1954 (Ref. 2; see also *Section I*) but unfortunately temporarily escaped attention. It was not until 1976 that Rao *et al.* (41) and our laboratory (42, 43) in 1978 independently published the saturable and energy-dependent transport of T_3 and T_4 into rat hepatocytes. Since then a whole series of reports from different laboratories have confirmed carrier-mediated, mostly energy- and Na^+-dependent transport of iodothyronines into a variety of cells from different species.

A. Transport into hepatocytes

In Table 2 the kinetics of thyroid hormone uptake by hepatocytes are summarized. In most studies two saturable processes have been discerned: a high-affinity, low-capacity and a low-affinity, high-capacity process (41–55). In the majority of the studies, the apparent K_m values of the high-affinity systems for T_4, T_3, or rT_3 uptake are in the nanomolar range (42–55). This process is thought to represent the translocation process across the plasma membrane as it is energy and temperature dependent (41–55). Studies testing the possible Na^+ dependence of the high-affinity uptake of iodothyronines have produced controversial results in rats (44–47, 50), confirmatory results in human hepatocytes (52), and negative results in trout hepatocytes (54, 55). The energy-, temperature-, and Na^+-independent, low-affinity uptake process may represent binding of thyroid hormone to cell surface-associated proteins (45). T_4 and T_3 mutually inhibit their high-affinity uptake processes in rat hepatocytes, but kinetic analysis of these inhibitions indicates that T_3 and T_4 cross the plasma membrane by different pathways (47, 55). This finding was confirmed by others who found differences in the dependence of the T_3 and T_4 transport systems on the cell phase of the rat hepatocyte and on sodium butyrate stimulation (56). Preliminary results in rat hepatocytes suggest that rT_3 shares the same transport system with T_4 (48), but kinetic studies of plasma iodothyronine clearance in humans suggest different plasma-to-liver transfer mechanisms for rT_3 and T_4 (57), in line with different binding sites for rT_3 and T_4 in (rat) liver plasma membrane (21). In addition to the metabolic condition of hepatocytes in culture, in particular with regard to ATP concentration, the free T_4 concentration in the medium is also a determinant for the amount of hormone that is taken up by the cell and subsequently metabolized (58). Stereospecificity of T_3 and T_4 uptake has been demonstrated in rat and trout liver cells (51, 54, 55).

B. Transport into other cell types

Many studies have confirmed carrier-mediated, often energy- and Na^+-dependent transport of thyroid hormones in various cell types from different species, *i.e.,* human (22, 59–62), rat (63–65), and trout (66, 67) erythrocytes; normal (68, 69) and clonal (70) rat pituitary cells, brain cells such as human glioma cells (71), rat glial cells (72), astrocytes (73), cerebrocortical neurons (74), and brain synaptosomes (75); mouse neuroblastoma cells (76), rat skeletal (77) and cardiac (78) myocytes; human (79, 80) and mouse (81) fibroblasts; human epithelial carcinoma cells (81); Chinese hamster ovary cells (81); human trophoblasts (82); human choriocarcinoma cells (83–86); rat adipocytes (87); human peripheral leukocytes (88, 89); and mouse thymocytes (90, 91) (Table 3).

1. T_3 transport. Similar to hepatocytes, apparent Michaelis-Menten (K_m) values for the high-affinity uptake of T_3 in other cell types are mostly in the nanomolar range. Some authors (22, 73), including our laboratory (80), have also detected a low-affinity T_3-binding site, like that present on hepatocytes, apparently depending on the use of protein (albumin)-containing incubation media and probably reflecting the association of protein-bound T_3 with/around the cells (45). When studied, the energy dependence of T_3 transport was invariably demonstrated in the different cell types. In contrast, the Na^+ dependence of this process differed between cell types. Thus, transport of T_3 in erythrocytes of human, rat, and trout origin (22, 59–67), in rat astrocytes (72, 73), and human choriocarcinoma cells (82–86) was not dependent on

TABLE 2. Kinetics of thyroid hormone transport into hepatocytes *in vitro* (mean values)

Species	K_m T_4	K_m T_3	K_m rT_3	Temperature dependent	Energy (ATP) dependent	Na^+ dependent	Stereo-specific	Ref.
Rat								
1[a]		52 nM		Yes	Yes			41
2[b]		144 nM		Yes	Yes			
Rat								
1[a]	1.2 nM[c]	21 nM[c]		Yes	Yes	Yes		42,45–47,50
2[b]	1.0 μM	1.8 μM		No	No			
Rat								
1[a]		86 pM		Yes	Yes	No		44
2[b]		726 pM		Yes	Yes	No		
Rat			≈6 nM[d]		Yes			48
Rat hepatoma		680 nM			Yes		Yes	51
Human		NR[e]		Yes	Yes	Yes		52
Human								
1[a]		3.6 nM			Yes			53
2[b]		503 nM						
Trout	0.52 μM[a]	74 nM[a]			Yes	No	Yes	54,55

[a] High-affinity uptake system.
[b] Low-affinity uptake system.
[c] T_4 and T_3 have different transport systems.
[d] rT_3 transport system possibly shared with T_4.
[e] Not reported.

the Na^+ gradient over the plasma membrane, whereas this was the case in rat pituitary cells (68–70), rat brain synaptosomes (73), rat neonatal cardiac myocytes (78), human fibroblasts (80), and mouse thymocytes (90, 91). In some cell types the influence of pH on transport was studied and found to be of importance, in the sense that T_3 uptake decreased when pH increased in mouse thymocytes (91), while the reverse was true in rat brain astrocytes (75). When studied, T_3 transport was invariably (stereo)specific, *i.e.,* in human and rat erythrocytes, human and rat nerve and brain cells, rat skeletal myoblasts, human choriocarcinoma cells, and mouse thymocytes (Table 3). In general, different L-iodothyronine analogs and the D-isomers of T_3 and T_4 were less potent in inhibiting T_3 and T_4 uptake than L-T_3 and L-T_4.

2. T_4 transport. T_4 transport into intact cells has been less well studied than T_3 transport (Table 3). The most probable explanation for this, at least in liver cells, is the greater requirement of an optimal energy charge of the cells under study for transport of T_4 than for uptake of T_3. This is explained by the much steeper slope of the relationship between cellular ATP concentration and the rate of T_4 (and rT_3) transport in hepatocytes than that of the relationship between ATP and T_3 transport (Fig. 1) (46). Even a small decrease in cellular ATP concentration results in a major reduction in T_4 (and rT_3) transport but only slightly affects T_3 uptake. This may also be the reason why some authors could not observe specific, energy-dependent transport of T_4 in liver cells (44, 92). Others (93) did find saturable but energy-independent uptake not only of T_4 but also of T_3 in rat hepatocytes under far from optimal cellular ATP conditions. In other cell types, such as erythrocytes, rat neonatal cardiac myocytes, rat brain cells, pituitary cells, and fibroblasts, some laboratories observed that, in contrast to T_3, T_4 was apparently taken up by diffusion only or not at all, whereas other laboratories did find (stereo)specific, mostly energy-dependent T_4 uptake in the same cell types (Table 3). It is not known whether these discrepancies are related to the different energy requirements of the T_4 and T_3 transport processes as mentioned above or due to other factors such as the use of different techniques.

C. Interactions of various compounds with thyroid hormone transport

1. Amino acids. Interrelationships between amino acid and thyroid hormone transport have been studied in different cell types from different species. It should be noted that the effects of amino acids on thyroid hormone transport cited below were usually obtained at physiological serum concentrations of free amino acids in the micromolar range.

a. Erythrocytes. In rat erythrocytes, the aromatic amino acids tryptophan (Trp), phenylalanine (Phe), and tyrosine (Tyr) competitively inhibited T_3 transport, while transport of Trp was similarly inhibited by T_3, D-T_3, T_4, and thyronine (T_0) (94). *N*-ethylmaleimide (NEM) irreversibly inhibited Trp and T_3 transport, and both ligands protected each others transport from inactivation by this compound. These data indicated common or closely linked transport systems for T_3 and for aromatic amino acids, *i.e.,* the system T amino acid transporter, at least in erythrocytes (94). Similar results were obtained for binding of T_3 and Trp to rat erythrocyte membranes (28). Further studies suggested a common carrier for T_3 and Trp, which also facilitates countertransport such that the uphill transport of T_3 is driven by heteroexchange with intracellular aromatic amino acids (95). Evidence for uptake of T_3 by the system T amino acid transporter or a closely linked transporter was also obtained using human and trout erythrocytes (62, 67). No such relationship was found between T_4 and system T amino acid transport in trout erythrocytes (67).

b. Other cell types. In rat hepatocyte sinusoidal membrane vesicles, Trp transport occurs via a NEM-resistant (system T) and a NEM-sensitive (system L) pathway, and T_3 and T_4 mainly inhibit Trp transport via system T (96). The inhibitory

TABLE 3. Kinetics of thyroid hormone uptake in different cell types *in vitro* (mean values)

Cells	K_m T_4	K_m T_3	Temperature dependent	Energy (ATP) dependent	Na^+ dependent	Stereo-specific	Ref.
Human erythrocytes							
1[a]		16 nM			Yes		22
2[b]		3.3 μM			No		
Human erythrocytes		128 nM		No	No		59
Human erythrocytes		248 nM					60
Human erythrocytes	Diffusion?	67 nM		No	No	Yes	61
Human erythrocytes		59.9 nM					62
Rat erythrocytes	No uptake	53 nM[c]	Yes		No	Yes	63,64
Rat erythrocytes		160 nM					65
Trout erythrocytes	0.1–1.1 nM	70–119 nM	Yes	No	No		66,67
Rat pituitary	NR[d]	400 nM[e]		Yes	Yes	Yes	68–70
Human glioma cells	0.46 nM	2.17 nM	Yes	Yes		Yes	71
Rat astrocytes	1.02 μM[g]	0.52 μM[g]		No	No	Yes	72,75
Rat brain synaptosomes							
1[a]	Diffusion?	50 pM	Yes	Yes	Yes		73
2[b]		3.1 nM					
Mouse neuroblastoma	6.07 nM	2.38 nM		Yes		Yes	74
Rat brain neurons	≈300 nM[f]	≈400 nM[f]				Yes	76
Rat skeletal myoblasts		17 nM	Yes	Yes		Yes	77
Rat neonatal cardiac myocytes	Diffusion?	NR[d]		Yes	Yes		78
Human fibroblasts	Diffusion?	108 nM					79
Human fibroblasts							
1[a]	1.9 nM	29 nM		Yes	Yes		80
2[b]	141 nM	650 nM					
Mouse fibroblasts		NR[d]		Yes			81
Human epithelial carcinoma		NR[d]		Yes			81
Hamster ovary		NR[d]		Yes			81
Human trophoblasts		755 nM					82
Human choriocarcinoma	59.4 nM[h]	378–586 nM[h]	Yes	Yes	No	Yes	83–86
Rat adipocyte	0.30 nM	0.29 nM					87
Human leukocytes		NR[d]					88,89
Mouse thymocytes	Diffusion?	0.8 nM		Yes	Yes	Yes	90,91

[a] High-affinity uptake system.
[b] Low-affinity uptake system.
[c] *trans*-Inhibition of T_3 in- and efflux by T_3.
[d] Not reported.
[e] T_4 and T_3 share same transport system.
[f] T_4 and T_3 have different transport systems.
[g] Na^+-H^+ exchanger dependent.
[h] K_m rT_3 3.04 μM.

activity of T_3 and T_4 is dependent on the thyroid status of the donor rat, *i.e.*, decreasing in the order hyperthyroid > euthyroid > hypothyroid. T_3 and T_4 share the same stereospecific uptake carrier in the rat pituitary (68, 69), and the potent inhibition of T_3 and T_4 uptake by leucine (Leu) suggests the involvement of amino acid transport system L (70). This system was also found to participate in T_3 and T_4 transport in mouse neuroblastoma cells (74) and in T_3 transport in rat astrocytes (97). In Ehrlich ascites cells, the neutral amino acids Phe, α-aminoisobutyric acid, and cycloleucine did not compete with transport of T_4, indicating that the system A, L, and ASC amino acid pathways were not involved (98). In rat hepatocytes, participation of the amino acid transport system A in uptake of T_3 and T_4 was ruled out (51, 99). A weak interaction was found between uptake of system L and T amino acids and uptake of T_3 in human JAR choriocarcinoma cells (100).

