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 (T₃), thyroxine (T₄) and reverse T₃ (rT₃) are carried by the same transport mechanism. Uptake of ¹²⁵I-T₃, ¹²⁵I-T₄ and ¹²⁵I-rT₃ was saturable and stereospecific and was inhibited by T₄ and rT₃. Efflux of ¹²⁵I-T₄ or ¹²⁵I-rT₃ 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 ¹²⁵I-T₃ and ¹²⁵I-rT₃, but not ¹²⁵I-T₄. We conclude that in JAR cells, uptake and efflux of ¹²⁵I-T₃ are mediated by saturable and stereospecific membrane transport processes. In contrast, the uptake, but not the efflux, of ¹²⁵I-T₄ and ¹²⁵I-rT₃ is saturable and stereospecific, indicating that uptake and efflux of T₄ and rT₃ 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, Kₘ, around 1 µM) which interacts with T₃, T₄ and rT₃, but not amino acids, and an amino acid transporter which takes up T₃, but not T₄ or rT₃. Efflux of T₄ and rT₃ 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 Vulsma 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 (T₃) (Mitchell et al. 1992a, 1994) and thyroxine (T₄) (Mitchell et al. 1995) in the human choriocarcinoma cell line, JAR. These transporters have calculated Michaelis constants (Kₘ) of 1 µM and 60 nM respectively. The T₃ transport mechanism in JAR cells is kinetically similar to that described by us in normal cultured trophoblast cells (Kₘ=0·8 µM (Mitchell et al. 1992b)). In JAR cells, T₃ and T₄ uptake are both dependent on intracellular energy but independent of the Na⁺ gradient across the cell membrane. The nuclear uptake of T₃ 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 T₃ occurs by a saturable process inhibited by external T₃, T₄ and tryptophan (Trp) (Mitchell et al. 1994). We observed a 50% reduction in the efflux rate of T₃ with external T₃ and Trp concentrations of around 2 µM 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 T₃, T₄ and reverse T₃ (rT₃) interact with the same transport mechanisms.

**Materials and Methods**

**Reagents**

Materials were purchased from the following sources: ¹²⁵I-T₃ (3300 µCi/µg), ¹²⁵I-rT₃ (790–1250 µCi/µg) and ¹²⁵I-T₄ (1250 µCi/µg) 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. Nitrendipine was a gift from Rohm and Haas Company, Philadelphia, PA, USA and nitrindipine 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% CO₂. 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 × 10⁵ 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 (Kₘ and maximum velocity) of initial cellular uptake of ¹²⁵I-T₃, ¹²⁵I-T₄ and ¹²⁵I-rT₃ 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%. ¹²⁵I-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 ¹²⁵I-T₃, 100 pM ¹²⁵I-rT₃ or 50 pM ¹²⁵I-T₄, with or without 10 µM excess of unlabelled thyroid hormones, 10 mM Trp or 100 µM mfenamic 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 T₃, rT₃ or T₄. To determine the kinetic parameters of uptake, the cells were incubated in the presence of 30 pM ¹²⁵I-T₃, 50 pM ¹²⁵I-T₄ or 100 pM ¹²⁵I-rT₃ and unlabelled T₃, T₄ or rT₃ (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 (Kᵢ) 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 ¹²⁵I-T₃ or 50 pM ¹²⁵I-T₄, 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. ¹²⁵I-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 ¹²⁵I-T₃, ¹²⁵I-T₄ and ¹²⁵I-rT₃ 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.
Results

The uptake of $^{125}\text{I-T}_3$, $^{125}\text{I-T}_4$ and $^{125}\text{I-rT}_3$ by cultures of JAR cells was saturable. $\text{T}_3$ uptake was reduced to $28.0 \pm 5.4\%$ of control values ($n=4$) by an excess of unlabelled $\text{rT}_3$ (Fig. 1A). $\text{T}_4$ uptake was reduced to $71.9 \pm 4.9\%$ ($n=7$) by unlabelled $\text{rT}_3$ (Fig. 1B) and $\text{rT}_3$ uptake was reduced by unlabelled $\text{T}_3$ 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 $\text{rT}_3$ or $\text{T}_3$ (Fig. 1A and B). Unlabelled $\text{rT}_3$ (10 µM) inhibited only $20.4 \pm 1.0\%$ ($n=9$) of $^{125}\text{I-T}_3$ uptake (Fig. 1C). Unlabelled $\text{T}_3$-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 $\text{rT}_3$ or $\text{T}_3$, total cellular uptake of $^{125}\text{I-T}_3$ was reduced to $49.2 \pm 1.0\%$ ($n=3$), 66.0 ± 7.1% ($n=4$) and 73.2 ± 13.9% ($n=4$) of control values respectively (Fig. 1A). Similarly, a 10 µM excess of unlabelled $\text{rT}_3$ and $\text{T}_3$ reduced total cellular uptake of $^{125}\text{I-T}_4$ to $77.2 \pm 4.0\%$ ($n=7$) and 78.5 ± 4.4% ($n=8$) (Fig. 1B).

