Comparison of mechanisms mediating uptake and efflux of thyroid hormones in the human choriocarcinoma cell line, JAR

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Abstract

We compared the specificities of transport mechanisms for uptake and efflux of thyroid hormones in cells of the human choriocarcinoma cell line, JAR, to determine whether triiodothyronine (T_3), thyroxine (T_4) and reverse T_3 (rT_3) are carried by the same transport mechanism. Uptake of ^{125}I - T_3 , ^{125}I - T_4 and ^{125}I - rT_3 was saturable and stereospecific, but not specific for T_3 , T_4 and rT_3 , as unlabelled L-stereoisomers of the thyroid hormones inhibited uptake of each of the radiolabelled hormones. Efflux of ^{125}I - T_3 was also saturable and stereospecific and was inhibited by T_4 and rT_3 . Efflux of ^{125}I - T_4 or ^{125}I - rT_3 was, in contrast, not significantly inhibited by any of the unlabelled thyroid hormones tested. A range of compounds known to interfere with receptor-mediated thyroid hormone uptake in cells inhibited uptake of ^{125}I - T_3

and ^{125}I - rT_3 , but not ^{125}I - T_4 . We conclude that in JAR cells uptake and efflux of ^{125}I - T_3 are mediated by saturable and stereospecific membrane transport processes. In contrast, the uptake, but not the efflux, of ^{125}I - T_4 and ^{125}I - rT_3 is saturable and stereospecific, indicating that uptake and efflux of T_4 and rT_3 in JAR cells occur by different mechanisms. These results suggest that in JAR cells thyroid hormones may be transported by at least two types of transporters: a low affinity iodothyronine transporter (Michaelis constant, K_m , around $1 \mu M$) which interacts with T_3 , T_4 and rT_3 , but not amino acids, and an amino acid transporter which takes up T_3 , but not T_4 or rT_3 . Efflux of T_4 and rT_3 appears to occur by passive diffusion in these cells.

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Introduction

The degree of thyroid hormone transfer from mother to child remains controversial, although the clinical studies of Vulsmä *et al.* (1989) indicate that, at least in the presence of a hypothyroid fetus, considerable maternal thyroid hormone can reach the fetus. The mechanisms and control of thyroid hormone transfer are, however, as yet undefined.

We have previously described carrier-mediated, saturable membrane transport mechanisms for uptake of triiodothyronine (T_3) (Mitchell *et al.* 1992a, 1994) and thyroxine (T_4) (Mitchell *et al.* 1995) in the human choriocarcinoma cell line, JAR. These transporters have calculated Michaelis constants (K_m) of $1 \mu M$ and $60 nM$ respectively. The T_3 transport mechanism in JAR cells is kinetically similar to that described by us in normal cultured trophoblast cells ($K_m=0.8 \mu M$ (Mitchell *et al.* 1992b)). In JAR cells, T_3 and T_4 uptake are both dependent on intracellular energy but independent of the Na^+ gradient across the cell membrane. The nuclear uptake of T_3 in JAR cells is time dependent and accounts

for around 5% of total cellular uptake after 120 min of incubation (A M Mitchell, unpublished observations).

Receptor-mediated uptake of thyroid hormones has been described in many types of cells (for recent reviews see Kragie (1994, 1996)). There have been, in contrast, few studies investigating mechanisms of efflux of thyroid hormones from cells (Hennemann *et al.* 1984, Osty *et al.* 1990a,b, Samson *et al.* 1992, Zhou *et al.* 1992). The finding of saturable efflux processes for thyroid hormones in rat (Osty *et al.* 1990b, Samson *et al.* 1992, Zhou *et al.* 1992) and human (Osty *et al.* 1990a) erythrocytes raised the possibility that similar saturable efflux processes are present in trophoblasts. These could contribute to the regulation of transplacental transfer of thyroid hormone. We have found evidence in JAR cells that efflux of T_3 occurs by a saturable process inhibited by external T_3 , T_4 and tryptophan (Trp) (Mitchell *et al.* 1994). We observed a 50% reduction in the efflux rate of T_3 with external T_3 and Trp concentrations of around $2 \mu M$ and $2 mM$ respectively. It is not known, however, whether uptake and release of thyroid hormones occur in these cells by the same or different transporters. In the present study, we

compared the specificities of transport mechanisms for uptake and efflux of thyroid hormones in JAR cells to determine whether T_3 , T_4 and reverse T_3 (rT_3) interact with the same transport mechanisms.

