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, but not specific for T3, T4 and rT3, as unlabelled l-stereoisomers of the thyroid hormones inhibited uptake of each of the radiolabelled hormones. Efflux of 125I-T3 was also 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 Vulisma 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 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% 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 methylamino 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 $^{125}$I-T$_3$, $^{125}$I-T$_4$ and $^{125}$I-rT$_3$ by cultures of JAR cells was saturable. T$_3$ uptake was reduced to 28.0 ± 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 ± 4.9% ($n=7$) by unlabelled l-T$_3$ (Fig. 1B) and rT$_3$ uptake was reduced by unlabelled rT$_3$ to 31.4 ± 2.4% ($n=3$) (Fig. 1C).

Uptake of $^{125}$I-T$_3$, $^{125}$I-T$_4$ and $^{125}$I-rT$_3$ was stereo-specific, 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 ± 1.0% ($n=9$) of $^{125}$I-rT$_3$ uptake (Fig. 1C). Unlabelled l-stereoisomers of thyroid hormones inhibited $^{125}$I-T$_3$ uptake (Fig. 1A), $^{125}$I-T$_4$ uptake (Fig. 1B) and $^{125}$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 $^{125}$I-T$_3$ was reduced to 49.2 ± 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 l-rT$_3$ and l-T$_3$ reduced total cellular uptake of $^{125}$I-T$_4$ to 77.2 ± 4.0% ($n=7$) and 78.5 ± 4.4% ($n=8$) (Fig. 1B).

Unlabelled l-T$_4$ and l-rT$_3$ (both 10 µM) reduced total uptake of $^{125}$I-rT$_3$ to 26.4 ± 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 $^{125}$I-T$_3$ uptake to 47.8 ± 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 $^{125}$I-T$_4$ uptake (Fig. 2B). Verapamil and nifedipine also reduced saturable uptake of $^{125}$I-rT$_3$ to 76.8 ± 4.3% ($n=4$) and 81.0 ± 2.6% ($n=4$) respectively.

Efflux of $^{125}$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 $^{125}$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 ± 4.2% ($n=5$) of that released in the absence of unlabelled T$_3$. Efflux of $^{125}$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 $^{125}$I-T$_3$, efflux of $^{125}$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 $^{125}$I-T$_4$, but in contrast to efflux of $^{125}$I-T$_3$, efflux of $^{125}$I-rT$_3$ was not inhibited by a 10 µM excess of extracellular unlabelled rT$_3$

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

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 $T_3$, $T_4$ and $rT_3$. It is also uncertain whether thyroid hormones enter and leave the cells by the same or different mechanisms. In this study, we further characterised thyroid hormone transport processes in JAR cells by examining interactions between uptake and efflux of $T_3$, $T_4$ and $rT_3$ 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 $T_3$ 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 $^{125}$I-$T_3$ (Mitchell et al. 1992a), $^{125}$I-$T_4$ (Mitchell et al. 1995) or $^{125}$I-$rT_3$ (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-$T_3$, $^{125}$I-$T_4$ and $^{125}$I-$rT_3$ in JAR cells to examine the specificity of thyroid hormone uptake processes. Of the thyroid hormones tested, $l-T_3$ (10 µM) was the most effective inhibitor of $^{125}$I-$T_3$ uptake, with inhibition of around 70% of total uptake. The transport mechanism for $^{125}$I-$T_3$ was, however, not specific for $l-T_3$ as it also interacted with $l-T_4$, Trp and $rT_3$. These latter compounds were not as effective in inhibiting $^{125}$I-$T_3$ uptake as $l-T_3$ itself and $l-T_4$ inhibited only about 30% of total $T_3$ uptake. Similarly, uptake of $^{125}$I-$T_4$ was not only inhibited by $l-T_4$ (around 30%), but also by $l-T_3$ and $rT_3$ (both around 20%). Although unlabelled $rT_3$ inhibited around 30% of $^{125}$I-$rT_3$ uptake, unlabelled $l-T_4$ and $l-T_3$ were more effective inhibitors of $^{125}$I-$rT_3$ uptake than $L-rT_3$, indicating that the uptake process was not specific for $rT_3$.

