ACTIVE TRANSPORT OF TRIIODOTHYRONINE (T_3) INTO ISOLATED RAT LIVER CELLS

E. P. KRENNING, R. DOCTER, H. F. BERNARD, T. J. VISSER and G. HENNEMANN
Department of Internal Medicine III and Clinical Endocrinology, Medical Faculty, Erasmus University, Rotterdam, The Netherlands

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1. Introduction

Since the receptors for thyroid hormones are located within the cell [1], these hormones have to be transported from the extracellular compartment through the plasma membrane into the cell. Only scarce and conflicting data concerning this transport mechanism are available. Both diffusion [2–4] and carrier-mediated processes [5–7] have been described or suggested. In view of the fact that amino acids [8] are transported through the plasma membrane by active or mediated processes, it is not likely that thyroid hormones enter the cell by diffusion only. In an attempt to gain more insight into the mechanism of cellular uptake of T_3 the present study was performed. As a model we have chosen non-proliferative cultures of parenchymal cells from adult rat liver. The use of primary cultures offers a distinct advantage over freshly prepared liver cell suspensions. Cells are given time to recover from the damage associated with the isolation, such as temporary changes in the inability to concentrate amino acids [9] and the increased catabolic state during the first 2–4 h after isolation [10]. In this report, we describe the basic characteristics of T_3 uptake in this system. A preliminary account of this work has been published [11].

2. Materials and methods

2.1. *Materials*

3,3′, 5-Triiodo-L-thyronine (L-T_3) L-thyroxine (L-T_4), piperazine-*N*,*N*′-bis[2-ethane sulfonic acid] (Pipes), *N*-2-hydroxy-ethyl piperazine-*N*′-2-ethane sulfonic acid (Hepes), *N*,*N*-bis [2-hydroxy ethyl]-2-amino ethane sulfonic acid (Bes), dinitrophenol (DNP), potassium cyanide (KCN), oligomycin collagenase (type I), bovine serum albumin (type V) (BSA), insulin and D-glucose were purchased from Sigma, St Louis, MO; fetal bovine serum (FBS), penicillin/streptomycin (P/S) and Ham's F10 from Flow Laboratories, Irvine.

All glass-ware was siliconized (Siliclad®, Clay Adams, New York, NY). Plastic culture dishes were purchased from Costar, Cambridge, MA.

The purity of [^{125}I] T_3 tracer (Radiochemical Centre, Amersham), spec. act. $> 1200\ \mu Ci/\mu g$, was verified by cellulose thin-layer chromatography [12] (acetone : 0.5 M acetic acid, 30 : 70).

2.2. *Methods*

Parenchymal cells were isolated from livers of male Wistar rats (150–300 g) according to the method in [13] with minor modifications. These consist of a preperfusion of the liver for 10 min with Ca^{2+}-free Hank's solution followed by perfusion with collagenase (0.05%) and Ca^{2+} (2 mM) in Hank's solution. About 2×10^6 cells in 4 ml culture medium (Ham's F10, 10.6 mM Pipes, 11.2 mM Bes, 8.9 mM Hepes, 12 mU/ml insulin, 15% FBS, 2 mM $CaCl_2$ and 10 U/ml P/S, pH 7.4) were inoculated into 60 mm dishes. After 4 h culture at 37°C the medium was replaced by 4 ml incubation medium (136.9 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 6.7 mM glucose and 1% BSA, pH 7.4). Incubations were performed in triplicate for 1 min at 21°C with [^{125}I] T_3 and increasing amounts of unlabelled T_3 in 4 ml incubation medium.

Uptake experiments were terminated by removing the medium and washing the monolayer with 4 ml incubation medium without albumin. This procedure resulted in an almost complete removal of the medium (99.96%), as tested with ^{131}I-labelled albumin.

For counting of radioactivity and DNA determination [14] cells were removed after lysing with 0.1 M NaOH. After incubation with T_3, viability of cells, as determined by the exclusion of Trypan blue, was over 90%. At each concentration of unlabelled T_3 the uptake data of 4–6 experiments were combined after correction for varying DNA concentrations. For all doses used, free T_3 and T_4 concentrations in the incubation medium were estimated by equilibrium dialysis [5].