Materials and Methods

Reagents

Materials were purchased from the following sources: $^{125}\text{I}-T_3$ (3300 $\mu\text{Ci}/\mu\text{g}$), $^{125}\text{I}-rT_3$ (790–1250 $\mu\text{Ci}/\mu\text{g}$) and $^{125}\text{I}-T_4$ (1250 $\mu\text{Ci}/\mu\text{g}$) from Du Pont Company, Wilmington, DE, USA; fetal calf serum from Commonwealth Serum Laboratories, Melbourne, Victoria, Australia; bicinchoninic acid (BCA) Protein Reagent from Pierce Chemicals, Rockford, IL, USA; six-well tissue culture plates from Costar, Cambridge, MA, USA. All other chemicals and cell culture media were from Sigma Chemical Co., St Louis, MO, USA. Nitrofen was a gift from Rohm and Haas Company, Philadelphia, PA, USA and nitrendipine was a gift from Bayer Pharmaceutical Division, West Haven, CO, USA.

Cell culture

The JAR cell line was purchased from American Type Culture Collection, Rockville, MD, USA and maintained in continuous culture at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 . Culture medium was RPMI 1640 supplemented with 10% (v/v) fetal calf serum. Cells were subcultured three times a week. For uptake and efflux experiments, 3×10^5 cells were plated into each well of the six-well tissue culture plates. Medium was changed 24 h after plating. Cells were cultured for 2–3 days. At the end of the uptake and efflux experiments, viability of the cells was assessed by the trypan blue exclusion test and was always over 90%.

Uptake and efflux studies

The procedures for uptake and efflux studies and the determination of the kinetic parameters (K_m and maximum velocity) of initial cellular uptake of $^{125}\text{I}-T_3$, $^{125}\text{I}-T_4$ and $^{125}\text{I}-rT_3$ were as previously described (Mitchell *et al.* 1994, 1995). Briefly, prior to all uptake experiments cells were incubated for 1 h in Hanks' balanced salts solution (HBSS). All incubations were carried out at 37 °C. To terminate uptake, incubation medium was aspirated, cells were washed twice in ice-cold HBSS and immediately lysed in 1 M NaOH. Cell-associated radioactivity was determined by counting the radioactivity in a γ -counter with a counting efficiency of 84%. ^{125}I -labelled thyroid hormone tracer taken up in the presence of analogue or inhibitor was expressed as a percentage of that taken up in the absence of the analogue or inhibitor. The specificity

of the uptake process was examined by incubating the cells for 30 min in the presence of either 30 pM $^{125}\text{I}-T_3$, 100 pM $^{125}\text{I}-rT_3$ or 50 pM $^{125}\text{I}-T_4$, with or without 10 μM excess of unlabelled thyroid hormones, 10 mM Trp or 100 μM mefenamic acid, verapamil, nifedipine or nitrendipine. Drugs were dissolved in ethanol and diluted in HBSS. The final concentration of ethanol did not exceed 0.4%. This had no effect on cellular uptake of T_3 , rT_3 or T_4 . To determine the kinetic parameters of uptake, the cells were incubated in the presence of 30 pM $^{125}\text{I}-T_3$, 50 pM $^{125}\text{I}-T_4$ or 100 pM $^{125}\text{I}-rT_3$ and unlabelled T_3 , T_4 or rT_3 (0–10 μM) for 2 min. Results from 12–15 determinations were pooled and data fitted to the Michaelis-Menten equation using a non-linear curve-fitting program (GraphPad Prism, GraphPad, San Diego, CA, USA). Inhibition constants (K_i) were calculated by fitting the data pooled from three to eight determinations to the one-site competition model using non-linear regression by GraphPad Prism software.