2. Drugs and other chemicals. As shown in Table 4, a variety of compounds has been demonstrated to inhibit thyroid hormone uptake in different cells. Despite their widely different properties, the inhibitory activity of most of these substances is suggested to be based on competition because of structural similarity with thyroid hormone (19, 48, 51, 53, 101, 103–107, 111, 112). The antiarrhythmic drug amiodarone is also known to inhibit binding of T_3 to its nuclear receptors on the basis of structural similarity (114). The concentration of amiodarone shown to inhibit uptake of thyroid hormone in rat hepatocytes was ≈1 μM, which is similar to therapeutical serum levels in humans (114). However, since in serum, amiodarone is primarily bound to albumin that circulates at a concentration of ≈4% but was used in the hepatocyte incubations at a concentration of 1%, the free amiodarone concentrations obtained *in vitro* may be higher than in treated humans. Nevertheless, *in vivo* kinetic data in patients treated with amiodarone also show decreased net tissue uptake of thyroid hormone (115). This decrease can be explained by inhibition of thyroid hormone transport into tissues and/or by inhibition of thyroid hormone binding to intracellular proteins. Cholecystographic agents usually reach serum concentrations between 100 and 700 μM in humans (116) and were tested *in vitro* (at lower albumin levels) at concentrations between 10 and 100 μM (48). These agents not only inhibit thyroid hormone transport into rat hepatocytes, supposedly on the basis of molecular structural similarity (19,

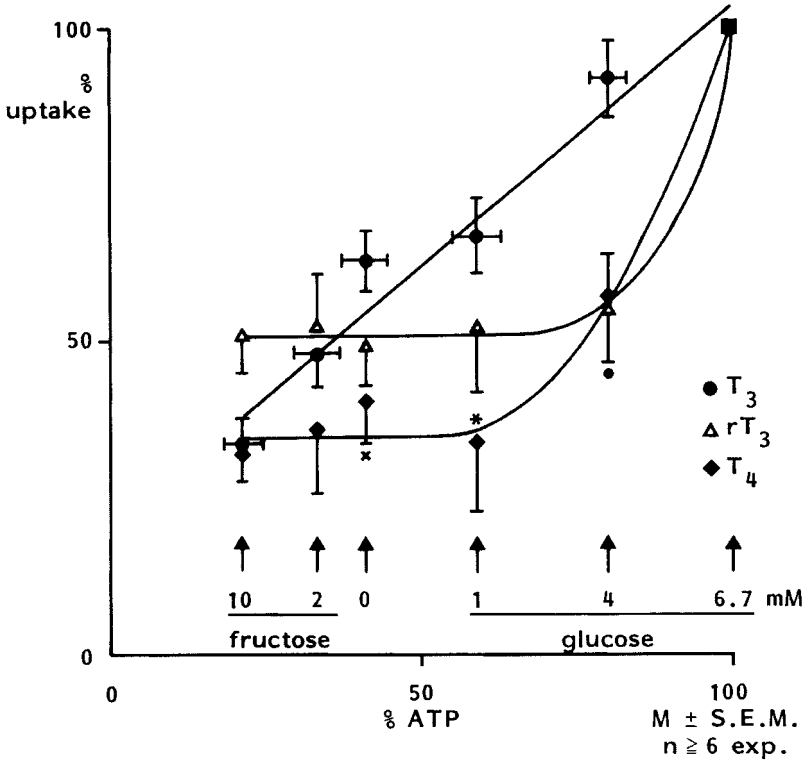

FIG. 1. Uptake of T_3 (●), rT_3 (△), and T_4 (◆) *vs.* ATP concentration in rat hepatocytes preincubated with different concentrations of glucose or fructose. [Reproduced with permission from E.P. Krenning *et al.*: *FEBS Lett* 140:229–233, 1982 (48).]

TABLE 4. Chemical inhibitors of thyroid hormone uptake into cells *in vitro*

Inhibitor	Cell type	Ligand	Supposed mechanism of inhibition	Ref.
Ouabain, monensin	Rat hepatocytes, rat skeletal muscle, pituitary	T_3	Abolition Na^+ gradient	48,49,68
KCN, dinitrophenol bacitracin, oligomycin	Rat hepatocytes, mouse thymocytes	T_3, T_4	ATP depletion	48,90
Vinblastin, colchicin cytochalasin	Rat hepatocytes, mouse thymocytes	T_3, T_4	ATP depletion + perturbation cytoskeleton	48,90
D- and L-propranonol	Rat hepatocytes, mouse thymocytes	T_3, T_4	ATP depletion + membrane stabilization	48,90
Amiodarone	Rat hepatocytes	T_3, T_4	Competitive	48,114
Cholecystographic agents	Rat hepatocytes	T_3, T_4	Competitive	20,51
Nifedipine, verapamil, diltiazem	Rat hepatocytes, rat myoblasts, human hepatocytes	T_3	Interaction with calmodulin (like-protein)	101,112
Bromosulphthalein, indocyanine green	Rat hepatocytes, rat brain astrocytes	T_3, T_4 T_3, T_4	Competitive	19,51
Bilirubin and conjugates	Rat hepatocytes	T_3	?	106,109
Diphenylhydantoin, phenylanthranilic acid, and phenylacetic acid derivatives	Rat hepatocytes, rat pituitary	T_3	Competitive	51,107,111,113
Phloretin	Human hepatocytes	T_3	Competitive	53
3,5-Dibromo-3′-pyridazinone-L-thyronine (L-94901)	Rat myoblasts, rat hepatocytes, rat neuroblasts	T_3	Un- or noncompetitive	102
Benzodiazepines	Human hepatocytes, human neuroblast, rat pituitary	T_3	Direct or indirect interaction with T_3 carrier	103–105
CMPF, indoxyl sulfate	Rat hepatocytes	T_4	Unknown	108
NEFA	Rat hepatocytes	T_4	Unknown	109,110

48), but also displace T_4 from the human liver *in vivo* (117). The non-bile acid cholephils, sulfobromophthalein, bilirubin, and indocyanine green, also inhibit thyroid hormone transport and binding in rat hepatocytes on the basis of structural similarity (19, 51). Diphenylhydantoin, the nonsteroidal antiinflammatory phenylanthranilic acids, flufenamic acid, meclofenamic acid, and mefenamic acid, and the structurally related compounds, 2,3-dimethyldiphenylamine and diclofenac, all competitively inhibit rat hepatocyte and pituitary uptake of thyroid hormone (51, 107, 111, 113). Analysis of the structure-activity relationship for inhibition of T_3 uptake in rat hepatocytes by the phenylanthranilic acids demonstrated that inhibitory potency was highly dependent on the hydrophobicity of the inhibitor (107). Phloretin, a glucose

transporter inhibitor that is structurally related to thyroid hormones, competitively inhibited T_3 uptake into human HepG2 hepatocarcinoma cells (53). Many of the inhibitors of thyroid hormone uptake discussed here also interact competitively with thyroid hormone-binding sites on serum proteins and nuclear T_3 receptors (51, 107, 113, 118). Amiodarone, cholecystographic agents, and bilirubin have been shown to interact with deiodinases (114, 119). The benzodiazepine drugs do not interact with nuclear T_3-binding sites, but inhibit T_3 uptake in different cell types from human and rat origin (Table 4) by competing for the T_3 carrier without being transported themselves (104). The structure-activity relationships were studied for inhibition of T_3 uptake in HepG2 cells by benzodiazepine and thyromimetic compounds. The results of these studies, along with computer-assisted molecular modeling techniques, predicted a "tilted crossbow" conformation of the inhibitor for interaction with the iodothyronine transporter (105).

The three different types of organic calcium channel blockers, nifedipine, verapamil, and diltiazem, inhibit T_3 uptake in different cell types (Refs. 101 and 112; Table 4). It is considered unlikely that the inhibitory effect is due to dependence of the uptake process on extracellular Ca^{2+}, on Ca^{2+} fluxes via voltage-dependent or receptor-operated calcium channels, or on the interaction of Ca^{2+} with PKC. A plausible mechanism for the inactivation of the uptake process is by interaction of the calcium blockers with calmodulin in the plasma membrane. Calmodulin is found in high concentrations in plasma membranes; it binds T_3 and may play a role as such in the translocation process of thyroid hormone (101). 3-Carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), indoxyl sulfate, and nonesterified fatty acids (NEFAs) are substances that circulate in increased amounts in patients with nonthyroidal illness (NTI) and inhibit thyroid hormone uptake in liver cells (Refs. 108–110; see *Section VII.B*).

Little information is available about stimulatory factors of thyroid hormone uptake *in vitro*. The histamine H1 receptor antagonist, telemastine, and phenobarbital enhance the specific, energy-dependent uptake of T_4 in rat hepatocytes but not in hepatocytes from guinea pig or beagle dog (120). The exact mechanism of this induction in rat hepatocytes is unknown but appears to be a primary effect on the plasma membrane transport system. Telemastine did not influence T_3 uptake in rat hepatocytes, underscoring the functional difference in the uptake systems of T_3 and T_4 in the liver (121).

In summary, transport of T_4 and T_3 has been studied extensively in human, rat, and trout hepatocytes. For both T_4 and T_3, high-affinity, low-capacity and low-affinity, high-capacity uptake processes have been identified. The high-affinity processes have apparent K_m values in the nanomolar range and represent the translocation of the hormones over the plasma membrane. This transport is temperature, energy, and Na^+ dependent, and rate limiting for subsequent hormone metabolism. T_4 and T_3 mutually inhibit their high-affinity uptake processes, but they are transported by different carriers. The low-affinity processes represent binding to cell surface-associated proteins and are not involved in transport. High-affinity, energy-dependent T_3 transport systems similar to those in hepatocytes have also been identified in many other cell types, although their Na^+ dependence varies. T_4 transport has been less well studied in other cell types, and results are variable, possibly because of its greater requirement for an optimal energy charge of the cells.

T_3 uptake in different cells (rat erythrocytes, pituitary cells, astrocytes, and mouse neuroblastoma cells) is inhibited by Trp, Phe, Tyr, and/or Leu, suggesting the involvement of system L or T amino acid transporters. A large variety of chemicals (Table 4) inhibit cellular uptake of thyroid hormones on the basis of structural similarity or by decreasing the cellular energy charge. Alternatively, inhibition is mediated by a decrease in the Na^+ gradient over the plasma membrane, or by other as yet unknown mechanisms. The inhibitory activities of amino acids and other compounds are in the concentration range observed in humans and may interfere with *in vivo* tissue uptake of thyroid hormone.

IV. Cellular Efflux of Thyroid Hormones

Efflux of thyroid hormones has been studied in a number of cell types from different species, *i.e.*, hepatocytes (122–124), erythrocytes (60, 61, 64, 125, 126), placenta cells (84, 127, 128), pituitary cells (129), FRTL-5 thyroid cells (130), NIH-3T3 cells (130), thymocytes (90), lymphocytes (131), and Ehrlich ascites cells (98).

