UPTAKE OF THYROID HORMONE BY ISOLATED RAT LIVER CELLS

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Summary

L-Triiodothyronine is taken up by isolated rat liver cells by a process which is saturable and exhibits sigmoidity. Two uptake systems make themselves evident: A system with high affinity with an apparent K_t value of 52 ± 22 nM and a system with low affinity with an apparent K_t value of 1446 ± 764 nM. Cells heated at 60°C or after freezing do not show saturability of uptake. KCN inhibits the uptake by the low affinity system. In the presence of L-thyroxine and L-tyrosine the uptake of L-triiodothyronine is increased. The results suggest transport of L-triiodothyronine by proteins in the plasma membrane of the liver cell.

Thyroid hormones are bound to intracellular binding proteins after entering target cells (1). The cell nucleus is the main locus of action, where the thyroid hormones influence gene expression (2). In addition thyroid hormones exert a direct effect upon the mitochondrion by stimulating oxygen consumption (3) and mitochondrial protein synthesis (4). The first step leading to the hormonal effects, is the translocation through the plasma membrane. This translocation is presumed to take place by passive diffusion. However, the following two considerations do not support the entrance of hormones into the cells by diffusion: 1. The pore radius of the plasma membrane as calculated by Stitzer and Jacquez (5) is between 3.5 and 5.5 Å whereas the approximate radius of the L-triiodothyronine molecule is between 7.2 and 7.5 Å. Hence L-triiodothyronine cannot pass freely through the pores in the membrane of the cell. 2. Since L-triiodothyronine is in a charged state, it is even more difficult for the molecule to be translocated through the plasma membrane by passive diffusion (5).

This initial study was carried out to find how L-triiodothyronine is transported into isolated rat liver cells with the ultimate aim to obtain information on the regulatory function of the plasma membrane in the transport of thyroid hormones.

Materials and Methods

L-Triiodothyronine and L-thyroxine were from Sigma Chemical Co., St. Louis, U.S.A.; L-tyrosine and Hepes (N-2'-hydroxy ethyl piperazine-2-ethane sulfonic acid) from Serva, Heidelberg, Germany and L-[3'-^{125}I]triiodothyronine (specific radioactivity 48 mCi/mg from Amersham-Buchler, Braunschweig, Germany. Silicone oil AR 20 and AR 200 were gifts from Wacker Chemie, München, Germany.

Isolation of rat liver parenchymal cells was carried out according to a modification (6) of the method of Berry and Friend (7). Viability of the cells was checked by exclusion of trypan blue and by measuring the ability to synthesize glucose from pyruvate. Investigation of the morphology of the isolated liver cells by electron microscopy was carried out as reported earlier (8). Cellular protein was estimated according to Lowry et al. (9), with bovine serum albumin as standard; 1 mg cellular protein corresponded to 654,000±16,000 cells.

The uptake of L-triiodothyronine by liver cells was measured by the oil-centrifugation technique described by Livingston and Lockwood (10). Increasing concentrations of nonlabeled L-triiodothyronine, made by serial dilution, and a constant amount of L-[3'-^{125}I]triiodothyronine (about 25,000 cpm) in 200 µl of Hepes buffer, pH 7.4(buffer composition: 143 mM NaCl, 6.72 mM KCl, 1.22 mM $CaCl_2$, 10 mM Hepes, 11 mM KH_2PO_4, 7 mM Na_2SO_4, 13.2 mM $MgCl_2$ and 6.6 mM NaOH), were incubated with 25 µl of liver suspension, equivalent to 100 to 150 µg cell protein, for 30 s at 23°C. After incubation and centrifugation the radioactivity associated with the pellet of cells was measured in a LKB gamma counter. The amount of L-triiodothyronine adsorbed extracellularly was estimated for each experiment by carrying out measurements of uptake of L-triiodothyronine at different times upto 45 s and extrapolating them to zero. The counts obtained at zero incubation time were regarded as blanks and subtracted from total uptake values.

Uptake values are the corrected mean of quintuplicate determinations; 10,000 cpm were counted to keep the error below 1%. The variation coefficient of quintuplicates was 4 to 6%. The K_t values were estimated from the Eadie-Scatchard plot using a program for the method of least squares to calculate the regression lines for the linear parts of the curves. The data were analyzed by the Student's t-test; a P value less than 0.05 was considered to be statistically significant.