The procedure to study efflux was as described previously (Mitchell *et al.* 1994). Briefly, cells were incubated for 45 min at 37 °C with 30 pM $^{125}\text{I}-T_3$ or 50 pM $^{125}\text{I}-T_4$, washed, and then incubated for 30 min at 37 °C in non-radioactive medium with or without 10 μM unlabelled thyroid hormone analogue, 100 μM nitrendipine or 10 mM unlabelled Trp. ^{125}I -labelled thyroid hormone tracer released in the presence of an analogue, Trp or nitrendipine during the 30 min incubation in non-radioactive medium was expressed as a percentage of that released in the absence of the analogue, Trp or nitrendipine.

We sought evidence of metabolism of $^{125}\text{I}-T_3$, $^{125}\text{I}-T_4$ and $^{125}\text{I}-rT_3$ by duplicate cultures of JAR cells during uptake experiments by analysing radioactivity present in the cells and in the medium after incubation with the tracers for 30 min at 37 °C. Similarly to previous studies, we found only minimal evidence of metabolism of the tracers by the cells.

Determination of the cellular protein content

The protein content of cell lysates was determined with the BCA reagent, which is a modification of the Biuret reaction, using BSA as a standard.

Statistical analysis

Statistical analysis was performed by one-way ANOVA followed by multiple comparison of means against a single control (Bonferroni's *t*-test) using the statistical software package, SigmaStat (Jandel Scientific, San Rafael, CA, USA). Results were expressed as means and standard errors of the mean, *n* is the number of independent determinations. A probability of <0.05 was regarded as significant.

Figure 1 Effect of thyroid hormone analogues on uptake of $^{125}\text{I-T}_3$ (A), $^{125}\text{I-T}_4$ (B) and $^{125}\text{I-rT}_3$ (C) in JAR cells. Cells were incubated for 30 min at 37 °C with 30 pM $^{125}\text{I-T}_3$, 50 pM $^{125}\text{I-T}_4$ or 100 pM $^{125}\text{I-rT}_3$ in the absence or in the presence of 10 μM unlabelled thyroid hormone analogue, or 10 mM unlabelled Trp. Values shown are means $\pm$ S.E.M. ($n=3-8$). * $P<0.05$.

Results

The uptake of $^{125}\text{I-T}_3$, $^{125}\text{I-rT}_3$ and $^{125}\text{I-T}_4$ by cultures of JAR cells was saturable. T_3 uptake was reduced to $28.0 \pm 5.4\%$ of control values ($n=4$) by an excess of unlabelled L-T_3 (Fig. 1A). T_4 uptake was reduced to $71.9 \pm 4.9\%$ ($n=7$) by unlabelled L-T_4 (Fig. 1B) and rT_3 uptake was reduced by unlabelled rT_3 to $31.4 \pm 2.4\%$ ($n=3$) (Fig. 1C).

Uptake of $^{125}\text{I-T}_3$, $^{125}\text{I-T}_4$ and $^{125}\text{I-rT}_3$ was stereospecific, as it was not significantly reduced by a 10 μM excess of unlabelled D-T_3 or D-T_4 (Fig. 1A and B). Unlabelled D-rT_3 (10 μM) inhibited only $20.4 \pm 1.0\%$ ($n=9$) of $^{125}\text{I-rT}_3$ uptake (Fig. 1C). Unlabelled L-steroisomers of thyroid hormones inhibited $^{125}\text{I-T}_3$ uptake (Fig. 1A), $^{125}\text{I-T}_4$ uptake (Fig. 1B) and $^{125}\text{I-rT}_3$ uptake (Fig. 1C). In the presence of 10 mM unlabelled Trp, or 10 μM L-rT_3 or L-T_4 , total cellular uptake of $^{125}\text{I-T}_3$ was reduced to $49.2 \pm 1.0\%$ ($n=3$), $66.0 \pm 7.1\%$ ($n=4$) and $73.2 \pm 13.9\%$ ($n=4$) of control values respectively (Fig. 1A). Similarly, a 10 μM excess of unlabelled L-rT_3 and L-T_3 reduced total cellular uptake of $^{125}\text{I-T}_4$ to $77.2 \pm 4.0\%$ ($n=7$) and $78.5 \pm 4.4\%$ ($n=8$) (Fig. 1B). Unlabelled L-T_4 and L-T_3 (both 10 μM) reduced total uptake of $^{125}\text{I-rT}_3$ to $26.4 \pm 2.7\%$ ($n=3$) and $17.8 \pm 1.3\%$ ($n=3$) respectively (Fig. 1C).

