DECREASED TRANSPORT OF THYROXINE (T_4), 3,3′,5-TRIIODOTHYRONINE (T_3) AND 3,3′,5′-TRIIODOTHYRONINE (rT_3) INTO RAT HEPATOCYTES IN PRIMARY CULTURE DUE TO A DECREASE OF CELLULAR ATP CONTENT AND VARIOUS DRUGS

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1. Introduction

T_4, the main secretory product of the thyroid gland, is deiodinated and conjugated in peripheral tissues [1,2]. Phenolic ring deiodination of T_4 accounts for ~80% of the total body production of T_3, the most biologically active iodothyronine [3]. The remainder is produced by the thyroid gland. The other main product of peripheral deiodination is rT_3, which is biologically inactive [4]. Initiation of biological activity by T_3 occurs after binding to nuclear receptors [5]. At least 70% of the liver nuclear bound T_3 is derived from the extracellular compartment and the remainder from local, intracellular deiodination of T_4 [6]. The liver, which contains ~30% of the total T_4 pool and 80% of all intracellularly located T_4, is an important organ for the production of thyroid hormone metabolites [7]. Studies related to membranal transport of iodothyronines into hepatic cells are important since they may increase our understanding of regulatory mechanisms involved in the ultimate delivery of thyroid hormone to intracellular active sites like metabolizing enzymes and receptors.

Uptake of T_3 and T_4 by rat hepatocytes in primary culture is mediated by distinct, ATP-dependent processes [8]. Additionally, they are saturably bound to the surface of the plasma membrane [8–10]. Study of the binding of T_3 and T_4 to purified rat liver plasma membranes has revealed 2 sets of saturable sites for both hormones of which the highest affinity components do not seem to reflect a common binding site [11–13]. In harmony with the concept of an active transport mechanism for the uptake of thyroid hormone by cells is the visualization by video intensification microscopy of receptor-mediated endocytosis of fluorescent T_3 in cultured fibroblasts [14,15]. We report here on a difference in ATP-dependency of the uptake of T_3 on the one hand and that of T_4 and rT_3 on the other by rat hepatocytes in primary culture. T_4 and rT_3 showed the most remarkable diminution of transport by small decreases in cellular ATP content. In addition, effects of propranolol, X-ray contrast agents, amiodarone and cytoskeleton-disrupting agents have been studied. The results indicate that besides changes in T_4 deiodination [1,2] and sulfoconjugation [16,17], decrease in cellular uptake of T_4 by tissues secondary to decreased cellular ATP concentrations or to the effects of some compounds may be a contributing factor to the clinical condition known as low T_3 syndrome. A preliminary account of this work has been published [18].

2. Materials and methods

The sources of most materials are mentioned in [8,9]. The purity of tri-[3,3′,-5′-^{125}I]iodothyronine (The Radiochemical Centre, Amersham), spec. act. >1200 μCi/μg, was verified by HPLC [19]. The following drugs were generous gifts; sodium ipodate (Schering, Berlin), sodium tyropanoate and iopanoic acid (Sterling-Winthrop Labs, NY), D- and L-propranolol (ICI, Rotterdam) and amiodarone (La Baz, Maassluis). Inhibitors of the cytoskeleton (vinblastine, colchicine, cytochalasine B) were purchased from Sigma (St Louis MO).

The experimental details of isolation (collagenase perfusion technique), culture and incubations of rat hepatocytes with thyroid hormone are in [8]. Uptake was studied in rat hepatocytes in monolayer culture

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(2×10^6 cells/dish) and were performed in quadruplicate/expt by incubations for 1 min at 37°C with ^{125}I-labelled thyroid hormone and additional amounts of unlabelled hormone in 4 ml incubation medium [8] containing 1% (for uptake of T_3 and rT_3) or 0.5% (T_4) bovine serum albumin. In some experiments, uptake studies were preceded by incubations in incubation medium for 30 min with various concentrations of glucose or fructose to decrease the cellular ATP content [20], 1 and 10 μM propranolol, 11 μM vinblastine, 25 μM colchicine or 25 μM cytochalasin B. In other experiments uptake was studied in the presence of iopanoic acid, sodium tyropanoate, sodium ipodate (10 and 100 μM) or amiodarone (1 μM).