Unlabelled $\text{rT}_3$ and $\text{T}_3$ (both 10 µM) reduced total uptake of $^{125}\text{I-rT}_3$ to $26.4 \pm 2.7\%$ ($n=3$) and 17.8 ± 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 ± 15.8% ($n=3$), 31.0 ± 1.8% ($n=3$) and 29.7 ± 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 ± 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 $\text{T}_3$ 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 $\text{T}_3$ (10 µM) was reduced to $67.3 \pm 4.2\%$ ($n=5$) of that released in the absence of unlabelled $\text{T}_3$. 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 $\text{T}_3$, 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 $\text{T}_3$.

Increasing concentrations of unlabelled $\text{T}_3$, $\text{T}_3$ or $\text{rT}_3$ (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 $\text{T}_3$ and $\text{rT}_3$, $^{125}\text{I-T}_4$ uptake by unlabelled $\text{T}_3$ and $\text{rT}_3$ and $^{125}\text{I-rT}_3$ uptake by unlabelled $\text{T}_3$ and $\text{rT}_3$, 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 $\text{T}_4$ and $\text{T}_3$ by JAR cells it has not been clear if shared or distinct transporters mediate
Figure 2 Effect of various inhibitors on saturable uptake of $^{125}$I-T$_3$ (A), $^{125}$I-T$_4$ (B) and $^{125}$I-rT$_3$ (C) in JAR cells. Cells were incubated for 2 min at 37°C with 30 pM $^{125}$I-T$_3$, 50 pM $^{125}$I-T$_4$ or 100 pM $^{125}$I-rT$_3$ in the absence or in the presence of 100 μM mefenamic acid, verapamil, nifedipine or nitrendipine. Values are means ± S.E.M. (n=3–4). *P<0·05.

Inhibitors

Figure 3 Effect of thyroid hormone analogues on efflux of $^{125}$I-T$_3$ and $^{125}$I-T$_4$ from JAR cells. Cells were incubated for 45 min at 37°C with 30 pM $^{125}$I-T$_3$ or 50 pM $^{125}$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 ± S.E.M. (n=4–5). *P<0·05.
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 characterized 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 (Kulanthaivel et al. 1991, Mitchell et al. 1992a). We previously found no significant deiodination of either ¹²⁵I-T₃ (Mitchell et al. 1992a), ¹²⁵I-T₄ (Mitchell et al. 1995) or ¹²⁵I-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 ¹²⁵I-T₃, ¹²⁵I-T₄ and ¹²⁵I-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 ¹²⁵I-T₃ uptake, with inhibition of around 70% of total uptake. The transport mechanism for ¹²⁵I-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 ¹²⁵I-T₃ uptake as L-T₃ itself and L-T₄ inhibited only about 30% of total T₃ uptake. Similarly, uptake of ¹²⁵I-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 ¹²⁵I-rT₃ uptake, unlabelled L-T₄ and L-T₃ were more effective inhibitors of ¹²⁵I-rT₃ uptake than L-rT₃, indicating that the uptake process was not specific for rT₃.

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

<table>
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<tr>
<th>Transporters</th>
<th>Kinetic parameter</th>
<th>Mean ± S.E.M.</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₃ and T₄</td>
<td>Kᵣ for T₃ uptake</td>
<td>0·39 ± 0·17</td>
<td>5</td>
<td>0·095</td>
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<td></td>
<td>Kᵣ for inhibition of T₃ uptake by T₃</td>
<td>1·10 ± 0·27</td>
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<tr>
<td></td>
<td>Kᵣ for T₄ uptake</td>
<td>0·75 ± 0·16</td>
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<td>0·686</td>
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<td>Kᵣ for inhibition of T₃ uptake by T₄</td>
<td>1·13 ± 0·74</td>
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<td></td>
</tr>
<tr>
<td>T₃ and rT₃</td>
<td>Kᵣ for T₃ uptake</td>
<td>0·39 ± 0·17</td>
<td>5</td>
<td>0·031</td>
</tr>
<tr>
<td></td>
<td>Kᵣ for inhibition of rT₃ uptake by T₃</td>
<td>1·23 ± 0·29</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kᵣ for rT₃ uptake</td>
<td>3·98 ± 0·55</td>
<td>10</td>
<td>0·003</td>
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<tr>
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<td>Kᵣ for inhibition of T₃ uptake by rT₃</td>
<td>1·55 ± 0·43</td>
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<tr>
<td>T₄ and rT₃</td>
<td>Kᵣ for T₄ uptake</td>
<td>0·75 ± 0·16</td>
<td>3</td>
<td>0·762</td>
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<tr>
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<td>Kᵣ for inhibition of rT₄ uptake by T₄</td>
<td>0·66 ± 0·19</td>
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<tr>
<td></td>
<td>Kᵣ for rT₄ uptake</td>
<td>3·98 ± 0·55</td>
<td>10</td>
<td>0·001</td>
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<tr>
<td></td>
<td>Kᵣ for inhibition of T₄ uptake by rT₃</td>
<td>1·05 ± 0·34</td>
<td>8</td>
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</table>