Mefenamic acid, verapamil, nifedipine and nitrendipine (all 100 μM) reduced saturable $^{125}\text{I-T}_3$ uptake to $47.8 \pm 5.8\%$ ($n=3$), $43.6 \pm 15.8\%$ ($n=3$), $31.0 \pm 1.8\%$ ($n=3$) and $29.7 \pm 2.1\%$ ($n=3$) of control uptake respectively in the absence of inhibitor (Fig. 2A), but failed to inhibit saturable $^{125}\text{I-T}_4$ uptake (Fig. 2B). Verapamil and nifedipine also reduced saturable uptake of $^{125}\text{I-rT}_3$ to $76.8 \pm 4.3\%$ ($n=4$) and $81.0 \pm 2.6\%$ ($n=4$) of control values respectively.

Efflux of $^{125}\text{I-T}_3$ from the cells was inhibited in the presence of unlabelled L-T_3 in the external medium (Fig. 3A), indicating saturability. The amount of $^{125}\text{I-T}_3$ released from the cells during 30 min incubation in the presence of external unlabelled L-T_3 (10 μM) was reduced to $67.3 \pm 4.2\%$ ($n=5$) of that released in the absence of unlabelled T_3 . Efflux of $^{125}\text{I-T}_3$ was also inhibited by the thyroid hormones and nitrendipine (Fig. 3A), but was not reduced by a 10 μM excess of unlabelled D-T_3 , indicating that efflux is also stereospecific. In contrast to efflux of $^{125}\text{I-T}_3$, efflux of $^{125}\text{I-T}_4$ from the cells was not significantly affected by any of the unlabelled thyroid hormones tested (Fig. 3B). As was seen for efflux of $^{125}\text{I-T}_4$, but in contrast to efflux of $^{125}\text{I-T}_3$, efflux of $^{125}\text{I-rT}_3$ was not inhibited by a 10 μM excess of extracellular unlabelled rT_3 .

Increasing concentrations of unlabelled T_4 , T_3 or rT_3 (0–10 μM) progressively inhibited the initial rates of specific uptake of $^{125}\text{I-T}_3$, $^{125}\text{I-T}_4$ and $^{125}\text{I-rT}_3$ (Fig. 4). The calculated K_i for the inhibition of $^{125}\text{I-T}_3$ uptake by unlabelled T_4 and rT_3 , $^{125}\text{I-T}_4$ uptake by unlabelled T_3 and rT_3 and $^{125}\text{I-rT}_3$ uptake by unlabelled T_3 and T_4 , and K_m for uptake of $^{125}\text{I-T}_3$, $^{125}\text{I-T}_4$ and $^{125}\text{I-rT}_3$ are listed in Table 1.

Discussion

Although we have previously characterised stereospecific, energy-dependent uptake of T_4 and T_3 by JAR cells it has not been clear if shared or distinct transporters mediate

Figure 2 Effect of various inhibitors on saturable uptake of $^{125}\text{I-T}_3$ (A), $^{125}\text{I-T}_4$ (B) and $^{125}\text{I-rT}_3$ (C) in JAR cells. Cells were incubated for 2 min at 37 °C with 30 pM $^{125}\text{I-T}_3$, 50 pM $^{125}\text{I-T}_4$ or 100 pM $^{125}\text{I-rT}_3$ in the absence or in the presence of 100 μM mefenamic acid, verapamil, nifedipine or nitrendipine. Values are means $\pm$ S.E.M. ($n=3-4$). * $P<0.05$.