We reported on absence of energy dependence of T_3 and T_4 efflux from cultured rat hepatocytes (122). Cellular efflux consisted of two components, representing release of hormone bound to the outer cell surface and of intracellularly located hormone. We also observed a lack of saturability of T_3 efflux after loading of rat hepatocytes using free T_3 concentrations up to 54 nM (122). However, further results suggested saturation of T_3 efflux after loading of the cells using a free T_3 concentration of 1.5 μM. Others also observed saturability of T_3 efflux, by both T_3 and T_4, from a poorly differentiated rat hepatoma cell line (HTC) (123). The same authors also demonstrated that verapamil inhibited thyroid hormone efflux from these cells as well as from isolated rat hepatocytes, cardiomyocytes, and fibroblasts (123). Furthermore, they observed increased verapamil-inhibitable T_3 efflux from HTC cells adapted for resistance to a permeable bile ester (HTC-R cells). The authors suggested that the carrier protein involved in export of thyroid hormone is related to the family of the multidrug resistance-related ABC transporters as these membrane proteins are overexpressed in HTC-R cells (123). The same group also found verapamil inhibition of T_3 efflux from FRTL-5 thyroid cells and NIH-3T3 cells (130). Others assessed T_4 and T_3 efflux from multidrug-resistant pituitary tumor cells but did not find kinetics to be different from control pituitary tumor cells (129). Neither was any effect detected by verapamil on thyroid hormone efflux in both cell types. Possible saturability of thyroid hormone efflux was not tested by these authors (129).

Efflux of T_3 from rat erythrocytes was found to be a saturable process that is stimulated by aromatic amino acid countertransport, much as T_3 uptake is stimulated by counter efflux of aromatic amino acids (61, 64). Efflux of T_4 from these cells occurred apparently by diffusion as is the case with T_4 and rT_3 efflux from human JAR choriocarcinoma cells, while

also in these latter cells efflux of T_3 is saturable (84, 128). No inhibitory effect on thyroid hormone efflux by neutral system A, L, and ASC amino acids was observed in Ehrlich ascites cells (98). In many of the *in vitro* studies discussed in this section, it has been shown that thyroid hormone-binding proteins, including T_4-binding globulin (TBG), transthyretin (TTR), albumin, and lipoproteins have a permissive effect on efflux of thyroid hormones, probably by facilitating diffusion of thyroid hormone through the water layer around the cell (122, 124, 126).

In summary, efflux of T_3 from rat hepatocytes, cardiomyocytes, and fibroblasts has shown to be a saturable but energy-independent process. The efflux carriers in these cells may be related to the multidrug resistance-related ABC transporter family. In rat erythrocytes, T_3 efflux is also saturable and is stimulated by aromatic amino acid counter transport. Neither T_4 efflux from these cells nor T_4 and rT_3 efflux from human JAR choriocarcinoma cells was found to be saturable, in contrast to the saturable efflux of T_3. Little is known about the role of efflux mechanisms in the regulation of intracellular hormone concentrations.

V. Transport of Thyroid Hormone into Isolated Organs

Transport of thyroid hormones into perfused organs isolated from animals has been extensively studied. The advantage of studying an isolated organ is that its function can be evaluated without interference from other influences in the intact organism. Compared with experiments using isolated cells, the study of intact organs better represents the function of the tissues *in vivo*, although conditions are still appreciably different from the (patho)physiological situation. The results of thyroid hormone uptake studies using perfused, isolated organs from different species will be discussed in this section.

A. Transport into the liver

Transport of thyroid hormones into the intact liver has been mostly studied using organs isolated from rats. In 1979, Jenning *et al.* (132) reported on the effect of starvation on T_3 production from T_4 taken up by the perfused rat liver. They found that the reduced T_3 production was not caused by impaired deiodination of T_4 to T_3 in the liver but by reduced transport of T_4 into the liver, underlining the regulatory role of transport of thyroid hormone in subsequent hormone metabolism (132). One of the explanations that these authors mentioned was that T_4 uptake was inhibited by decreased activity of a "specific" transport system. We extended these studies to T_3 and also found inhibition of T_3 uptake in the intracellular compartment of livers from fasted *vs.* normally fed rats perfused with medium lacking glucose, insulin, and cortisol (133). This inhibition was reverted to normal by a 30-min preperfusion of fasted livers with medium containing a combination of glucose, insulin, and/or cortisol but not by the individual additions. On the basis of these results, we explained the diminished T_3 uptake by a decrease in cellular ATP induced by fasting, which was restored by preperfusion with energy-rich medium (133). Further studies using fructose in the perfusate to (transiently) lower cellular ATP stores in the rat liver showed a parallel decrease in T_4 uptake in the intracellular compartment of the liver, thus underscoring the regulatory role of the energy charge of the cell in the transport process (Fig. 2, Ref. 134). Similar to the results in cultured rat hepatocytes, we found that, in addition to the energy state of the liver, the free hormone concentration in the perfusion medium determined the amount of hormone taken up by the intracellular compartment of the liver (135). Studies using livers from amiodarone-treated animals indicated that transport of T_4, but not of T_3, was inhibited (136), in agreement with hepatocyte studies (47, 55) showing that T_4 and T_3 are transported differently across the liver plasma membrane. Efflux of T_3 from the isolated perfused trout liver was stimulated by addition of T_4, epinephrine, or TSH to the perfusion medium, and efflux of T_4 was stimulated by addition of T_4 to the medium. The stimulating effect of extracellular thyroid hormone on efflux of T_4 and T_3 may be caused by inhibition of reuptake, stimulation of an exchange mechanism, and/or displacement of hormone from intracellular binding sites (137, 138). However, the stimulation of T_3 efflux by epinephrine and TSH remains unexplained.

B. Transport into other organs

As the choroid plexus is known to synthesize TTR (139, 140), the specific role that this tissue plays in transport of thyroid hormone to brain cells was evaluated. Isolated choroid plexus of the rat was found to accumulate T_4 and T_3 from surrounding medium by a nonsaturable process (141). The authors proposed a positive role of choroid plexus-derived TTR in the transport of thyroid hormones from the blood to the cerebrospinal fluid (CSF) and subsequently to brain cells. Others found partly saturable uptake of T_4 in the choroid plexus of the rabbit (142). Measurement of T_3 uptake at the blood face of isolated sheep choroid plexus showed both saturable and nonsaturable transport (143). T_3 uptake lacked stereospecificity and was Na^+ independent, but was inhibited by T_4 and by large neutral amino acids. Uptake of T_3 at the CSF side of sheep choroid plexus was also partially saturable and independent of the Na^+ gradient over the plasma membrane (143).

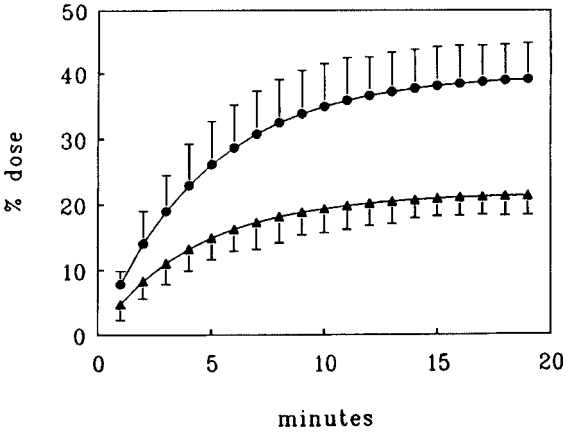

FIG. 2. T_4 liver uptake (in % dose) in rat livers during glucose (●) and glucose/fructose (▲) perfusion. [Reproduced with permission from M. de Jong *et al.*: *Am J Physiol* 266:E768-E775, 1994 (134).]

Incubation of whole soleus muscle isolated from rats showed stereospecific, energy- and Na^+-dependent uptake of T_3, but T_4 uptake was considered to be a diffusion process (49, 144). Addition of insulin to the incubation medium stimulated T_3 uptake but did not affect T_4 uptake (145). T_3 uptake in the perfused rat heart showed a saturable process with an apparent K_m value of 80 μM (146). This value is about 1 order of magnitude higher than the apparent K_m values obtained in *in vitro* studies using isolated cardiomyocytes (Tables 2 and 3). This difference may be explained by the fact that T_3 uptake in the perfused rat heart was determined after a single capillary passage that proceeds within seconds and differs fundamentally from techniques in which initial uptake rates in cells are measured over a period of minutes. The question is if the former method represents uptake of the ligand by the cardiomyocytes, since this assumes that the hormone has already passed the endothelium after such a short time lapse. Another explanation, of course, is that the experiments using cultured cells provide data that are more remote from the *in vivo* situation than data obtained from isolated organ studies. In contrast to the rat liver (132), fasting did not decrease uptake of T_4 by the isolated perfused rat kidney, but T_4 uptake was decreased in kidneys of diabetic rats (147, 148).

In summary, uptake of T_4 and T_3 is decreased in isolated livers from fasted *vs.* fed rats perfused with the same "energy-poor" medium. Changing the perfusate to an energy-rich medium restores uptake in 30 min, suggesting restoration of cellular ATP. Perfusion of fed livers with fructose results in a lowering of cellular ATP and a parallel decrease in thyroid hormone uptake. Analysis of transport in livers from amiodarone-treated rats showed that in the intact liver, T_3 and T_4 are also taken up by different mechanisms. Apart from the cellular energy charge, the free and not the protein-bound fraction of thyroid hormone determines the amount of hormone taken up by the cellular compartment of the liver. Uptake of T_4 and T_3 in isolated rat or sheep choroid plexus was found to be nonsaturable by some investigators but partly saturable by others. Saturable transport of T_3, but not of T_4, was observed in the isolated rat soleus muscle. Saturable T_3 transport was also found in the perfused rat heart.

VI. *In Vivo* Plasma Membrane Transport of Thyroid Hormones in Animals

To assess plasma membrane transport of thyroid hormones to different organs *in vivo*, animals were injected with tracer amounts of labeled hormones after which entry of hormones into the isolated organs was analyzed.

A. Brain

Several questions related to transport of thyroid hormone to the brain have been addressed. One aspect is whether entry of thyroid hormone into brain proceeds via a passive process or via a carrier-mediated mechanism. When dogs were injected intravenously with tracer T_4, allowing entry in the brain via the blood-brain barrier (BBB) and the CSF, brain uptake was saturable under conditions of T_4 loading, indicating that transport occurred via a carrier-mediated process (149). In mice, transport of T_3 into the brain was saturable but, under the conditions of the experiment, no saturation of T_4 transport was observed. Efflux of both T_3 and T_4 from the brain appeared to proceed by a carrier-mediated mechanism (150).

Another point of interest is to what extent transport through the BBB and the choroid plexus-CSF barrier (CP-CSFB) contributes to overall brain uptake of thyroid hormone. To investigate this, rats were injected either intravenously or intrathecally with radioactive thyroid hormones. When administered intravenously, hormones have access to the brain via both the BBB and the CP-CSFB. However, hormone injected intrathecally represents entry into brain cells via the CP-CSFB. After injection of radioactive hormones via these two routes and subsequent autoradiography of the brain, distribution of thyroid hormone over brain areas could be documented as well as the contribution of the BBB and the CP-CSFB to brain accessability (151–153). These studies demonstrated that T_3 and T_4 enter the brain mainly via the BBB for distribution throughout the brain, but that localization in the ependymal cells and in the circumventricular organs occurs via the CP-CSFB. In contrast, rT_3 is excluded by the BBB but has limited access to the brain via the CP-CSFB (Fig. 3).