Figure 4 Interaction between uptake systems for thyroid hormones in JAR cells. Cells were incubated for 2 min at 37 °C with 30 pM ¹²⁵I-T₃, 50 pM ¹²⁵I-T₄ or 100 pM ¹²⁵I-rT₃ in the presence of increasing concentrations (0–10 µM) of an appropriate unlabelled thyroid hormone analogue. Values are means ± S.E.M. (n=3–10).
We examined the specificity of thyroid hormone uptake processes in JAR cells by looking at the effects on saturable uptake of 125I-T3, 125I-T4 and 125I-rT3 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 T3 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 T3 uptake in rat hepatoma and myoblast cell lines (Topliss et al. 1993)). All inhibited saturable T3 uptake in JAR cells by around 50–70%, but had no effect on T4 uptake, suggesting that T3 was transported by two systems, only one of which interacted with T4. In addition, verapamil and nifedipine also inhibited rT3 uptake by around 30%. The inhibition of T3 and rT3 uptake by calcium channel blockers raised the possibility that T3 and rT3 uptake in JAR cells occurs by a calcium-related mechanism, as was found for T3 uptake in a rat hepatoma and myoblast cell lines (Topliss et al. 1993).

T3, T4 and rT3 all inhibited uptake of the radiolabelled hormones in a dose-dependent way (Fig. 4) with the calculated Ki within the micromolar range (Table 1). 1-T3 and 1-T4 inhibited uptake of 125I-T4 with similar potencies, but 1-T4 was less effective in inhibiting uptake of 125I-T3 than was 1-T3 itself. Assuming that 1-T4 was interacting with the saturable component of 125I-T3 uptake (which accounts for around 70% of total uptake of 125I-T3), 1-T4 inhibited less than half of the saturable uptake of 125I-T3. This was similar to the dose-dependent (Ki=2·9 mM) but incomplete inhibition (around 75%) of T3 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 T3, T4 and rT3, and is not inhibited by Trp, and another transporter which takes up T4, is inhibited by Trp, and is likely to be an aromatic amino acid transporter. The iodothyronine transporter which takes up T3, T4 and rT3 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 T3, rT3 and T4 are transported by the same or different carriers in non-placental tissues. In liver, T3 and T4 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, rT3, T4 and T3 share the same transporter (Everts et al. 1994), while in rat hepatocytes rT3 and T4 share the same transporter, which is different from that for T3 (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 T3, T4 and rT3 (Kaptein 1997).

There have been few studies of thyroid hormone efflux from cells. T3 appears to diffuse passively from rat hepatocytes (Hennemann et al. 1984). Efflux of T3 from rat erythrocytes is inhibited by excess T3 (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 T3 but not of T4 from human erythrocytes is also saturable (Osty et al. 1990a). There is evidence in hepatocytes that T3 uptake is energy dependent, while T4 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 T3 and T4 in JAR cells to investigate whether release of T3 and T4 is mediated by the same or different mechanisms. 1-T3 (33%), 1-T4 (23%), nitrendipine (26%) and Trp (50%) inhibited efflux of 125I-T4. Efflux of T4 was, in contrast, not affected by any of the compounds tested, suggesting that in these cells different mechanisms mediated the release of 125I-T4 and 125I-T3. These results are similar to those described in human erythrocytes, where T3 efflux is subject to trans-inhibition while T4 efflux is not. It has been proposed that T3 efflux is mediated by facilitated diffusion, while T4 efflux results from passive diffusion (Osty et al. 1990a). Efflux of rT3 in JAR cells was also not saturable.

The results of the present study indicated that in JAR cells both the uptake and release of T3 occur by saturable processes that exhibit similar specificities for the other thyroid hormones. This suggests that, the same transporter mediates uptake and efflux of T3. Uptake of T4 was also saturable but appeared to proceed by a mechanism distinct from that for T3. Efflux of rT3 and T4, in contrast to efflux of T3, was not saturable. This suggests that, in JAR cells, different transporters mediate uptake and release of T4. As T3, but not T4 or rT4 efflux was saturable, it is unlikely that in JAR cells T3, rT3 and T4 exit by a common carrier.

In liver, inhibition of membrane transport of T3 (which has a Ks 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 T3 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 125I-T3 in JAR cells. In contrast, the uptake, but not the efflux, of 125I-T4 and 125I-rT3 was saturable and stereospecific, indicating that uptake and efflux of T4 and rT3 in JAR cells proceeded via different mechanisms. An iodothyronine transporter mediates uptake of T4 and rT3, and contributes...
to uptake of T₃. Efflux of T₄ and rT₃, 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, T₃ may also be transported in JAR cells by a carrier which is inhibited by Trp and dihydropyridines, does not interact with T₄ and rT₃, and is likely to be an aromatic amino acid transporter.

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