The procedure of calculation of transport was based on the principle of the mutual inhibition by substrates for their energy-dependent uptake systems as in [8]. Unpublished observations (E. P. K., R. D., H. F. B.) showed transport of reverse T_3 with K_m 6 nM at 37°C, which can be inhibited by T_3. To parallel uptake studies of T_4, T_3 and rT_3 the following free amounts of iodothyronines were added to inhibit transport of the particular iodothyronine (in parentheses): 6.8 μM T_3 (T_4); 0.8 μM T_4 (T_3); and 2.9 μM T_3 (rT_3). The difference in total uptake and uptake in the parallel incubation yields a measure of the active transport. Measurements of free hormone concentrations and ATP content was done as in [8].

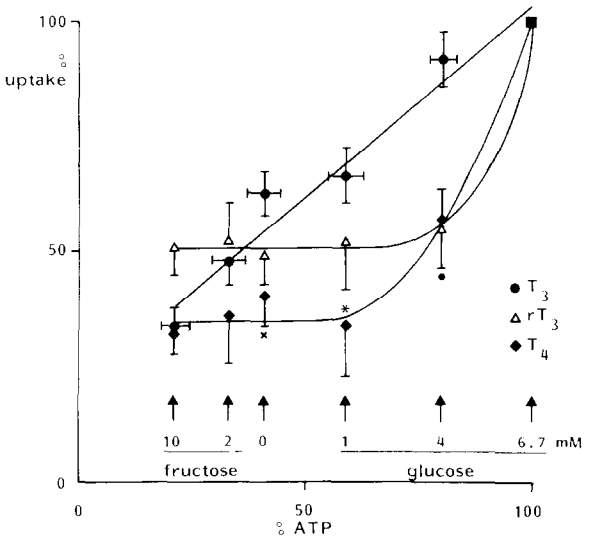

Fig.1. Active transport (in % of control) of iodothyronines into rat hepatocytes in primary culture as function of cellular ATP content (in % of control). Values from incubations with 6.7 mM glucose are expressed as 100%. The absolute control values amounted to 0.67 T_4, 36 T_3 and 3.8 rT_3 pmol . 35 μg DNA^{-1} . min^{-1} and 64 nmol ATP/35 μg DNA. ATP content was varied by pre-exposure of the cells to the indicated concentrations of glucose or fructose. Thereafter the monolayers were exposed to the following free hormone levels for 1 min at 37°C: 0.1 nM T_4, 9 nM T_3 and 1.2 nM rT_3. Each uptake value represents the mean ± SEM of ⩾6 expt (in quadruplicate) and ATP values are from ⩾16 expt (at least in duplicate). Statistical evaluation of T_3 and T_4 transport with Student's *t*-test results in: $^{\cdot}p < 0.005$; $^{*}p < 0.025$; $^{x}p = 0.025$.

3. Results

3.1. *Effect of changes in ATP content on T_4, T_3 and rT_3 transport*

Monolayers of hepatocytes were preincubated for 30 min with different concentrations of glucose or fructose to vary the ATP content of the liver cells (fig.1). A curvilinear relationship is found between transport of T_4 or rT_3 and ATP, in contrast to a linear correlation with T_3. This transport was studied with hormone concentrations which are below the K_m of the transport system of the particular iodothyronine ([8], this paper) to study transport in the absence of saturation of the uptake mechanism. The interrelationships between T_4 and T_3 transport and between T_4 and rT_3 transport are shown in fig.2. In fig.2 the ratios of the percentage of inhibition of T_4 transport relative to that of T_3 or rT_3 are calculated and related to the ATP content. For instance, with a preincuba-

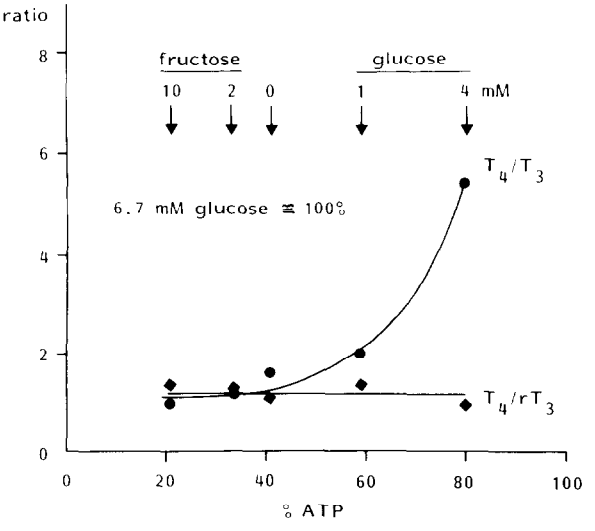

Fig.2. Ratios of the amount of inhibition of T_4–T_3 transport and T_4–rT_3 transport as function of cellular ATP content. Data are taken from fig.1.