Also, by *in vivo* injection of tracer hormones, the question of whether TTR has a special role in transport of thyroid hormone to the brain via the CP-CSFB was addressed. Results of studies in rats and sheep, showing accumulation of thyroid hormone in the choroid plexus, led to the proposal of a model for T_4 transport from the bloodstream into the CSF, involving uptake of T_4 by the choroid plexus, binding

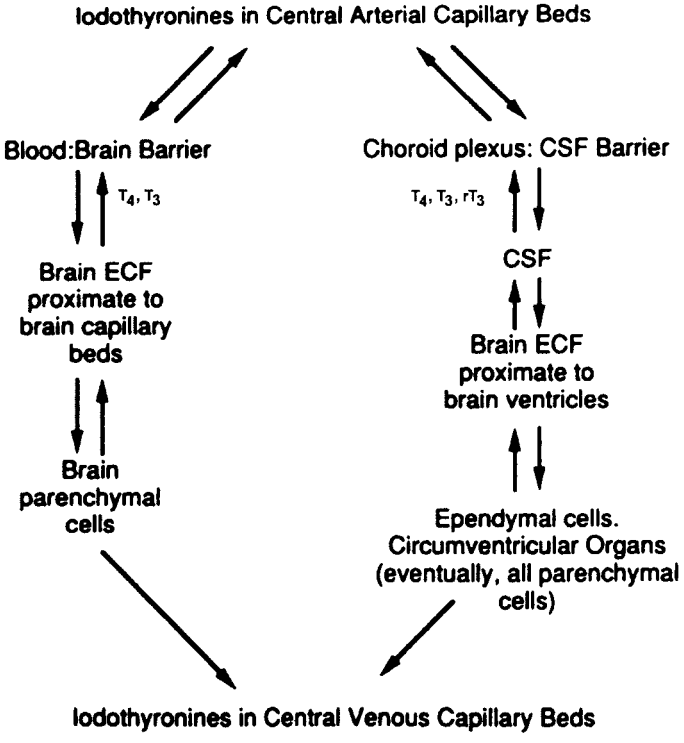

FIG. 3. Routes of iodothyronine transport between blood and brain. According to autoradiographic results, rT_3 crosses the CP-CSFB but not the BBB, whereas T_3 and T_4 cross both BBB and CP-CSFB. [Derived from Ref. 153.]

of the hormone to newly synthesized TTR, and secretion of the complex into the CSF (140, 154–156). Recent studies in the TTR-null mouse mutant showed that total lack of TTR seems to have no consequences for normal development and fertility (157, 158). In these mice, serum levels of free T_4, free T_3, and TSH were normal as were the type I and II deiodinase activities (being very sensitive to the thyroid status of the tissue) in liver and brain, respectively (157). Analysis of tracer hormone kinetics showed that T_4 tissue content of liver and kidney was little affected, but was decreased in the brain. T_3 content of these tissues was normal. The low T_4 content of brain was explained on the basis of absence of TTR-T_4 complexes, apparently without repercussion for normal local T_3 production from T_4. These studies show that TTR is not essential for sufficient transport of thyroid hormones into brain and other organs. It seems that as long as the free hormone concentration is kept constant, probably by virtue of the presence of other thyroid hormone-binding proteins in blood and other body fluids, no apparent harm is done to tissue metabolism. In this respect, it is noteworthy that a similar situation exists in humans with complete TBG deficiency, who also show no apparent biological abnormality (159). However, it is remarkable that genetic abnormalities associated with complete TTR deficiency have so far not been documented in humans or animals.

B. Other organs

The liver is another organ that has been studied in animals for plasma membrane transport of thyroid hormone. Pardridge *et al.* published a series of *in vivo* studies in the rat (for review see Ref. 160). From their studies the authors concluded that thyroid hormone delivery to the liver "occurs via the free intermediate mechanism, *i.e.*, protein-bound hormone debinding is an obligatory intermediate step in the transport process." Although they found that transport of T_4 into rat brain via the BBB is a saturable process, they could not find saturability of plasma membrane transport in rat liver, and suggested that this occurred via passive diffusion. The authors used for their studies a single capillary pass technique for analysis of initial kinetics of transport (160). The model used by Pardridge *et al.* and their interpretation of the data were strongly contested (161, 162). The main criticism concerned the rate-limiting role in the transport process that was attributed to the dissociation of hormone from serum binding proteins. No such role could be envisaged, both on theoretical and experimental basis, by these opponents. Others documented hepatic uptake in mice, injected *in vivo* with radioactive T_3, using autoradiography (163). Excess unlabeled T_3 resulted in 90% inhibition of liver uptake of labeled T_3. Time sequence autoradiographic analysis showed that the plasma membrane is initially labeled before internalization of T_3 occurs (163). These results clearly document *in vivo* specific binding of T_3 to the liver plasma membrane as an initial step to internalization of the hormone. *In vivo* injection of rats with radiolabeled T_4 and subsequent measurement of uptake in heart and lung tissue, isolated at different time intervals, showed that T_4 transport in these organs was also saturable, in accordance with a carrier-mediated transport mechanism (164).

In summary, brain entry of T_4 in dogs appears to proceed via a carrier-mediated mechanism. This was also found for brain uptake of T_3, but not of T_4, in the mouse. It was further shown in the rat that T_3 and T_4 mainly enter the brain via the blood-brain barrier for distribution throughout the brain, and via the CP-CSF barrier for restricted distribution in circumventricular areas. Although it has been envisaged for a long time that TTR expressed in the choroid plexus plays an essential role in the transport of thyroid hormones into the brain, total lack of the protein in TTR knock-out mice has no effect on concentrations of plasma free thyroid hormones and TSH or on tissue thyroid hormone status. *In vivo* studies have shown saturable T_3 uptake into rat liver and saturable T_4 uptake into mouse lung and heart.

VII. Plasma Membrane Transport in Humans

A. Introduction

In healthy individuals, about 80% of plasma T_3 is produced outside the thyroid gland, the remaining 20% being secreted directly by the thyroid (165). In the extrathyroidal pathway, T_3 is produced by outer ring deiodination of T_4, and in this process the type I deiodinase in the liver (and kidneys) plays an important role (165, 166). Another organ that may be involved in this pathway in humans is skeletal muscle, expressing the type II deiodinase that also catalyzes the conversion of T_4 to T_3 (167). To reach the intracellular T_3-producing enzymes, T_4 must cross the plasma membrane of these tissues. It has been established in rats that the extent to which nuclear receptor-bound T_3 is derived from plasma T_3 and from local T_3 production from T_4 varies among the tissues. Thus, for instance, nuclear T_3 in cerebral cortex is derived for ≈80% from local conversion of T_4, in pituitary for ≈50%, in skeletal muscle for ≈40%, and in liver for only ≈5% (168, 169). In other words, for exertion of biological activity by nuclear T_3, both T_4 and T_3 must cross the plasma membrane of target cells. It follows that the activity of these transport processes may have an important influence on the regulation of the biological activity of thyroid hormone. Although the exact contribution of the different sources of nuclear T_3 in human tissues is unknown, it will also depend to varying degrees on plasma membrane transport of T_3 and its precursor T_4.

Many reports have dealt with the measurement of thyroid hormone distribution and metabolism in humans. However, few of these are concerned with analysis of unidirectional transport of thyroid hormones into tissues. To study regulation of biological processes, it is in general necessary to analyze these under circumstances of perturbation of the physiological steady state. This is certainly also true for the study of the regulation of thyroid hormone transport into tissues. Both in starvation and in so-called nonthyroidal illness (see *Section VII.C*), plasma T_3 production is decreased. As the diminution in plasma T_3 production may be substantial and thyroidal secretion of T_3 contributes only little to total plasma T_3, the main cause of this diminution in T_3 production must consequently be located in the extrathyroidal pathway. Both starvation and nonthyroidal illness have been used as models to study regulation of thyroid hormone penetration

into target tissues. Two possibilities have been suggested to be responsible for the lowered T_3 production in these situations, *i.e.*, a decrease in outer ring deiodinase activity in plasma T_3-producing tissues and/or a decrease of T_4 transport into these tissues as substrate for T_3 production. There is evidence in animals, but not in humans (170), that outer ring deiodination is indeed lowered in starvation and in nonthyroidal illness, but this aspect will not be further discussed here. For further orientation, the reader is referred to Ref. 171. In this section we will discuss plasma membrane transport of thyroid hormones in human tissues both in starvation and in nonthyroidal illness.

B. In starvation

In caloric deprivation, as in nonthyroidal illness (see *Section VII.C*), abnormalities in serum thyroid function parameters are invariably present. The most constant and thus characteristic abnormality is a low serum T_3 concentration; hence the term "low T_3 syndrome" for this entity. Serum T_4 and TSH are usually normal, whereas serum rT_3 is usually elevated (for a review see Ref. 172). To our knowledge the first published study that was primarily designed to evaluate unidirectional transport of thyroid hormones into tissues before and during caloric deprivation in man was published in 1986 by our laboratory (170). In this study T_4 and T_3 kinetics were studied using a three-pool model of thyroid hormone distribution and metabolism in 10 obese but otherwise healthy subjects before dieting and while on a 240-kcal diet. During caloric restriction, unidirectional transport of T_4 and T_3 into the rapidly equilibrating tissues (liver) was decreased by 50% and 25%, respectively, when corrected for changes in free hormone concentration. The decrease in plasma T_3 production amounted to 42%, about equaling the reduction in T_4 transport into the liver. T_4-to-T_3 conversion rate decreased by an insignificant 8%. Therefore, the lowered T_3 production during caloric deprivation is largely, if not fully, explained by a decrease of T_4 entry into T_3-producing tissues. The fasting-induced decrease in liver T_4 transport may be explained, at least in part, by a decrease in the energy charge of liver cells. This explanation is based on at least two points. First, it has been shown that starvation leads to ATP depletion of the liver as assessed by ^{31}P-magnetic resonance spectroscopy (173). Second, tissue T_4 transport was much more affected by caloric deprivation than transport of T_3, similar to findings of T_4 and T_3 transport in cultured rat hepatocytes deficient in ATP (Ref. 48 and Fig. 1). To further substantiate the effect of the intracellular ATP concentration on hepatic T_4 uptake *in vivo* in humans, liver T_4 uptake was measured in four healthy human volunteers, using T_4 tracer plasma kinetics, before and after an intravenous bolus injection of fructose, which is known to transiently decrease liver ATP levels. Obviously, hepatic ATP could not be measured, but fructose was found to induce an increase in serum lactic acid and uric acid concentrations, reflecting a decrease in liver ATP. After fructose administration there was a temporary decrease in liver T_4 uptake that normalized after fructose was metabolized and hepatic ATP concentrations were restored, as reflected by the normalization of serum lactic acid and uric acid levels (134). In contrast to the transient effect of fructose, transport of T_4 into the liver remained suppressed when the same subjects were studied on a calorie-restricted diet (Fig. 4). As will be discussed in *Section VII.C*, NEFAs that circulate in increased concentrations during caloric restriction have an additional inhibitory effect on T_4 uptake by the liver.

We also studied renal handling of T_4 and T_3 in humans during fasting (174). The results suggested inhibition of T_4 and T_3 uptake at the basolateral membrane of the tubular cells in the kidney. As to the cause of this inhibition, several factors were proposed, including a decreased energy state of the cells, the existing acidosis, and/or inhibition of transport by the increased serum NEFA concentration.