Results and Discussion

Isolated liver cells exhibiting 90% viability by the trypan blue test produced 86 µg glucose x mg protein^{-1} x 30 min^{-1} when incubated with 10 mM pyruvate. The liver cells were viable for 4 h, when kept at 0°C in an atmosphere of O_2 in Hepes buffer.

The dependence of uptake of L-triiodothyronine on time is presented in Fig. 1. The initial rapid uptake is probably due to adsorption of L-triiodothyronine on the cell membrane. A similar phenomenon has also been described for the uptake of L-thyroxine by Ehrlich ascites cells (5,11). The uptake is linear with time

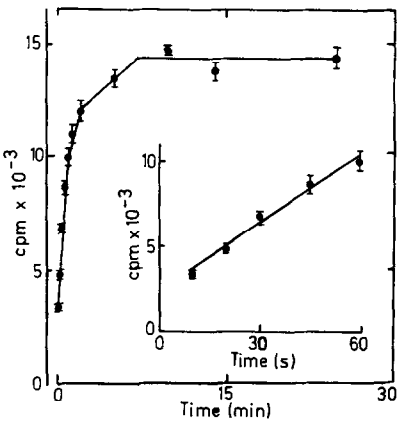

Fig. 1. Uptake of L-triidothyronine by isolated rat liver cells in dependence of time.

Cells equivalent to 150 µg of protein were incubated with L-[3'-^{125}I]triiodothyronine (about 25,000 cpm) and 88.9 nM non-labeled hormone at 23°C. Incubations were terminated by the rapid oil-centrifugation technique at the indicated time intervals and the uptake of radioactivity by cells was measured as described in the text.

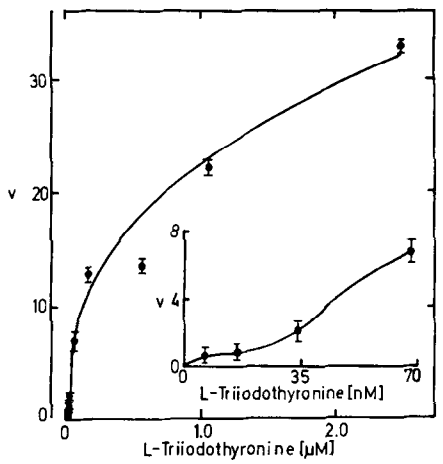

Fig. 2. Kinetics of uptake of L-triiodothyronine by isolated rat liver cells.

Tubes contained increasing concentrations of nonlabeled L-triiodothyronine and a constant amount (25,000 cpm) of L-[3'-^{125}I]triiodothyronine in Hepes buffer, pH 7.4. Cells equivalent to 100 µg of protein were incubated at 23°C for 30 s. Termination of the incubation and measurement of radioactivity is described in the text; v, denotes uptake of L-triiodothyronine, expressed as pmol x mg protein^{-1} x min^{-1}. The inset shows the initial rates of uptake at lower concentrations (7 and 70 nM) of L-triiodothyronine.

upto 60s after which it slows down and reaches a plateau after 7 min. Further investigations were carried out with an incubation period of 30 s to ensure working under initial rates of uptake. Uptake measured in dependence of protein showed linearity upto 150 µg cellular protein, using 88.9 nM L-triidothyronine.

Uptake in dependence of L-triiodothyronine concentrations is shown in Fig. 2. As can be seen, the uptake shows saturability and exhibits sigmoidity at lower concentrations (inset). When the data is analyzed by the Eadie-Scatchard plot (Fig. not shown), the presence of 2 uptake systems becomes apparent: one, a high affinity system between 7 and 35 nM L-triiodothyronine with a K_t of 52 ± 22 nM (n=4) and the other between 70 and 2500 nM with an apparent K_t of 1446 ± 764 nM (n=4). In view of the sigmoid uptake pattern the affinity constants should be considered only as approximations. The break in the curve occurred in all 4 experiments between 35 and 70 nM. The rather large standard deviation might be due to biological variations, since freshly isolated liver cells were used for each experiment. Controls were carried out in the absence of the respective agent used. The saturability of the uptake system by L-triiodothyronine points to the fact that a protein might be involved in the transport of the thyroid hormone into the liver cells. If this would be the case, denaturation of the protein should lead to abolishment of the saturable uptake. When cells were heated for 10 min at 60°C saturability of uptake was abolished and uptake was linear with increasing L-triiodothyronine concentrations (7 to 2500 nM), whereas the controls exhibited saturability. Thus the saturability of uptake observed by the non-heated cells is lost by heat denaturation indicating, that some protein component in the plasma membrane may be responsible for the uptake of the thyroid hormone. The question arises, whether a properly functioning membrane is a prerequisite for a saturable uptake or whether saturable uptake can still be measured after rendering the membrane permeable in which case only the binding due to intracellular proteins should be observed. Intracellular binding proteins maintain their binding properties even after long storage periods; the integrity of the cell and probably the surrounding plasma membrane, however, is considerably affected after freezing. Thus by freezing cells, one might be able to distinguish between the uptake by the plasma membrane and the binding to intracellu-