tion with 4 mM glucose, the fall in ATP content compared to control is 20%. This resulted in ~5-times more inhibition of T_4 transport than of T_3 (ratio is 5), whereas the inhibition of T_4 and rT_3 transport is about equal (ratio is 1.2, which value does not change with lower ATP levels). Thus, it seems that T_4 and rT_3 transport are in a similar way dependent on ATP concentration in contrast to T_3 transport and it is the small decrease in cellular ATP content, that results in a greater inhibition of T_4 and rT_3 transport compared to T_3.

3.2. *Effects of structurally related compounds on T_4 and T_3 transport*

Uptake of thyroid hormone in the presence of 10 or 100 μM sodium ipodate, sodium tyropanoate, iopanoic acid or 1 μM amiodarone results in a statistically significant decrease in transport of T_3 and T_4 (table 1). A lower concentration of bovine serum albumin in the incubation medium is used for T_4 uptake studies to increase the percentage uptake, as the free fraction of T_4 is much lower compared to T_3 or rT_3. This may have implications for the free amounts (not tested) of the above compounds, and therefore comparison of the decreases in T_3 and T_4 transport by these compounds in this experiment is not opportune.

3.3. *Effects of D- and L-propranolol and inhibitors of the cytoskeleton function on T_3 and T_4 transport*

Pretreatment of monolayers for 30 min with vinblastine, colchicine or cytochalasin B leads to a diminished transport of T_3 and T_4 (table 1). Similarly, the isomers of propranolol show this effect (table 1). These agents are able to decrease the cellular ATP content markedly (table 1). When similar ATP-decreases were compared between the experiments as illustrated in fig.1 and table 1 no significant differences in inhibition of T_4 transport were present. However, T_3 transport is significantly more diminished (one way analysis of variance, [21], all p levels <0.025) in the experiments from table 1.

Table 1
Effect of preincubation for 30 min with D, L-propranolol, vinblastine, colchicine and cytochalasin B and of incubation for 1 min with sodium tyropanoate, sodium ipodate, iopanoic acid and amiodarone on the active transport of T_4 and T_3 into rat hepatocytes in primary culture

Agent	μM	T_4			T_3			ATP		
		% control	(n)	SEM	% control	(n)	SEM	% control	(n)	SEM
Sodium tyropanoate	10	51	(5)	8[a]	53	(4)	21[a]			
Sodium tyropanoate	100	19	(5)	13[a]	39	(6)	11[a]		n.s.	
Iopanoic acid	10	58	(5)	11[b]	57	(3)	10[a]			
Iopanoic acid	100	22	(5)	14[a]	44	(5)	14[a]		n.s.	
Sodium ipodate	10	49	(5)	9[a]	53	(3)	13[a]			
Sodium ipodate	100	32	(4)	10[a]	45	(5)	4[a]		n.s.	
Amiodarone	1	20	(3)	9[a]	31	(3)	8[a]		n.s.	
Vinblastine	11	26	(3)	13[a]	45	(4)	7[a]	43	(4)	5
Colchicine	25	14	(3)	2[a]	34	(5)	5[a]	37	(4)	8
Cytochalasin	25	17	(3)	2[a]	23	(4)	9[a]	37	(4)	7
D-Propranolol	1	43	(6)	7[a]	45	(4)	8[a]	42	(3)	5
D-Propranolol	10	19	(6)	7[a,c]	23	(7)	6[a,d]	41	(4)	6
L-Propranolol	1	44	(6)	7[a]	53	(4)	13[a]	45	(3)	12
L-Propranolol	10	21	(6)	4[a]	30	(5)	7[a,e]	39	(4)	8

[a,b] p-Values for difference with control ([a] $p < 0.001$, [b] $p < 0.005$)
[c,d] Difference with 1 μM D-isomer ([c] $p < 0.05$; [d] $p < 0.001$)
[e] Difference with 1 μM L-isomer ([e] $p < 0.01$); n.s., not significant