Figure 3 Effect of thyroid hormone analogues on efflux of $^{125}\text{I-T}_3$ and $^{125}\text{I-T}_4$ from JAR cells. Cells were incubated for 45 min at 37 °C with 30 pM $^{125}\text{I-T}_3$ or 50 pM $^{125}\text{I-T}_4$, washed, and then incubated for 30 min at 37 °C in non-radioactive medium with or without 10 μM unlabelled thyroid hormone analogue, or 10 mM unlabelled Trp. Values shown are means $\pm$ S.E.M. ($n=4-5$). * $P<0.05$.

Figure 4 Interaction between uptake systems for thyroid hormones in JAR cells. Cells were incubated for 2 min at 37 °C with 30 pM ^{125}I -T₃, 50 pM ^{125}I -T₄ or 100 pM ^{125}I -rT₃ in the presence of increasing concentrations (0–10 μM) of an appropriate unlabelled thyroid hormone analogue. Values are means $\pm$ S.E.M. ($n=3$ –10).

uptake of T₃, T₄ and rT₃. It is also uncertain whether thyroid hormones enter and leave the cells by the same or different mechanisms. In this study, we further character-

ised thyroid hormone transport processes in JAR cells by examining interactions between uptake and efflux of T₃, T₄ and rT₃ and compounds which interfere with uptake of thyroid hormones in other types of cells. We chose the cytotrophoblast-derived cell line, JAR, as a convenient model of the human trophoblast. Although these cells are transformed and may exhibit characteristics different from those found in normal trophoblast cells, they do retain membrane transport processes for taurine and T₃ which are kinetically identical to those of normal trophoblast (Kulanthavel *et al.* 1991, Mitchell *et al.* 1992a). We previously found no significant deiodination of either ^{125}I -T₃ (Mitchell *et al.* 1992a), ^{125}I -T₄ (Mitchell *et al.* 1995) or ^{125}I -rT₃ (Mitchell *et al.* 1998) under the experimental conditions used in the present study. The lack of deiodination by these cells makes them a useful model for study of membrane transport of thyroid hormones.

We studied the effects of unlabelled thyroid hormones on uptake of ^{125}I -T₃, ^{125}I -T₄ and ^{125}I -rT₃ in JAR cells to examine the specificity of thyroid hormone uptake processes. Of the thyroid hormones tested, L-T₃ (10 μM) was the most effective inhibitor of ^{125}I -T₃ uptake, with inhibition of around 70% of total uptake. The transport mechanism for ^{125}I -T₃ was, however, not specific for L-T₃ as it also interacted with L-T₄, Trp and rT₃. These latter compounds were not as effective in inhibiting ^{125}I -T₃ uptake as L-T₃ itself and L-T₄ inhibited only about 30% of total T₃ uptake. Similarly, uptake of ^{125}I -T₄ was not only inhibited by L-T₄ (around 30%), but also by L-T₃ and rT₃ (both around 20%). Although unlabelled rT₃ inhibited around 30% of ^{125}I -rT₃ uptake, unlabelled L-T₄ and L-T₃ were more effective inhibitors of ^{125}I -rT₃ uptake than L-rT₃, indicating that the uptake process was not specific for rT₃.

Table 1 Kinetic parameters of uptake of ^{125}I -labelled thyroid hormones and of inhibition of ^{125}I -labelled thyroid hormone uptake by unlabelled thyroid hormones in JAR cells