L-Triiodothyronine (nM)	Uptake (%)	Significance (P)
5.7	146	>0.05
28.6	120	>0.05
119.8	66	<0.001
557	89	<0.05
1000	81	<0.001

Table 1. Influence of KCN on the uptake of L-triiodothyronine by rat liver cells.

Cells were treated for 10 min at 23°C with 2 mM KCN and incubated in the presence of 0.2 mM KCN with increasing concentrations of L-triiodothyronine and 25,000 cpm of L-[3'-^{125}I]triiodothyronine. Incubation and measurement of radioactivity was carried out as mentioned in the text. Uptake by cells not treated with KCN and incubated in the absence of KCN is taken as 100 %.

lar thyroid hormone binding proteins. This hypothesis was tested by an experiment in which liver cells were frozen at -20°C for 12 to 18 h, unthawed and incubated with increasing concentrations of L-triiodothyronine. It could be seen, cells that were frozen, did not show saturability of uptake when compared to cells that were freshly isolated, pointing to the involvement of the plasma membrane in the uptake process, which is independent of binding to intracellular proteins under the experimental conditions used. From analysis of the data in Fig. 2 it was found that L-triiodothyronine concentration in the cells was 13 to 28 fold higher than the external concentration. The question arose, whether this concentrative process was in some way dependent on metabolic energy. This was checked by an experiment in which isolated liver cells were treated for 10 min with 2 mM KCN at 23°C and incubated for 30 s at 23°C in the presence of 0.2 mM KCN. As can be seen from Table 1, uptake by the low affinity system (above 100 nM) is inhibited significantly by KCN whereas the high affinity system is not significantly affected. This result shows that metabolic energy is involved in some way in the transport of thyroid hormone into the liver cell. This finding agrees with the observations of Christensen et al. (11), who also found that KCN inhibited the accumulation of L-triiodothyronine by Ehrlich ascites cells.

The effect of L-thyroxine on the uptake of L-triiodothyronine by liver cells was tested to get some information on the specificity of uptake of the two closely related hormones. The results

L-Triiodothyronine (nM)	Compound tested	Uptake (%)	Significance (P)
7.6	L-Thyroxine	207	<0.001
19.1		145	<0.01
38.2		110	<0.05
70		104	>0.05
139		100	>0.05
202		105	>0.05
577		104	>0.05
910		114	>0.05
7.0	L-Tyrosine	171	<0.001
17.5		158	<0.01
35.0		254	<0.001
133		100	>0.05
195		118	>0.05
570		104	>0.05
903		127	<0.05

Table 2. Influence of L-thyroxine and L-tyrosine on the uptake of L-triiodothyronine by rat liver cells.

Increasing concentrations of L-triiodothyronine and 25,000 cpm L-[3'-^{125}I]triiodothyronine were incubated in the presence and absence of 500 nM L-thyroxine or 500 nM L-tyrosine for 30 s at 23°C. Incubation and measurement of radioactivity was carried out as mentioned in the text. Uptake in the absence of the two compounds is taken as 100 %.

presented in Table 2 show that in the presence of 500 nM L-thyroxine, the uptake of L-triiodothyronine at lower concentrations (7 to 38 nM) is significantly increased, indicating that the high affinity uptake system is being influenced by L-thyroxine. The low affinity uptake system remains unaffected. L-Tyrosine which is an analogue of thyroid hormones also increases the uptake of L-triiodothyronine by the high affinity system (Table 2). It appears therefore that L-triiodothyronine and L-thyroxine or L-tyrosine may not be sharing the same transport protein in the plasma membrane to get inside the cell. The present results demonstrate that thyroid hormones are transported into the cell of rat liver by components in the plasma membrane which show properties of proteins. The sigmoid pattern of uptake points to the presence of multiple components operating during the uptake process. Whether the transport proteins(s) regulate(s) the entry of thyroid hormones, remains to be investigated.

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