C. In nonthyroidal illness

NTI may be defined as any acute or chronic illness, not related to the thyroid gland, that is accompanied by an abnormal pattern of thyroid function parameters. Other terms that are synonymously being used are the "low T_3 syndrome" because serum T_3 is invariably low in NTI, and the "euthyroid sick syndrome" because patients are usually clinically euthyroid despite the low serum T_3 and sometimes also

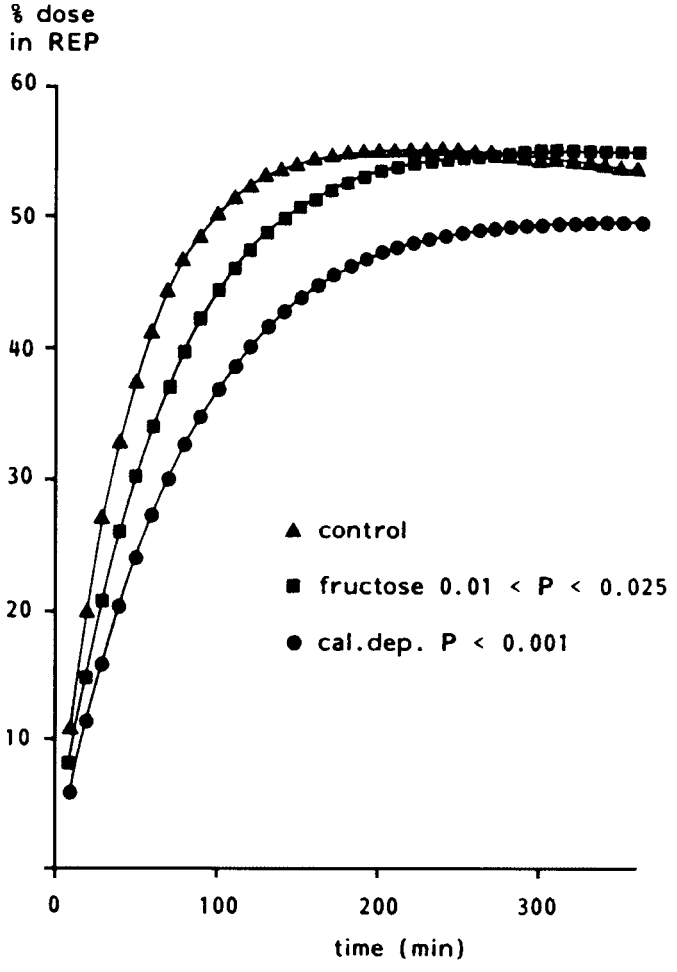

FIG. 4. Computed kinetics of T_4 uptake into the rapid equilibrating pool (REP, representing largely liver) in four obese volunteers, before (▲) and during (●) caloric deprivation and after intravenous fructose (■). [Derived from Refs. 134 and 170.]

low T_4 levels. With an increase in severity of disease there is a progressive decrease in serum T_3 and, in most diseases, an increase in serum rT_3 that eventually plateaus. Serum T_4 is usually normal but may be slightly increased in mild disease and lowered in critical illness (Fig. 5). Serum TSH is usually normal but may be depressed in severe illness (175, 176). Many studies of thyroid hormone distribution and turnover kinetics in patients with NTI have been reported (for reviews see Refs. 171,175–177). In general, they show that T_4 production rates are normal, except in severe illness when it is decreased, but that T_4 transport into tissues is decreased. Plasma rT_3 production, virtually all originating in type III deiodinase-containing tissues, such as brain (177), is normal in NTI, while the plasma rT_3 clearance, almost exclusively by the liver (178), is decreased. Plasma T_3 production rates are invariably decreased in proportion to the severity of disease, while plasma T_3 clearance is generally little affected (175). Few studies, mostly by Kaptein *et al.* (179–183), reported on the analysis of unidirectional T_4 transport into tissues during NTI to determine its possible contribution to low plasma T_3 production. Thus, in a group of 11 patients with acute critical illness, T_4 transport into tissues was inhibited by ≈50% and T_3 plasma production decreased by ≈70%. From this analysis it is not known to what extent inhibition of T_4 transport occurs in T_3-producing tissues, predominantly the liver (see *Section VII.A*). In another study in 15 patients with NTI due to various causes (180), these authors found an inhibition of T_4 transport into the rapidly equilibrating pool (representing liver and kidneys) by ≈30% and into the slowly equilibrating pool (representing the remaining tissues) by ≈65%. Plasma T_3 production rates were not reported in this study. In patients with chronic renal failure, tissue transport of T_4 was inhibited by ≈50%, but no data were presented for T_3 production (181). In contrast to most patients with NTI, who show normal plasma rT_3 production but decreased plasma rT_3 clearance (see *Section VII.A*) and thus elevated rT_3 plasma concentrations, this and other studies (for review see Refs. 182 and 183) demonstrate that patients with CRF have normal plasma rT_3 levels, clearance rates, and production rates. The fact that plasma T_4 clearance is much more affected than that of T_3 is in agreement with similar findings in fasting humans (see *Section VII.B*), and suggests that hepatic ATP depletion may also be important here, which does not seem illogical since NTI patients are mostly, if not always, in a negative energy balance.

We also considered the possibility of circulating inhibitors of thyroid hormone uptake in NTI. In the presence of serum from patients with severe NTI, T_4 uptake by rat hepatocytes was ≈50% lower than in the presence of serum from healthy controls, without any direct effect on the deiodination process (184). Further characterization of the factors responsible for this inhibition identified several compounds circulating at increased serum concentrations in patients with NTI, including CMPF and indoxyl sulfate in patients with renal failure (108), and bilirubin and NEFAs in nonuremic critically ill patients (109). It also appeared that in mild NTI and during caloric restriction in obese subjects, serum NEFAs are increased to levels that inhibit hepatocyte uptake of T_4 (110). Remarkably, T_4 uptake in the rat pituitary is not inhibited by concentrations of CMPF, indoxyl sulfate, and bilirubin that inhibit T_4 uptake in hepatocytes (185, 186). In addition, T_3 and T_4 uptake was normal in rat pituitary cells with low ATP concentration due to culture in an energy-poor medium. These phenomena indicate different effects of pathophysiological factors on the common pituitary transporter for both T_4 and T_3 (Table 3, Refs. 68–70) compared with the specific T_4 transporter in the liver. We hypothesized that this differential transport handling may serve to maintain low T_3 production in starvation and NTI, by allowing T_3, T_4, and the bioactive metabolites triiodothyroacetic acid and 3,5-diiodothyronine (187, 188), which circulate at increased levels in NTI (189, 190), and possibly also 3,3′,5,5′-tetrathyroacetic acid (191, 192), to enter the pituitary to prevent any compensatory increase in TSH (193). As a low T_3 level is asso-

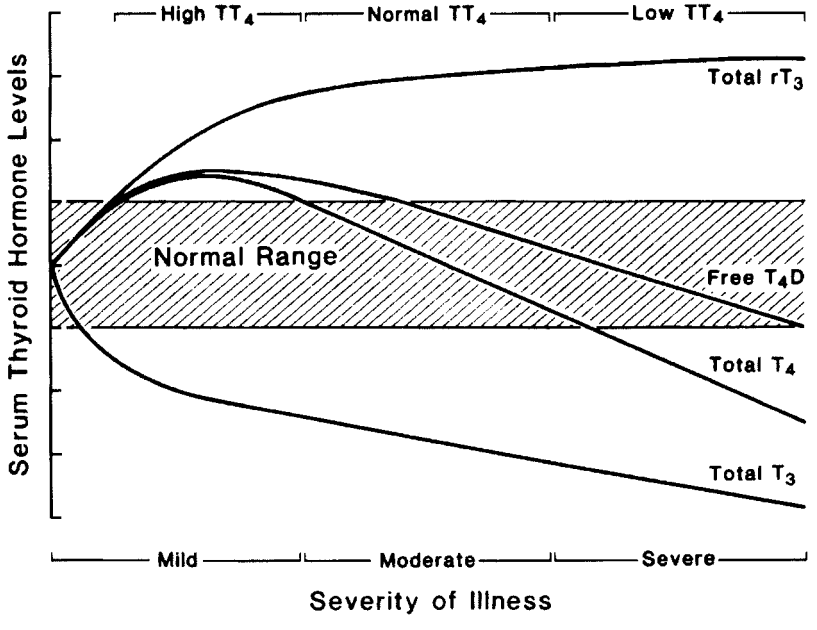

FIG. 5. Schematic representation of the changes in serum thyroid hormone levels in patients with nonthyroidal disorders relative to the severity of the illness. [Reproduced with permission from E. M. Kaptein. In: Thyroid hormone metabolism. New York: Marcel Dekker, 1986 (175).]

ciated with conservation of energy and possibly also protein, it is considered by some as a defense mechanism in situations of stress. This point, however, is controversial as conflicting results have been obtained in studies of this protein-sparing effect. For further orientation about this subject, the reader is referred to Ref. 171.

In summary, most plasma T_3 is produced by conversion of T_4 in peripheral tissues, in particular the liver. Nuclear receptor-bound T_3 in different tissues is derived to varying extents from plasma T_3 or from local deiodination of T_4. Thus, the exertion of the biological activity of thyroid hormone requires the transport of T_4 and T_3 across the plasma membrane. Analyses of thyroid hormone kinetics in humans during caloric restriction revealed a 50% inhibition of hepatic T_4 transport, roughly equal to the 40% decrease in plasma T_3 production, whereas the T_4-to-T_3 conversion in the liver was not affected. These findings suggest a rate-limiting role of hepatic T_4 transport for plasma T_3 production. The inhibition of T_4 transport was ascribed to hepatic ATP depletion by fasting. Liver ATP depletion by fructose infusion in humans indeed leads to a concomitant decrease of hepatic T_4 transport. In nonthyroidal illness, apart from a decrease of liver ATP, increased plasma concentrations of compounds such as CMPF, indoxyl sulfate, bilirubin, and NEFAs may inhibit T_4 transport into the human liver, thereby contributing to the low plasma T_3 production in this condition. NEFA concentrations are also elevated in starvation and may thus contribute to decreased hepatic T_4 uptake and T_3 production during caloric deprivation.

VIII. Requirements for a Regulatory Role of Plasma Membrane Transport in the Bioavailability of Thyroid Hormone

Although it has been amply discussed in the previous sections that in most, if not all, cells thyroid hormones cross the plasma membrane by a carrier-mediated (often energy-dependent) mechanism, its significance for the regulation of the bioavailability of thyroid hormone has not yet been addressed. This will be done in the following sections.

Certain requirements must be fulfilled before it can be concluded that the process of transport across the plasma membrane of target cells is potentially regulatory for the bioavailability of thyroid hormone and thus may have a role in the regulation of thyroid hormone bioactivity. These requirements are depicted in Table 5 and are discussed below.

A. Specificity of plasma membrane transport

Specificity of transport indicates that only structurally related substances are being transported or compete with the transport system. These systems are saturable and usually have limited capacity. Specificity of thyroid hormone transport into target cells has been substantiated for many cell types from many species as discussed in the different sections above. In some, but not all, cell types two systems have been detected for uptake of iodothyronines (Tables 1–3). If two systems were identified, the high-K_m site was attributed to binding of thyroid hormone to protein trapped in the water layer around the cell or associated with the cell surface (45). There is little doubt that in most cell types stereospecific transport of thyroid hormone across the plasma membrane occurs. The reported K_m values of transport varied but were mostly in the nanomolar range (Tables 2 and 3). The use of different conditions and techniques as well as the tissue-specific distribution of different transporters (see *Section IX*) may account for this variation. A point of apparent discrepancy is the fact that some laboratories could not identify a specific T_4 transport system whereas others could. This fact is probably related to the phenomenon that T_4 transport into cells, at least into the hepatocyte, is much more sensitive to suboptimal cellular ATP concentrations than T_3 transport (Ref. 48, *Section III.B.2*, and Fig. 1). When studies of T_4 transport are not focused on this aspect (92, 93), T_4 transport may become undetectable.