3. Results

As illustrated in fig.1, uptake of T_3 was almost linearly related with time up to 1 min. Therefore all uptake studies were performed using 1 min incubations. The total uptake of T_3 (fig.2) was not linearly related to the free concentration of T_3, indicating that in addition to diffusion* carrier-mediated processes were involved. The contributions of the saturable processes were negligible (< 8.5%)** at very high concentrations of T_3 ([FT_3] 48 μM) and since diffusion is linearly related to the free hormone concentration the line, representing uptake by means of diffusion, was constructed through this highest measured uptake point and the origin. By subtracting the diffusion line from the total uptake curve, the uptake curve of the carrier mediated processes was obtained.

By plotting these uptake data against the free T_3 concentration in a double reciprocal plot, fig.3 was obtained. In fig.3 the regression line for the 5 highest data points is shown. From the inset it can be seen that the five lowest data points are significantly ($p < 0.001$) below this regression line, indicating that a second saturable uptake system is present. To

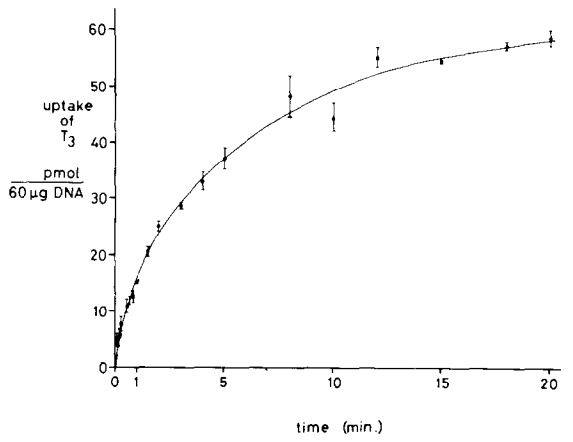

Fig.1. Uptake of T_3 ([FT_3] 5.5 nM) by rat liver cells as function of time of incubation at 21°C. For details see section 2.

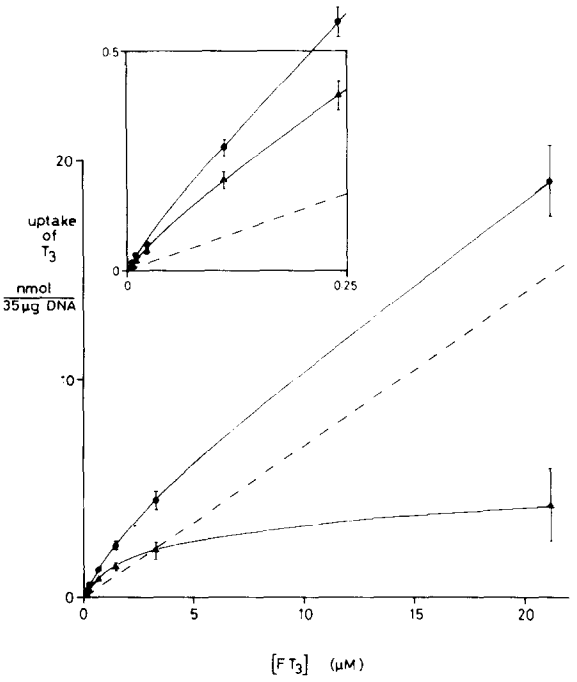

Fig.2. Uptake of T_3 (mean ± SEM) by rat liver cells during incubation for 1 min at 21°C using increasing concentrations of T_3. The inset is a magnification of the area near the origin. (●——●) Represents total uptake, (- - - -) diffusion and (▲——▲) uptake by carrier-mediated processes (n=25).