![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-$T_3$, 50 pM $^{125}$I-$T_4$ or 100 pM $^{125}$I-$rT_3$ 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).](image)

### 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

<table>
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<th>Transporters</th>
<th>Kinetic parameter</th>
<th>Mean ± S.E.M. (µM)</th>
<th>$n$</th>
<th>$P$</th>
</tr>
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<tr>
<td>$T_3$ and $T_4$</td>
<td>$K_m$ for $T_3$ uptake</td>
<td>0·39 ± 0·17</td>
<td>5</td>
<td>0·095</td>
</tr>
<tr>
<td></td>
<td>$K_i$ for inhibition of $T_4$ uptake by $T_3$</td>
<td>1·10 ± 0·27</td>
<td>7</td>
<td></td>
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<tr>
<td></td>
<td>$K_m$ for $T_4$ uptake</td>
<td>0·75 ± 0·16</td>
<td>3</td>
<td>0·686</td>
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<td>$K_i$ for inhibition of $T_3$ uptake by $T_4$</td>
<td>1·13 ± 0·74</td>
<td>4</td>
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</tr>
<tr>
<td>$T_3$ and $rT_3$</td>
<td>$K_m$ for $T_3$ uptake</td>
<td>0·39 ± 0·17</td>
<td>5</td>
<td>0·031</td>
</tr>
<tr>
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<td>$K_i$ for inhibition of $rT_3$ uptake by $T_3$</td>
<td>1·23 ± 0·29</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$K_m$ for $rT_3$ uptake</td>
<td>3·98 ± 0·55</td>
<td>10</td>
<td>0·003</td>
</tr>
<tr>
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<td>$K_i$ for inhibition of $T_3$ uptake by $rT_3$</td>
<td>1·55 ± 0·43</td>
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<tr>
<td>$T_4$ and $rT_3$</td>
<td>$K_m$ for $T_4$ uptake</td>
<td>0·75 ± 0·16</td>
<td>3</td>
<td>0·762</td>
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<tr>
<td></td>
<td>$K_i$ for inhibition of $rT_4$ uptake by $T_4$</td>
<td>0·66 ± 0·19</td>
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<td>$K_m$ for $rT_4$ uptake</td>
<td>3·98 ± 0·55</td>
<td>10</td>
<td>0·001</td>
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<tr>
<td></td>
<td>$K_i$ for inhibition of $T_4$ uptake by $rT_3$</td>
<td>1·05 ± 0·34</td>
<td>8</td>
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</table>
We examined the specificity of thyroid hormone uptake processes in JAR cells by looking at the effects on saturable uptake of $^{125}$I-T$_3$, $^{125}$I-T$_4$ and $^{125}$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). $^{125}$I-T$_3$ and $^{125}$I-T$_4$ inhibited uptake of $^{125}$I-T$_4$ with similar potencies, but $^{125}$I-T$_4$ was less effective in inhibiting uptake of $^{125}$I-T$_3$ than was $^{125}$I-T$_3$ itself. Assuming that $^{125}$I-T$_4$ was interacting with the saturable component of $^{125}$I-T$_3$ uptake (which accounts for around 70% of total uptake of $^{125}$I-T$_3$), $^{125}$I-T$_4$ inhibited less than half of the saturable uptake of $^{125}$I-T$_3$. This was similar to the dose-dependent ($K_i$=2.9 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$_4$, 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 isolated hepatocytes 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$_4$ 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. 1-T$_3$ (33%), 1-T$_4$ (23%), nitrendipine (26%) and Trp (50%) inhibited efflux of $^{125}$I-T$_4$. 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 $^{125}$I-T$_3$ and $^{125}$I-T$_4$. 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$_3$ 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 $K_i$ 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 $^{125}$I-T$_3$ in JAR cells. In contrast, the uptake, but not the efflux, of $^{125}$I-T$_4$ and $^{125}$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

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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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