TABLE 5. Characteristics of plasma membrane transport of thyroid hormone required for its potential function in the regulation of thyroid hormone bioavailability

1. Specificity of plasma membrane transport
2. Absence of significant diffusion
3. Plasma membrane transport is subject to regulation
4. Transport is rate-limiting for subsequent metabolism

B. Absence of significant diffusion

If a significant proportion of thyroid hormone transport across the plasma membrane would take place by diffusion, it is obvious that this would diminish the role of the plasma membrane in the regulation of hormone uptake. There is substantial evidence, on both theoretical and experimental grounds, that little or no diffusion occurs in the transport process. Thus, although overall iodothyronines are lipophilic compounds, the highly polar zwitter-ionic nature of the alanine side chain prevents passage of the molecule through the hydrophobic inner core of the lipid bilayer of the plasma membrane. Experimental evidence has also been provided that diffusion hardly takes place if at all. Thus, using an electron spin resonance stop-flow technique, it was shown that a spin-label derivative of T_3 does not flip-flop at any appreciable rate in phospholipid bilayers and that, after partitioning into the membrane, it remains in the outer half of the bilayer (194). In other words, if no specific transport sites were present in the membrane of target cells, thyroid hormones would not be able to cross the plasma membrane. Using a monoclonal antibody raised against a rat hepatocyte surface epitope involved in thyroid hormone transport, a concentration-dependent inhibition of the transport of T_3 and T_4 was observed, with 100% inhibition at a low (1:100) antiserum dilution (40). The same monoclonal antibody also strongly inhibited uptake of T_4, T_3, and rT_3 in cultured human hepatocytes (52). In rat anterior pituitary cells and also in *Xenopus laevis* oocytes, minimal, if any, uptake of T_3 sulfate (T_3S) was detected, in contrast to specific uptake of T_4 and T_3 in these cell types (195, 196). However, injection of rat liver mRNA induced uptake of T_3S in these oocytes (Ref. 197 and Fig. 6). These observations indicate that diffusion plays no role in transmembrane transport of sulfated iodothyronines.

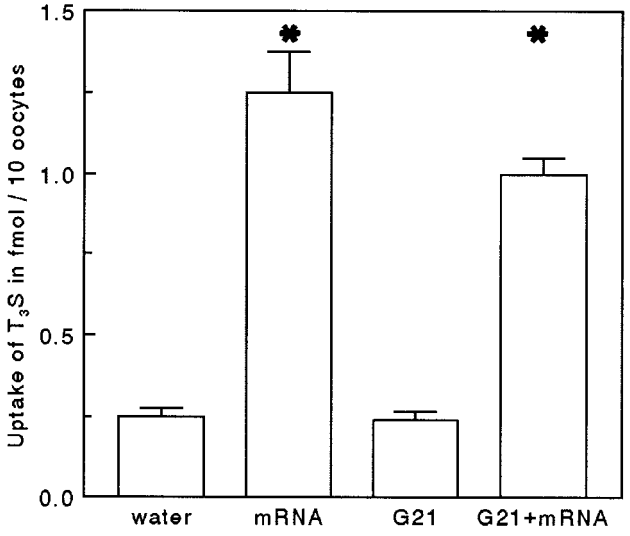

FIG. 6. Initial uptake of T_3S in *X. laevis* oocytes injected with water (control), fractionated rat liver mRNA, cRNA for rat liver type 1 deiodinase (G21), or both (G21 + mRNA). Values are means ± SEM; *, $P < 0.001$ *vs.* water. [Modified reproduction with permission from R. Docter *et al.*: *Endocrinology* 138:1841–1846, 1997 (197). © The Endocrine Society.]

C. Plasma membrane transport is subject to regulation

As the serum concentrations of free T_3 and free T_4 are in the picomolar range, whereas their apparent K_m values for the plasma membrane transporters are in the nanomolar range, no regulation of transport occurs by the process of saturation. However, as pointed out above (see *Sections III, V,* and *VII*) thyroid hormone transport into cells, except maybe for erythrocytes, is dependent on the energy state of the cell and often on the Na^+ gradient over the plasma membrane. Thus, cellular ATP and the Na^+ gradient may be important factors in the regulation of the activity of thyroid hormone transporters (Tables 2 and 3), while thyroid hormone uptake will also depend on the number of transporters located in the cell membrane. The latter is determined not only by the balance between the rates of synthesis and degradation of these proteins but also by mechanisms regulating their translocation between intracellular organelles and the plasma membrane. Circulating inhibitors such as CMPF, indoxyl sulfate, bilirubin, NEFAs, and amino acids (Refs. 108–110 and *Section III.C.1*) are also involved in the regulation of thyroid hormone uptake, especially in starvation and nonthyroidal illness. However, in tissues in which thyroid hormone is taken up by amino acid transporters that mediate exchange between extra- and intracellular ligands, hormone uptake is subject not only to *cis*-inhibition by extracellular amino acids but also to *trans*-stimulation by intracellular amino acids.

The possible effects of thyroid state on the rate of thyroid hormone uptake has been studied in rat liver. When livers of hypothyroid rats were perfused, uptake of T_3 was not different from normal, but T_3 metabolism was decreased. In livers of hyperthyroid rats, uptake of T_3 was decreased and T_3 metabolism was increased. These data suggest an adaptation mechanism at the cellular level to maintain tissue T_3 levels when T_3 supply is abnormal (198). When expression of mRNA of thyroid hormone transporters in rat liver was studied, using *Xenopus laevis* oocytes as the expression system, no thyroid state-dependent differences were seen in the expression of these transporters, not excluding, however, any regulation of transporter activity at the translational or posttranslational level (199).

Thus, although questions remain, a number of factors, both intracellular and circulating, have been identified that determine the amount of thyroid hormone taken up by target cells.

D. Transport is rate limiting for subsequent metabolism

Plasma membrane transport is rate limiting for cellular thyroid hormone metabolism if any change in transport results in proportional alterations in subsequent metabolism. This implies that influx of thyroid hormones is independent of intracellular metabolic capacity. When rat hepatocytes in primary culture were incubated with T_4, T_3, or rT_3 in the presence of an iodothyronine transport-blocking monoclonal antibody or ouabain to lower the Na^+ gradient over the plasma membrane, a decreased clearance from the medium of these iodothyronines was found that paralleled a decreased iodide production (Table 6). As it was shown that the added compounds had no effect on intracellular deiodinase activity, it was concluded that the decreased iodide production was caused by the inhibition of iodothyronine uptake (50). In addition it was reported from different laboratories that compounds that inhibit T_3 uptake at the plasma membrane level, and do not influence nuclear binding of T_3 *per se*, effected a decrease in nuclear occupancy that paralleled the inhibition of uptake, indicating that cellular uptake controls T_3 access to its receptors (77, 122, 200). These findings were obtained using rat pituitary tumor cells, hepatocytes, and skeletal myoblasts. Furthermore, uptake of T_3S induced in *X. laevis* oocytes by injection of fractionated rat liver mRNA was not affected by coinjection with cRNA coding for type I deiodinase. Thus, an increase in the capacity of oocytes to

TABLE 6. Remaining iodothyronine and iodide released in medium after incubation of rat hepatocytes in monolayer culture with T_4, T_3, or rT_3 in the absence (control) or presence of uptake inhibitors ER-22 (monoclonal antibody) and ouabain

	Percentage (mean ± SEM)	
	Iodothyronine	Iodide
T_4		
Control	82.9 ± 0.8	12.7 ± 0.4
ER-22	90.7 ± 1.2[a]	6.9 ± 0.6[a]
Ouabain	92.2 ± 1.2[a]	7.0 ± 0.6[a]
T_3		
Control	32.0 ± 1.4	51.5 ± 0.6
ER-22	64.8 ± 1.4[a]	24.6 ± 0.6[a]
Ouabain	66.2 ± 1.4[a]	21.5 ± 0.6[a]
rT_3		
Control	45.8 ± 0.9	54.1 ± 0.5
ER-22	62.8 ± 1.1[a]	36.9 ± 0.7[a]
Ouabain	56.8 ± 1.6[a]	41.0 ± 0.9[a]

[a] Significantly different from control, $P < 0.001$. [Reproduced with permission from G. Hennemann *et al.*: *Endocrinology* 119:1870–1872, 1986 (50). © The Endocrine Society.]

metabolize T_3S did not affect T_3S uptake (Fig. 6 and Ref. 197). Obviously, the rate of T_3S metabolism was stimulated by both induction of T_3S transport and induction of deiodinase activity. A remarkable finding was reported by our laboratory in support of the clinical relevance of inhibited hepatic T_4 transport as a cause for a decrease in T_3 production (58, 201). When rat hepatocytes in primary culture were incubated with T_4 in the presence of serum from patients with NTI, a strong correlation ($r = 0.69$) was observed between residual transport of T_4 into the hepatocytes and the serum T_3 concentration in these subjects (Fig. 7). In other words, the more inhibition of T_4 transport exerted by the serum, the lower the serum T_3 concentration of that particular patient.

There is evidence that *in vivo* inhibition of T_4 transport into the liver is also rate-limiting for total plasma T_3 production in humans. In a female in her 60s, an increased serum free T_4 concentration was present in combination with a low plasma T_3 concentration in the absence of NTI or any abnormality of serum thyroid hormone-binding proteins (202). Iodothyronine kinetic studies revealed that T_4 uptake (and content) in the rapidly equilibrating compartment, comprising mainly the liver (and kidneys), was inhibited, but uptake in the slowly equilibrating compartment, consisting of the other tissues, was normal (Fig. 8). T_3 uptake was normal in both compartments. Plasma T_3 production was subnormal, but the ratio of T_3 production over hepatic T_4 uptake or T_4 content was normal. It was concluded from these data that the lowered plasma T_3 production was caused by inhibition of T_4 uptake into the liver, leading to a decrease in substrate available for conversion to T_3, whereas the liver capacity to produce and secrete T_3 was unimpaired (202). We have identified this abnormal serum thyroid hormone profile also in another subject (203). In this latter subject, serum TBG was elevated and normalized upon administration of physiological amounts of T_3. As TBG may be elevated in hypothyroidism, this suggests that the lowered T_3 production caused hypothyroidism at the level of the liver. These human studies suggest that inhibition of T_4 transport into the liver, leading to lowered T_3 production, has biological consequences.

In summary, to play an important role in the regulation of tissue thyroid hormone bioavailability, the mechanism of transport of thyroid hormone over the plasma membrane must fulfill certain requirements (Table 5). Thus, plasma membrane transport should be specific, subject to regulation, and rate limiting for subsequent thyroid hormone metabolism. This implies that there is only limited or no diffusion into target cells such that influx of hormone is largely effected by specific transporters. Collectively, the studies discussed in this section have demonstrated that this is indeed the case in liver and many other tissues. Hepatic uptake of thyroid hormone is regulated by the energy charge of the cells, and also by compounds that circulate at increased levels in humans during starvation (NEFAs) and nonthyroidal illness (NEFAs, CMPF, indoxyl sulfate, and bilirubin). The reduced T_4 transport into the liver is a major cause for the decreased plasma T_3 production in these conditions.