* In our kinetic model adsorption can not be differentiated from diffusion

** Taking account of these contributions, resulted in minor differences of the calculated kinetic parameters

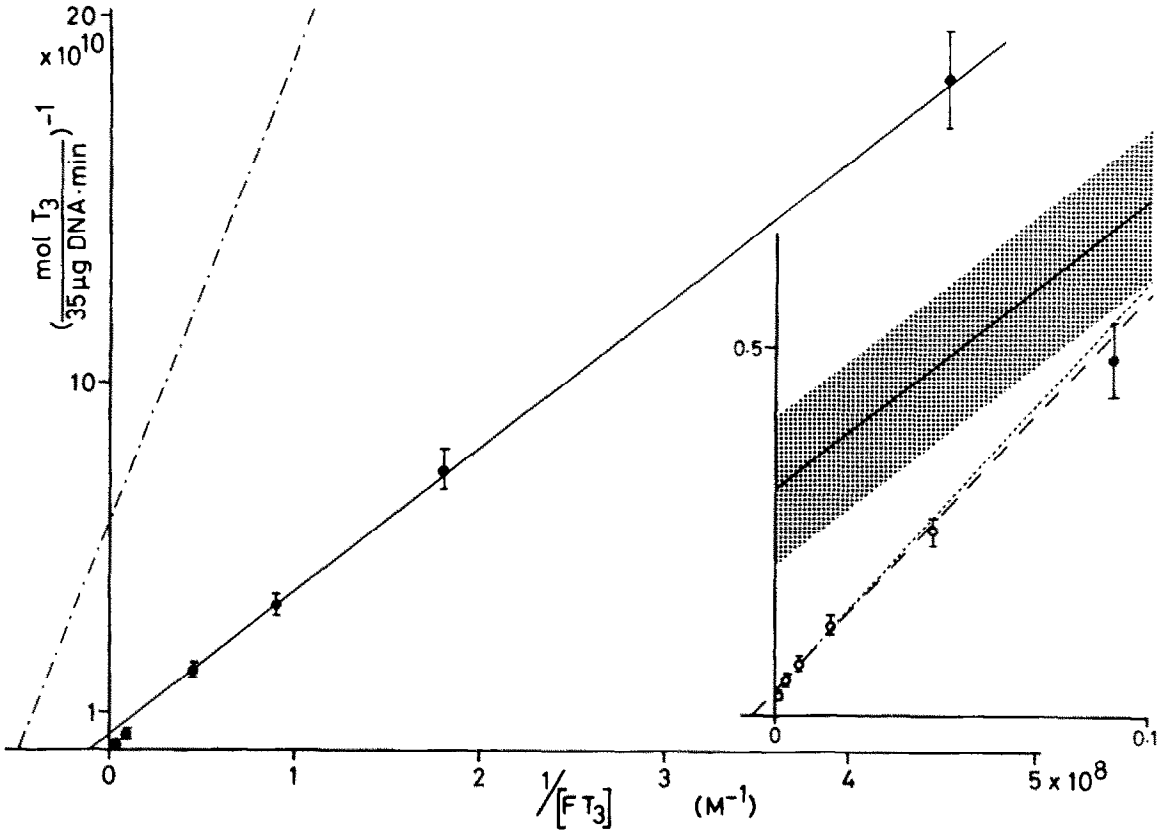

Fig.3. Double reciprocal plot of the T_3 uptake by rat liver cells corrected for diffusion, against the free T_3 concentration. Data are pooled from 25 experiments. The lowest point in the left panel represents a cluster of the 5 data points (○) shown on a magnified scale in the inset. (——) regression line for the data points (●) (mean ± SEM) representing the high affinity system, with 95% confidence limits (shaded area). (- - - -) regression line for the data points (○) representing the low affinity system. (. – . –) and (. . . . .) regression lines of the high and the low affinity systems, respectively, after cross-correction according to [16].

Table 1
Characteristics of the uptake of T_3 by rat liver cells; the effect of temperature, addition of T_4 and preincubations with metabolic inhibitors

Test		n	K_{m1} (µM)	V_{max1} (nmol/35 µg DNA/min)	K_{m2} (nM)	V_{max2} (pmol/35 µg DNA/min)
21°C		25	1.8 (1.1–2.5)[a]	3.3 (2.0–4.8)[a]	21 (9– 29)[a]	16 (6–25)[a]
0°C		4	1.5 (0.9–1.9)	2.8 (1.2–4.9)	–	– [b]
37°C		4	2.8 (2.5–3.1)	4.1 (3.1–5.0)	61 (34–108)	48 (26–91)[b]
[FT_4]	20 µM	6	1.9 (1.1–2.5)	2.4 (1.3–3.8)	–	–
[KCN]	2 mM	6	2.0 (1.9–2.7)	2.7 (2.0–4.5)	–	– [b]
[DNP]	2 mM	4	2.3 (2.2–2.4)	4.2 (3.5–4.9)	–	– [b]
[Oligomycin]	100 µM	4	1.8 (1.6–1.9)	2.4 (2.0–2.7)	–	– [b]

