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Uptake of Reverse T_3 in the Human Choriocarcinoma Cell Line, JAr

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The uptake and efflux of reverse triiodothyronine (rT_3) in JAr cells were investigated. Uptake of ^{125}I-rT_3 was time dependent and reversible with a saturable component of around 70 per cent of total uptake after 30 min of incubation. Efflux was not saturable. Kinetic analysis of the initial specific uptake rates revealed an uptake process with a Michaelis constant of 3.04 ± 0.53 μM (mean ± SEM, $n=15$) and a corresponding maximum velocity of 9.65 ± 2.49 pmol/min/mg protein ($n=15$). Uptake of rT_3 was stereospecific, but not specific for rT_3, as unlabelled L stereoisomers of thyroid hormone analogues were more effective as inhibitors of ^{125}I-rT_3 uptake than rT_3. Unlabelled T_3 and thyroxine (T_4) (10 μM) reduced cellular uptake of ^{125}I-rT_3 by around 82 and 74 per cent, respectively. The calculated inhibition constants K_i were 1.23 ± 0.29 μM ($n=4$) and 0.66 ± 0.19 μM ($n=4$) for T_3 and T_4, respectively. Similarly, rT_3 reduced cellular uptake of ^{125}I-T_3 and ^{125}I-T_4 by 34 and 23 per cent, respectively. The calculated inhibition constants K_i were 1.75 ± 0.55 μM ($n=8$) and 1.08 ± 0.36 μM ($n=8$) for the inhibition of ^{125}I-T_3 and ^{125}I-T_4 uptake, respectively. Reverse T_3 inhibited efflux of ^{125}I-T_3 from the cells by around 20 per cent, but did not inhibit efflux of ^{125}I-T_4. These results suggest that uptake of rT_3 in JAr cells may occur via a single, saturable membrane carrier, which also interacts with T_3 and T_4, while efflux of rT_3 may occur by passive diffusion.
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INTRODUCTION

In the placenta, thyroxine (T_4) is inactivated to reverse T_3 (rT_3) and triiodothyronine (T_3) to 3,3′-diiodotyrosine (3,3′-T_2) by a type III deiodinase (Salvatore et al., 1995) and large amounts of rT_3 appear in the maternal and fetal circulations of the human placental lobule perfused with T_4 via the maternal circuit (Mortimer et al., 1996).

Nothing is known of trophoblast cell membrane handling of rT_3. We have previously shown that human trophoblast has a cell membrane transport mechanism for T_3 (Mitchell, Manley and Mortimer, 1992a) and have extended these studies to demonstrate that in the human choriocarcinoma cell line, JAr, there are carrier-mediated, saturable membrane transport mechanisms for T_3 and T_4 which interact with certain amino acids (Mitchell, Manley and Mortimer, 1992b, 1994; Mitchell et al., 1995). As structurally similar amino acids can mutually inhibit amino acid transport (Yudilevich and Sweiry, 1985; Christensen, 1990), we postulated that rT_3, present at a higher concentration in fetal than in maternal plasma (Yoshida et al., 1987), may regulate trophoblast transport of thyroid hormone. The aim of the present study, using JAr cells as a model of the human trophoblast, was to examine whether membrane transport mechanisms mediating uptake and efflux of rT_3 could be demonstrated and to determine whether rT_3 modulates the uptake and efflux of T_3 and T_4.

MATERIALS AND METHODS

Reagents

Materials were purchased from the following sources: ^{125}I-rT_3 (790–1250 μCi/μg), ^{125}I-T_3 (3300 μCi/μg) and ^{125}I-T_4 (1250 μCi/μg) from Du Pont Company, Wilmington, DE, USA; fetal calf serum from Commonwealth Serum Laboratories, Melbourne, Victoria, Australia, BCA Protein Reagent from Pierce Chemicals, Rockford, IL, USA; six-well tissue culture plates from Costar, Cambridge, MA, USA; and LH-20 Sephadex from Pharmacia LKB Biotechnology, Uppsala, Sweden. All other chemicals and cell culture media were from Sigma Chemicals, St Louis, MO, USA.

Methods

The JAr cell line was purchased from American Type Culture Collection, Rockville, MD, USA. The procedures for maintenance of cultures and preparation of cells for uptake experiments were as described previously (Mitchell, Manley and Mortimer, 1992b). Briefly, cells were maintained in continuous culture at 37°C in a humidified atmosphere of 95 per cent air

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and 5 per cent CO_2. Culture medium was RPMI 1640 supplemented with 10 per cent (v/v) fetal calf serum. Cells were subcultured three times a week. For uptake 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 uptake experiments, viability of the cells was assessed by the trypan blue exclusion method and was always over 90 per cent.