IX. Identification of Thyroid Hormone Transporters

A. Organic anion transporters

Recently, we have explored the possibility to clone iodothyronine transporters from rat liver using *X. laevis* oocytes as an expression system (197, 204–207). A modest increase in T_4 and T_3 uptake was induced by injection of oocytes with rat liver mRNA, in particular the 0.8–2.1 kb size fraction, above the background iodothyronine uptake by native oocytes (197). Much lower background uptake was observed with the sulfonated iodothyronine derivatives, T_3 sulfate (T_3S), T_4 sulfate (T_4S), T_3 sulfamate (T_3NS), and T_4 sulfamate (T_4NS), resulting in much larger relative inductions by injection with rat liver mRNA (197, 204). Uptake of these water-soluble derivatives was competitively inhibited by T_4 and T_3, suggesting that they are alternative ligands for the iodothyronine transporters (197, 204). Since the sulfonated compounds are organic anions, we tested the hypothesis that hepatic uptake of iodothyronine derivatives is mediated, at least in part, by organic anion transporters, in particular

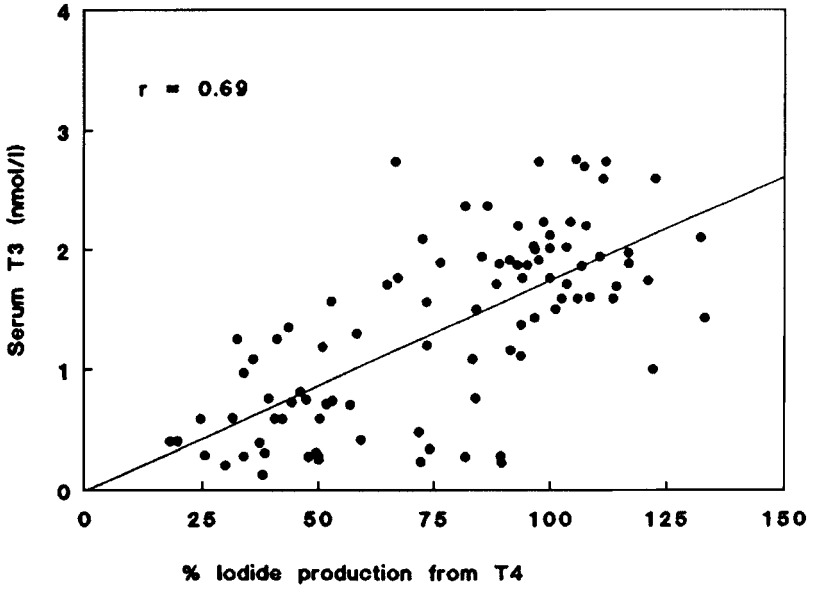

FIG. 7. Relationship between iodide production from T_4 (corrected for differences in free hormone concentration) in the presence of 10% NTI serum, expressed as percentage of iodide production in the presence of 10% serum of healthy controls and serum T_3. [Reproduced with permission from R. A. Vos *et al.*: *J Clin Endocrinol Metab* 80:2364–2370, 1995 (58). © The Endocrine Society.]

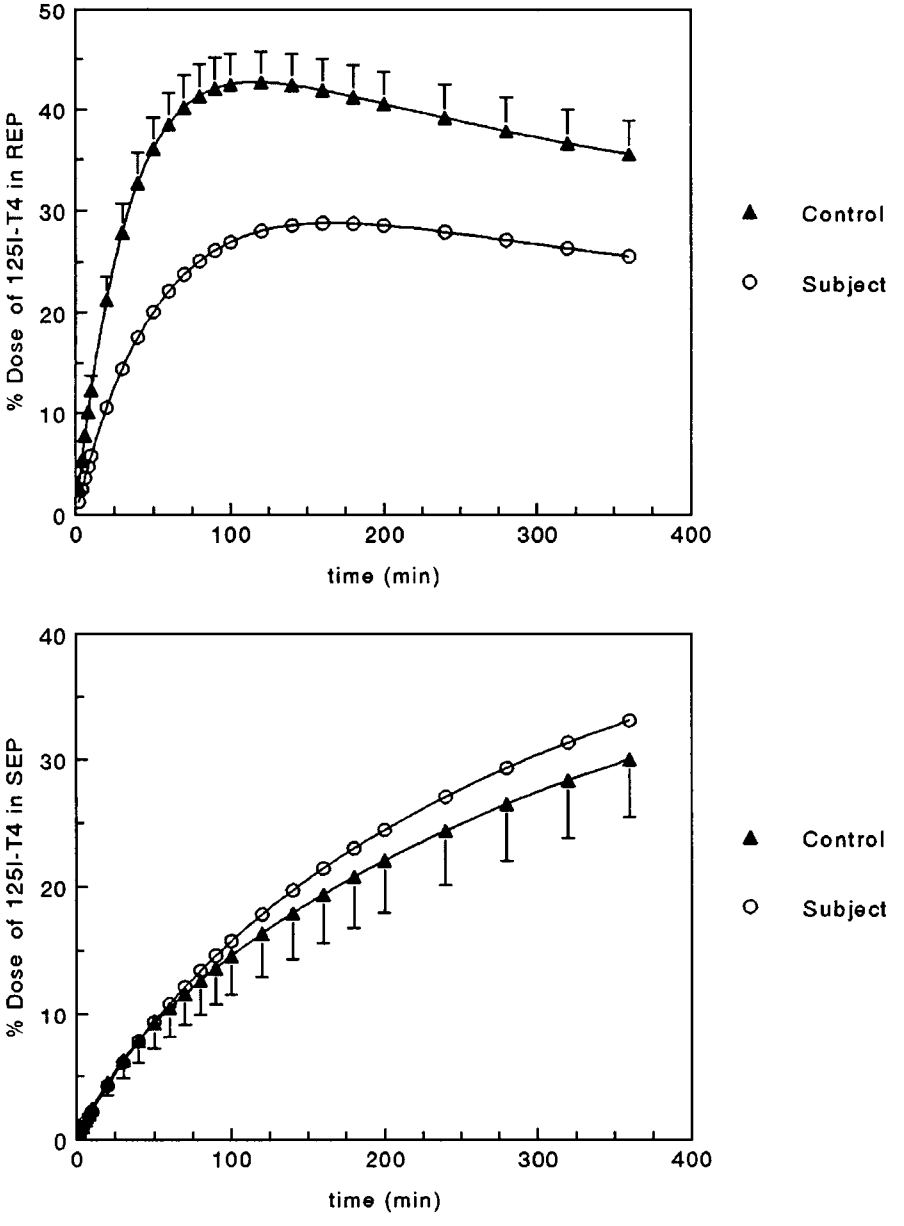

FIG. 8. T_4 uptake into the rapidly equilibrating pool (REP, *upper panel*) and the slowly equilibrating pool (SEP, *lower panel*) of a subject with reduced peripheral T_3 production (○) and control subjects (▲) during the first 400 min of T_4 tracer kinetics. Values are the mean ± SEM. [Reproduced with permission from G. Hennemann *et al.*: *J Clin Endocrinol Metab* 77:1431–1435, 1993 (202). © The Endocrine Society.]

Na^+/taurocholate-cotransporting polypeptide (NTCP) and the (Na^+-independent) organic anion transporting polypeptides (OATPs) (208, 209).

Human and rat NTCP are 349- to 362-amino acid proteins containing seven putative transmembrane domains and two glycosylation sites with an apparent molecular mass of ≈50 kDa (208–211). This transporter is now also known as solute carrier family 10, member 1 (SLC10A1). NTCP is only expressed in hepatocytes, where it is localized selectively to the basolateral cell membrane (208, 209). It is the major transporter of conjugated bile acids in liver, but it also mediates uptake of unconjugated bile acids and a number of non-bile acid amphipathic compounds, including estrogen conjugates such as estrone 3-sulfate (208, 209). A homologous bile acid transporter is expressed in ileum and kidney, where it is localized to the apical cell membrane (212–215). The OATPs constitute a large family of homologous Na^+-independent transporters, which are now comprised in the solute carrier family 21 (SLC21). Seven members of this family have been identified in rats, *i.e.*, rOATP1–5 (216–221), rOAT-K1 (222), and splice variant rOAT-K2 (222, 223), and the PG transporter rPGT (224); eight members in humans, *i.e.*, hOATP-A to -F (225–230), hOATP8 (231), and hPGT (232); and two members in mice, *i.e.*, mOATP1 (233, 234) and mPGT (235). rOATP1 was the first identified member of this transporter family, representing a 670-amino acid protein with 12 transmembrane domains and 2 glycosylation sites with an apparent molecular mass of 80 kDa (208, 209). The other OATP transporters have similar structures. The tissue distribution of the OATPs varies among the different members, *e.g.*, rOATP1 and rOATP2 are expressed in liver, kidney, and brain, rOATP4 and hOATP-C (*alias* hLST-1, liver-specific transporter) are expressed exclusively in liver, and rOAT-K1 and -K2 are expressed selectively in kidney. Like NTCP, the

OATPs expressed in liver are localized to the basolateral cell membrane. It is interesting to note that in brain both rOATP1 and rOATP2 show prominent localization in the choroid plexus, which may be an important gate of thyroid hormone to the brain (209). The OATPs are multispecific transporters, mediating the uptake of a wide variety of amphipathic ligands, not only anionic (*e.g.*, conjugated and unconjugated bile acids, conjugated steroids, bromosulfophthalein), but also neutral (*e.g.*, steroids, cardiac glycosides), and even cationic (*e.g.*, ajmalinium) compounds (208, 209). For different OATPs, it has been demonstrated that they facilitate the exchange of intra- and extracellular anions (236, 237). Intracellular reduced glutathione (GSH) is an important intracellular ligand, the efflux of which down its large electrochemical gradient provides the driving force for uptake of extracellular ligands (236). Figure 9 shows the phylogenetic tree of the OATP transporter family.

We observed marked stimulation of the uptake of native iodothyronines as well as their sulfamate and sulfate derivatives after injection of oocytes with cRNA for rNTCP, hNTCP, rOATP1, rOATP2, or hOATP-A (206, 207). The Na^+ dependence of the NTCPs and the Na^+ independence of the OATPs were confirmed with all these ligands. Significant transport of T_4 and T_3 has also been reported by others for rOATP2 (218), rOATP3 (218), and rOATP4 (219), but not its splice variant rLST-1 (220), for hOATP-C, *alias* hLST-1 (227, 228), and human and rat hOATP-E (238). The degree of stimulation of iodothyronine uptake varied among the different OATP family members, *e.g.*, rOATP1 showed highest iodothyronine transport with T_4 and rT_3, and hOATP-A with T_3 as ligand (207). Apparent K_m values were determined for T_4 and T_3 transport by rOATP2, rOATP3, and hOATP-C, and found to be in the micromolar range (218, 227). Together, these data suggest that tissue uptake of thyroid hormone may be mediated in part by different Na^+-dependent and Na^+-independent organic anion transporters, although the NTCPs and OATPs do not represent the high-affinity iodothyronine transporters detected in different tissues. Studies of the induction of iodothyronine transport by injection of *Xenopus* oocytes with liver mRNA size fractions have indicated the existence of a major Na^+-dependent transporter in addition to rNTCP and rOATP1 (204, 207).