The free hormone concentrations used are mentioned in fig.1, and are below the K_m of the transport system. Statistical evaluation was performed according to the one way analysis of variance [21]. n, no. expt (in quadruplicate)

4. Discussion

By kinetic analysis of their mutual inhibition of transport, active transport of T_3 and T_4 were shown to take place via different pathways [8]. According to these results, the ATP dependency of these transport mechanisms is different, such that small decreases in ATP content result in a larger inhibition of T_4 transport and after a certain amount of decrease in ATP, T_4 transport becomes ATP-independent. Unpublished observations (E. P. K., R. D., H. F. B.) have shown that similarly to T_4 [8] and T_3 [9], rT_3 is taken up by a high affinity system (K_m ~6 nM), which is energy-dependent (this study). Transport of T_4 and rT_3 are in a similar way dependent on ATP, which might indicate that T_4 and rT_3 share a common transport pathway. Apparently, in addition to the intracellularly located deiodinase activity [1,2] and conjugating enzymes [16,17], rat hepatocytes in primary culture are able to regulate the peripheral metabolism of iodothyronines by influencing the rate of their entry. Diminished entry of T_4 and rT_3 by a lowered ATP content in (patho-)physiological conditions results in decreased production of T_3 and degradation of rT_3, respectively. In concert with a diminished activity of the 5′-deiodinase activity in various conditions [22], these differences in transport can play a role in the genesis of the alterations of serum iodothyronine concentrations in the low T_3 syndrome. In T_4 perfusion of livers from fasted rats, T_4 uptake was diminished up to 40% [23]. Many compounds [24] (e.g., X-ray contrast agents, propranolol, amiodarone) can induce a low T_3 syndrome, which has been explained by inhibition of the activity of 5′-deiodinase. However, 50% inhibition of this activity in isolated hepatocytes [25] or homogenates [26] has been achieved with ~500 μM D,L-propranolol, ~3 orders of magnitude higher than in vivo plasma levels of subjects given the usual therapeutic dosage [27]. Furthermore, a membrane-stabilizing effect seems to be achieved with concentrations of propranolol, which are ~1 order of magnitude higher than the usual therapeutic plasma level [27]. This makes it less probable that the observed effect of therapeutic amounts of D-propranolol (which is devoid of β-blocking activity) on T_3 formation from T_4 in T_4 substituted subjects is merely by this membrane stabilizing action, as suggested in [28,29]. This suggestion was based on the fact that quinidine inhibited the formation of T_3 in a similar way. The ATP-lowering effect of local anaesthetics (including quinidine) is described in [30]. Pretreatment of monolayers of hepatocytes with D- and L-propranolol in the therapeutic concentration range, results in a marked lowering of the ATP content and in an inhibition of T_4 and T_3 entry by ≥50%. Consequently, at first sight it seems that the common denominator of the observed similar effects of both isomers is diminished active transport of iodothyronine by reduced ATP levels. However, at similar ATP levels, inhibition of T_3 transport by propranolol is more pronounced as compared to control. The reason for this finding is unclear.

Similarly, X-ray contrast agents and amiodarone added to the incubation medium with T_4 or T_3 lead to a decreased uptake in our system. Because of the structural similarities between these compounds and thyroid hormone, their effect can be explained by competitive inhibition of uptake, as has been shown for T_4 conversion [26].

X-Ray contrast agents are able to inhibit uptake of T_4 and T_3 by rat liver slices [31]. In vivo, sodium tyropanoate can displace T_4 from the liver [32] and provoke an acute increase in serum T_4 [33]. In addition to displacement from liver cytosol binding proteins, competitive inhibition of inward transport of T_4 through the plasma membrane may be another effect of tyropanoate [32].

In many plasma-membrane processes, like carrier-mediated transport, the cytoskeleton plays an essential role, and can be perturbed by agents like colchicine, vinblastine and cytochalasin B. In our system these agents decreased thyroid hormone transport too, suggesting a direct relation of the uptake mechanism and the cytoskeleton. However, these compounds decreased the ATP content as well, which in part may also explain our findings. Both effects are in agreement with [14,15], that the mechanism by which thyroid hormone is taken up is endocytosis, which is an energy–cytoskeleton-dependent (and time-consuming) process [34].

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