	Kinetic parameter	Mean $\pm$ S.E.M. uptake (μM)	<i>n</i>	<i>P</i>
Transporters				
T ₃ and T ₄	<i>K_m</i> for T ₃ uptake	0.39 $\pm$ 0.17	5	} 0.095
	<i>K_i</i> for inhibition of T ₄ uptake by T ₃	1.10 $\pm$ 0.27	7	
	<i>K_m</i> for T ₄ uptake	0.75 $\pm$ 0.16	3	} 0.686
	<i>K_i</i> for inhibition of T ₃ uptake by T ₄	1.13 $\pm$ 0.74	4	
T ₃ and rT ₃	<i>K_m</i> for T ₃ uptake	0.39 $\pm$ 0.17	5	} 0.031
	<i>K_i</i> for inhibition of rT ₃ uptake by T ₃	1.23 $\pm$ 0.29	4	
	<i>K_m</i> for rT ₃ uptake	3.98 $\pm$ 0.55	10	} 0.003
	<i>K_i</i> for inhibition of T ₃ uptake by rT ₃	1.55 $\pm$ 0.43	9	
T ₄ and rT ₃	<i>K_m</i> for T ₄ uptake	0.75 $\pm$ 0.16	3	} 0.762
	<i>K_i</i> for inhibition of rT ₃ uptake by T ₄	0.66 $\pm$ 0.19	4	
	<i>K_m</i> for rT ₃ uptake	3.98 $\pm$ 0.55	10	} 0.001
	<i>K_i</i> for inhibition of T ₄ uptake by rT ₃	1.05 $\pm$ 0.34	8	

We examined the specificity of thyroid hormone uptake processes in JAR cells by looking at the effects on saturable uptake of $^{125}\text{I-T}_3$, $^{125}\text{I-T}_4$ and $^{125}\text{I-rT}_3$ of a series of compounds which have been reported to interfere with thyroid hormone uptake in other cells. These included mefenamic acid (a non-steroidal anti-inflammatory drug which inhibits T_3 uptake into cultured rat hepatoma cells (Topliss *et al.* 1989)), and verapamil, nifedipine and nitrendipine (voltage-dependent calcium channel blockers of the dihydropyridine class which inhibit T_3 uptake in rat hepatoma and myoblast cell lines (Topliss *et al.* 1993)). All inhibited saturable T_3 uptake in JAR cells by around 50–70%, but had no effect on T_4 uptake, suggesting that T_3 was transported by two systems, only one of which interacted with T_4 . In addition, verapamil and nifedipine also inhibited rT_3 uptake by around 30%. The inhibition of T_3 and rT_3 uptake by calcium channel blockers raises the possibility that T_3 and rT_3 uptake in JAR cells occurs by a calcium-related mechanism, as was found for T_3 uptake in a rat hepatoma and myoblast cell lines (Topliss *et al.* 1993).

T_3 , T_4 and rT_3 all inhibited uptake of the radiolabelled hormones in a dose-dependent way (Fig. 4) with the calculated K_i within the micromolar range (Table 1). L-T_3 and L-T_4 inhibited uptake of $^{125}\text{I-T}_4$ with similar potencies, but L-T_4 was less effective in inhibiting uptake of $^{125}\text{I-T}_3$ than was L-T_3 itself. Assuming that L-T_4 was interacting with the saturable component of $^{125}\text{I-T}_3$ uptake (which accounts for around 70% of total uptake of $^{125}\text{I-T}_3$), L-T_4 inhibited less than half of the saturable uptake of $^{125}\text{I-T}_3$. This was similar to the dose-dependent ($K_i=2.9\text{ mM}$) but incomplete inhibition (around 75%) of T_3 uptake by Trp reported previously (Mitchell *et al.* 1994). These results suggest that uptake of thyroid hormones in JAR cells may be mediated by two transporters: an iodothyronine transporter which takes up T_4 , T_3 and rT_3 and is not inhibited by Trp, and another transporter which takes up T_3 , is inhibited by Trp, and is likely to be an aromatic amino acid transporter. The iodothyronine transporter which takes up T_3 , T_4 and rT_3 may be similar to the thyroid hormone transporter described in rat anterior pituitary cells (Everts *et al.* 1995).

There are conflicting reports as to whether T_3 , rT_3 and T_4 are transported by the same or different carriers in non-placental tissues. In liver, T_3 and T_4 uptake has been reported to be mediated by different transporters (Krenning *et al.* 1981), or the same carrier system (Blondeau *et al.* 1988). In rat anterior pituitary cells, rT_3 , T_4 and T_3 share the same transporter (Everts *et al.* 1994), while in rat hepatocytes rT_3 and T_4 share the same transporter, which is different from that for T_3 (Krenning *et al.* 1982). Recent evidence from serum tracer kinetic studies in humans suggests that the liver and other rapidly equilibrating tissues possess separate transport processes for T_3 , T_4 and rT_3 (Kaptein 1997).