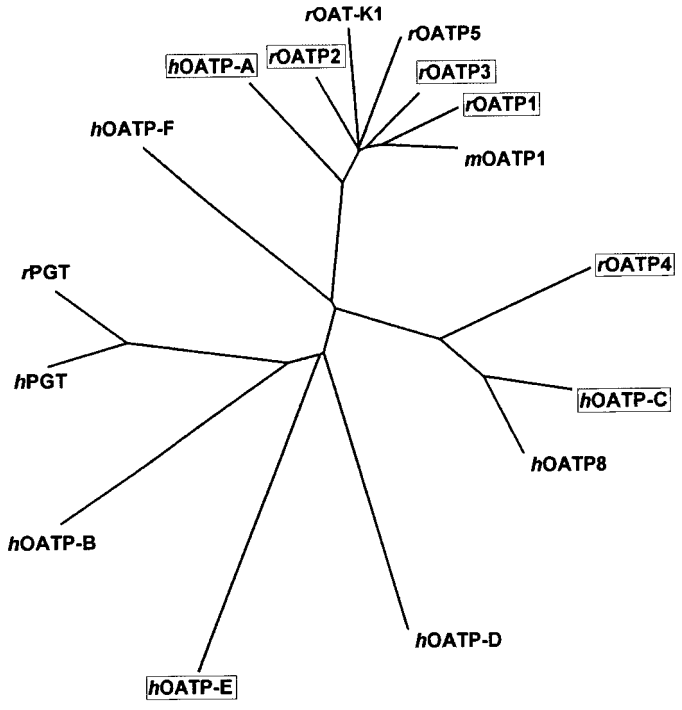

FIG. 9. Phylogenetic tree of the family of human, rat, and mouse OATP organic anion transporters, based on the alignment of the amino acid sequences using the ClustalW program (http://www.ebi.ac.uk), and constructed using the TreeView program (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). *Boxed* transporters have been shown to transport iodothyronines.

B. Amino acid transporters

A large number of amino acid transporters has been characterized in recent years, including the 4F2-related heterodimeric transporters (239, 240). The 4F2 or CD98 cell surface antigen is expressed in many tissues, especially on activated lymphocytes and tumor cells, and has recently been identified as a family of amino acid transporters (239, 240). These transporters are now comprised in the solute carrier family 7 (SLC7). These heterodimeric transporters consist of a common 4F2 heavy chain (4F2hc) linked through a disulfide bond to one member of a family of homologous light chains, seven of which have now been cloned (239–262). 4F2hc is a glycosylated protein with a single transmembrane domain, whereas the light chains are not glycosylated and have 12 transmembrane domains (239, 240). However, most investigators agree that one of the light chains ($b^{0,+}AT$) dimerizes preferentially with rBAT (for "related to basic amino acid transport"), another heavy chain homologous to 4F2hc (256–259). Cystine is an important ligand for the rBAT/$b^{0,+}$AT transporter (Table 7), and mutations in the rBAT heavy chain have been identified in patients with type I cystinuria (263), while mutations in the $b^{0,+}AT$ light chain have been found in patients with non-type I cystinuria (256–258). The characteristics of the different heterodimeric amino acid transporters are summarized in Table 7. The several 4F2 and rBAT-related heterodimeric transporters facilitate exchange of extra- and intracellular amino acids (239, 240).

We have studied possible transport of iodothyronines (T_4, T_3, rT_3, and 3,3′-T_2) by four heterodimeric amino acid transporters, consisting of h4F2hc and either hLAT1, mLAT2, hy^+LAT1, or hy^+LAT2 in *Xenopus* oocytes (264). The LAT1 and LAT2-containing heterodimers represent isoforms of the system L amino acid transporters, which mediate the Na^+-independent uptake of neutral amino acids. The 4F2hc/LAT1 transporter shows preference for large neutral (branched chain and aromatic) amino acids such as Leu, Tyr, Trp, and Phe, whereas 4F2hc/LAT2 also transports small neutral amino acids such as Gly, Ala, Ser, and Thr (241–250). The heterodimers containing the y^+LAT1 or y^+LAT2 light chains mediate the Na^+-dependent transport of neutral amino acids such as Leu as well as the Na^+-independent transport of basic amino acids such as Arg, which is characteristic of the system y^+L amino acid transporters (251–255).

Iodothyronine uptake in *Xenopus* oocytes was not affected

TABLE 7. Characteristics of heterodimeric amino acid transporters

Light chain	Heavy chain	Amino acids transported	Localization	Ref.
LAT1	4F2hc	Large neutral (Na^+-independent) *e.g.*, Leu, Phe, Tyr iodothyronines	*e.g.*, Brain, spleen, testis, placenta, stomach, skeletal muscle	241–245
LAT2	4F2hc	Broad, neutral (Na^+-independent) iodothyronines	*e.g.*, Kidney, intestine, placenta, brain, liver, skeletal muscle	246–250
y^+LAT1	4F2hc	Basic (Na^+-independent), *e.g.*, Arg, Lys, and neutral (Na^+-dependent), *e.g.*, Leu	*e.g.*, Kidney, intestine	251–254
y^+LAT2	4F2hc	Basic (Na^+-independent), *e.g.*, Arg, Lys, and neutral (Na^+-dependent), *e.g.*, Leu	*e.g.*, Brain, intestine, heart, kidney, testis	252,254,255
$b^{0,+}$AT	rBAT	Broad, basic, and neutral (Na^+-independent), *e.g.*, Lys, Arg, cystine, Leu	*e.g.*, Kidney, intestine	256–259
xCT	4F2hc	Cystine, Asp, Glu	Macrophage, brain	260
Asc-1	4F2hc	Small neutral amino acids, *e.g.*, Gly, Ala, Ser, Thr, Cys	*e.g.*, Brain, placenta, kidney, skeletal muscle, heart	261,262

by coexpression of 4F2hc and either y^+LAT1 or y^+LAT2, although the Na^+-dependent transport of Leu, Phe, and Tyr and the Na^+-independent uptake of Arg were markedly increased (264). This indicates that thyroid hormone transport is not mediated by 4F2-related, system y^+L amino acid transporters. However, coinjection of oocytes with cRNA for both 4F2hc and LAT1, but not for each subunit alone, resulted in marked increases in (Na^+-independent) uptake of the system L ligands Leu, Phe, Tyr, and Trp, and of the different iodothyronines. At subsaturating ligand concentrations, the rate of iodothyronine uptake by the h4F2hc/hLAT1 transporter decreased in the order 3,3′-T_2>T_3~rT_3>T_4. Apparent K_m values were found to be in the micromolar range, being lowest for T_3 (0.8 μM), which is the lowest value reported for a ligand of the h4F2hc/hLAT1 transporter (241–245). Both apparent K_m (8 μM) and V_{max} values were highest for 3,3′-T_2 (264). Significant but smaller increases in uptake of the different iodothyronines were observed in oocytes coexpressing 4F2hc and LAT2 (264). In addition, Ritchie *et al.* (265) have reported on the stimulation of T_3 transport in oocytes injected with cRNA for 4F2hc and for the IU12 *Xenopus* LAT1 homolog. These results, therefore, strongly confirm previous findings suggesting that thyroid hormone uptake in different cell systems is mediated by L type amino acid transporters (see *Section III.C*). However, the T type amino acid transporter thought to be involved in the uptake of thyroid hormone in erythrocytes (94) has yet to be characterized.

In contrast to the ubiquitous expression of the 4F2 heavy chain, the LAT1 and, in particular, LAT2 light chains show restricted tissue distributions (239–250). This suggests the existence of additional light chains involved in the uptake of aromatic amino acids and iodothyronines in tissues that do not express LAT1 or LAT2, one of which may be the subunit for the system T transporter. It has not been tested whether iodothyronines are transported by the rBAT/$b^{0,+}$AT heterodimeric transporter. Perhaps, other light chains combine with rBAT and mediate transport of iodothyronines. Iodothyronines may also be ligands for completely different classes of neutral (aromatic) amino acid transporters, such as the recently cloned Na^+-dependent $B^{0,+}$ transporter (266).

In summary, recent studies have identified plasma membrane transporters that are capable of mediating cellular uptake of thyroid hormone. These include 1) the rat and human Na^+-dependent organic anion transporter (NTCP), which is expressed exclusively in the basolateral liver cell membrane, 2) different members of the rat and human Na^+-independent organic anion transporter (OATP) families, which show different tissue distributions, and 3) the L type heterodimeric amino acid transporters, comprised of the human 4F2 heavy chain and the LAT1 or LAT2 light chains, which are expressed in different, largely extrahepatic tissues. The physiological relevance of these transporters for tissue thyroid hormone uptake, however, remains to be established.

X. Summary and Conclusions

There is little doubt that thyroid hormones and their analogs are transported into target cells via plasma membrane carriers. Although variations exist in reported K_m values, explained in part by differences in laboratory techniques and conditions, but also by different tissue distribution of the various transporters, it seems that the mechanism of saturation does not play a role in the regulation of thyroid hormone access to cells. Most laboratories report apparent K_m values in the nanomolar range (Tables 1–3) that are 3 orders of magnitude higher than serum free hormone concentrations. However, other factors have been identified that are involved in regulating thyroid hormone cellular uptake. Cellular factors include the energy charge, in particular cellular ATP concentrations, the number of carriers per cell, and the Na^+ gradient over the plasma membrane. Extracellular factors comprise the free hormone concentration, and possibly competition by circulating amino acids. Several groups of amino acids were shown to inhibit thyroid hormone transport at physiological serum concentrations. Also substances circulating in increased concentrations in NTI and starvation, such as CMPF, indoxyl sulfate, bilirubin, and NEFAs, and several drugs may influence thyroid hormone tissue uptake.

Strong evidence exists that plasma membrane transport of thyroid hormone is rate limiting for subsequent thyroid hormone metabolism. As in man about 80% of plasma T_3 is produced outside the thyroid gland from T_4 in plasma T_3-producing tissues, regulation of uptake of T_4 in these tissues is potentially determinant for overall plasma T_3 production and thus exertion of thyroid hormone activity at the tissue

level. This process probably plays a major role in the lowered T_3 production in NTI and starvation in man, in contrast to the situation in the rat, where a diminished T_4 production plays an important if not major role in the cause of the low T_3 syndrome (267, 268). The contributions of plasma-derived T_3 and of local T_3 production from T_4 differs between tissues. Thus, not only regulation of T_4 uptake but also of T_3 uptake at the level of the plasma membrane is important for overall regulation of thyroid hormone bioactivity. Plasma membrane carriers for thyroid hormone may be different in different organs. For instance, in the liver there are probably different carriers for T_3, T_4, and rT_3, whereas in the pituitary only one transport mechanism has been identified for both T_3 and T_4. Transport mechanisms may also differ in various tissues and species with regard to Na^+ dependence and maybe other, as yet unidentified, factors. Few publications deal with cellular efflux of thyroid hormone. When tested, T_3 efflux is found to be a saturable process, albeit at supraphysiological hormone levels. Efflux of thyroid hormone, even if carrier mediated, seems to be independent of the energy charge of the cell. This suggests that carrier-mediated efflux of thyroid hormone does not play a major role in the regulation of the cellular free hormone concentration.

A very recent development is the identification of different thyroid hormone transporters belonging to different families. This field is developing rapidly; nonetheless, information in the following areas is insufficient: 1) how and to what extent these transporters compete for thyroid hormone transport; 2) how they are distributed over the different tissues; and 3) in what way other ligands for these transporters interact with thyroid hormone transport into tissues. Insufficient information is also available about the rank order of physiological importance of the different transporters. Once more, knowledge has been accumulated about this aspect, but studies must be done on the regulation at the molecular level of the activity of physiologically important thyroid hormone transporters and the mechanisms by which they regulate bioavailability of thyroid hormone.

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