[a] Mean (range)
[b] Zero V_{max} after cross-correction for uptake by the low affinity system

calculate the K_m and V_{max} values of both systems the method in [16] was used which corrects for the contribution of one system when the parameters of the other are calculated. In table 1 the results are summarized. At 21°C a low affinity system with a K_m of 1.8 μM and a V_{max} of 3.3 nmol/35 μg DNA/min, and a high affinity system with a K_m of 21 nM and a V_{max} of 16 pmol/35 μg DNA/min were found. Increasing the temperature to 37°C had only a slight effect on the K_m of the low affinity system but increased both K_m and V_{max} of the high affinity system. If the temperature was lowered to 0°C the high affinity system was not measurable. When T_4 ([FT_4] 20 μM) was present during the incubations, uptake of T_3 via the high affinity system was not measurable, indicating that T_4 interfered with T_3 uptake. The low affinity system remains unaffected by T_4. When the cells were preincubated with 2 mM KCN, 2 mM DNP or 0.1 mM oligomycin for 30 min at 37°C the high affinity system was blocked. These treatments resulted in a decrease of total intracellular ATP of ~80% (not detailed here).

4. Discussion

The results clearly show that there are two saturable uptake systems for T_3 in cultured rat liver parenchymal cells. The high affinity system is blocked by KCN, DNP and oligomycin, probably as a result of their effects on intracellular ATP concentration. The effects of temperature on this system could also support this energy dependency, although other explanations like changes in membrane fluidity are possible. The fact that KCN, DNP or oligomycin do not influence the uptake characteristics of the low affinity system makes it unlikely that in this case an energy-dependent process is involved. Since initial T_3 uptake kinetics have been analyzed, it is assumed that transport and/or binding at the level of the cell membrane is being measured. The energy dependency of the high affinity system strongly suggests that at least part of the transport through the cell membrane takes place via this pathway. The low affinity energy-independent system may represent binding at the membrane level. This is supported by the finding that temperature changes do not affect the 'V_{max}' of this system. Cytosolic thyroid hormone binding proteins are probably not involved, since binding of T_3 to these sites is inhibited by T_4 [17] as opposed to the lack of effect of T_4 on the low affinity system.

References

[1] Bernal, J. and Refetoff, S. (1977) Clin. Endocrinol. 6, 227–249.
[2] Lein, A. and Dowben, R. M. (1961) Am. J. Physiol. 200, 1029–1032.
[3] Freinkel, N., Ingbar, S. H. and Dowling, J. T. (1957) J. Clin. Invest. 36, 25–38.
[4] Tata, J. R. (1964) in: The Thyroid Gland (Pitt-Rivers, R. and Trotter, W. B. eds) p. 163, Butterworths, London.
[5] Stitzer, K. and Jacquez, J. A. (1975) Am. J. Physiol. 229, 172–178.
[6] Rao, G. S., Eckel, J., Rao, M. L. and Breuer, H. (1975) Biochem. Biophys. Res. Commun. 73, 98–104.
[7] Pliam, N. B. and Goldfine, I. D. (1977) Biochem. Biophys. Res. Commun. 79, 166–173.
[8] Oxender, D. L. (1972) Ann. Rev. Biochem. 41, 777–814.
[9] Schreiber, G. and Schreiber, M. (1973) Subcell. Biochem. 2, 307–353.
[10] Seglen, P. O. (1977) Biochim. Biophys. Acta 496, 182–191.
[11] Docter, R., Krenning, E. P., Bernard, H. F., Visser, T. J. and Hennemann, G. (1977) Endocrinology 100, T-16.
[12] Zappi, E. (1967) J. Chromatogr. 31, 241–242.
[13] Berry, M. N. and Friend, D. S. (1969) J. Cell. Biol. 43, 506–520.
[14] Burton, K. (1956) Biochem. J. 62, 315–323.
[15] Sterling, K. and Brenner, M. A. (1956) J. Clin. Invest. 45, 153–163.
[16] Spears, G., Sneyd, J. G. T. and Loten, E. G. (1971) Biochem. J. 125, 1149–1150.
[17] Visser, T. J., Bernard, H. F., Docter, R. and Hennemann, G. (1976) Acta Endocrinol. 82, 98–104.