There have been few studies of thyroid hormone efflux from cells. T_3 appears to diffuse passively from rat hepatocytes (Hennemann *et al.* 1984). Efflux of T_3 from rat erythrocytes is inhibited by excess T_3 (10 μM) (Osty *et al.* 1990b, Samson *et al.* 1992) or 5 mM Trp (Zhou *et al.* 1992) in the external medium, suggesting a saturable membrane carrier subject to trans-inhibition. Efflux of T_3 but not of T_4 from human erythrocytes is also saturable (Osty *et al.* 1990a). There is evidence in hepatocytes that T_3 uptake is energy dependent, while T_3 efflux is not, suggesting that membrane carriers mediating uptake and efflux of thyroid hormones may be distinct (Hennemann *et al.* 1984). It is not known whether similar carriers are present in the intact liver *in vivo*.

We examined the effect of thyroid hormone analogues on efflux of T_3 and T_4 in JAR cells to investigate whether release of T_3 and T_4 is mediated by the same or different mechanisms. L-T_3 (33%), L-T_4 (23%), nitrendipine (26%) and Trp (30%) inhibited efflux of $^{125}\text{I-T}_3$. Efflux of T_4 was, in contrast, not affected by any of the compounds tested, suggesting that in these cells different mechanisms mediated the release of $^{125}\text{I-T}_4$ and $^{125}\text{I-T}_3$. These results are similar to those described in human erythrocytes, where T_3 efflux is subject to trans-inhibition while T_4 efflux is not. It has been proposed that T_3 efflux is mediated by facilitated diffusion, while T_4 efflux results from passive diffusion (Osty *et al.* 1990a). Efflux of rT_3 in JAR cells was also not saturable.

The results of the present study indicated that in JAR cells both the uptake and release of T_3 occur by saturable processes that exhibit similar specificities for the other thyroid hormones. This suggests that, the same transporter mediates uptake and efflux of T_3 . Uptake of T_4 was also saturable but appeared to proceed by a mechanism distinct from that for T_3 . Efflux of rT_3 and T_4 , in contrast to efflux of T_3 , was not saturable. This suggests that, in JAR cells, different transporters mediate uptake and release of T_4 . As T_3 , but not T_4 or rT_3 , efflux was saturable, it is unlikely that in JAR cells T_3 , rT_3 and T_4 exit by a common carrier.

In liver, inhibition of membrane transport of T_3 (which has a K_m similar to values described by us in this and previous studies) significantly decreases intracellular deiodination of the hormone (Hennemann *et al.* 1986). Although, as yet, only T_3 membrane transport has been demonstrated in normal trophoblast, we postulate that in the placenta cell membrane transport may regulate access of extracellular hormones to intracellular deiodinases, thereby modulating placental thyroid hormone transfer.

In conclusion, we have demonstrated the presence of saturable, stereospecific membrane transport processes mediating both the uptake and efflux of $^{125}\text{I-T}_3$ in JAR cells. In contrast, the uptake, but not the efflux, of $^{125}\text{I-T}_4$ and $^{125}\text{I-rT}_3$ was saturable and stereospecific, indicating that uptake and efflux of T_4 and rT_3 in JAR cells proceeded via different mechanisms. An iodothyronine transporter mediates uptake of T_4 and rT_3 , and contributes

to uptake of T_3 . Efflux of T_4 and rT_3 , which is not saturable, may occur by passive diffusion, though it could occur via the iodothyronine transporter if this transporter is not subject to trans-inhibition by substrate. In addition to the iodothyronine transporter, T_3 may also be transported in JAR cells by a carrier which is inhibited by Trp and dihydropyridines, does not interact with T_4 and rT_3 , and is likely to be an aromatic amino acid transporter.

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