The procedures for uptake and efflux studies and the determination of the kinetic parameters (Michaelis constant K_m, and maximum velocity V_{max}) of initial cellular uptake of ^{125}I-rT_3 were as previously described for T_3 and T_4 (Mitchell, Manley and Mortimer, 1994; Mitchell et al., 1995), except that ^{125}I-rT_3 (100 pM) and rT_3 (0–10 μM) were used. Briefly, prior to 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 with ice-cold HBSS and immediately lysed in 1 M NaOH. Cell-associated radioactivity was determined by counting the radioactivity of the cell lysates in a Packard γ-counter with a counting efficiency of 84 per cent. To study time course of cellular uptake, cells were incubated for 30 min in HBSS containing 100 pM ^{125}I-rT_3 with or without an excess (10 μM) unlabelled rT_3. At intervals of 1, 2, 5, 10, 15, and 30 min, medium was aspirated and cells were lysed. An uptake curve for the saturable process was obtained by subtracting non-saturable uptake in the presence of an excess of unlabelled ligand from the total uptake in the absence of an unlabelled ligand. To study efflux of ^{125}I-rT_3 from the cells, the cells were incubated with ^{125}I-rT_3 for 30 min, washed and then incubated in fresh HBSS with and without 10 μM excess unlabelled rT_3 for 30 min. After 1, 2, 5, 10, 15 and 30 min of incubation medium was aspirated and replaced with fresh HBSS to prevent re-uptake of the hormone released into the medium. The cells were pre-incubated without an excess of unlabelled rT_3 in order to allow the entry of ^{125}I-rT_3 into the cells via the saturable uptake mechanism. If the cells were pre-incubated with a 10 μM excess of unlabelled rT_3, in addition to the trace amount of ^{125}I-rT_3, the saturable uptake mechanism which mediates uptake of rT_3 into the cells, would be saturated. Consequently, any cell-associated radioactivity present at the end of the pre-incubation period under these experimental conditions would reflect ^{125}I-rT_3 bound non-specifically to the cell surface, and not rT_3 taken up into the cells. Under such experimental conditions it would have been, therefore, impossible to study efflux of intracellular hormone from the cells.

To determine the kinetic parameters of uptake, the cells were incubated in the presence of 100 pM ^{125}I-rT_3 and unlabelled rT_3 (0–10 μM) for 2 min. Results from 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).

The specificity of rT_3 uptake process was examined by incubating the cells for 30 min in the presence of 100 pM ^{125}I-rT_3 with or without an excess (10 μM) of unlabelled L-rT_3, D-rT_3, L-T_4, L-T_3, D-T_4, D-T_3, L-tryptophan (Trp) or L-phenylalanine (Phe). ^{125}I-rT_3 tracer taken up in the presence of an analogue was expressed as a percentage of that taken up in the absence of an analogue. The procedure to examine the effect of unlabelled rT_3 on the uptake of ^{125}I-T_3 and ^{125}I-T_4 was similar, except that the cells were incubated for 30 min with either 30 pM ^{125}I-T_3, or 50 pM ^{125}I-T_4 with and without an excess (10 μM) unlabelled rT_3. Results were expressed as a percentage of initial uptake rate in the presence of an inhibitor compared with the control value in the absence of an inhibitor. Inhibition constants (K_i) were calculated by fitting data to the one-site competition model using non-linear regression by GraphPad PRISM software.

Evidence of metabolism of ^{125}I-rT_3 by duplicate cultures of JAr cells during uptake experiments was sought by analysing radioactivity present in the cells and in the medium after incubation with 100 pM ^{125}I-rT_3 for 30 min at 37°C by LH-20 Sephadex chromatography as described by Otten, Mol and Visser (1983). Cells were lysed in ethanol and media were extracted with tertiary amyl alcohol. Free iodine, iodothyronine degradation products and intact rT_3 were eluted from the column with 0.1 mM HCl, 10 per cent ethanol in NaOH and 50 per cent ethanol in NaOH, respectively.

The protein content of the cell lysates was determined with the bicinchoninic acid reagent (Pierce Chemicals) which is a modification of the Biuret reaction using bovine serum albumin as a standard.

Results are expressed as mean ± SEM, and n is the number of independent determinations. Statistical analysis was performed using Student's t-test using the statistical software package Sigma Stat (Jandel Scientific, San Rafael, CA, USA). A probability of <0.05 was regarded as significant.

RESULTS

Uptake of ^{125}I-rT_3 in the human choriocarcinoma cell line JAr was time dependent and increased during 30 min incubation to a value of about 3 per cent of added ^{125}I-rT_3 (Figure 1). Uptake was saturable, being reduced in the presence of a 10 μM excess of the unlabelled ligand. After 30 min of incubation, saturable ^{125}I-rT_3 uptake was around 70 per cent of total uptake. The early phase of the uptake curve was approximately linear allowing the use of the measurement at 2 min as an estimate of the initial uptake rate.

Uptake of ^{125}I-rT_3 was reversible, with the label being progressively released from the cells during the incubation in the non-radioactive medium (Figure 2). Efflux of ^{125}I-rT_3 from the cells was, however, not saturable, as it was not reduced in the presence of an excess (10 μM) of extracellular rT_3. Uptake of ^{125}I-rT_3 was significantly ($P<0.05$) reduced by 68.6 ± 2.4 per cent ($n=9$) after 30 min of incubation with unlabelled L-rT_3 (10 μM) while unlabelled D-rT_3 (10 μM) inhibited only 20.4 ± 1.0 per cent ($n=9$) of ^{125}I-rT_3 uptake, indicating that uptake was stereospecific. Unlabelled L-stereoisomers of thyroid hormones were more effective inhibitors of ^{125}I-rT_3

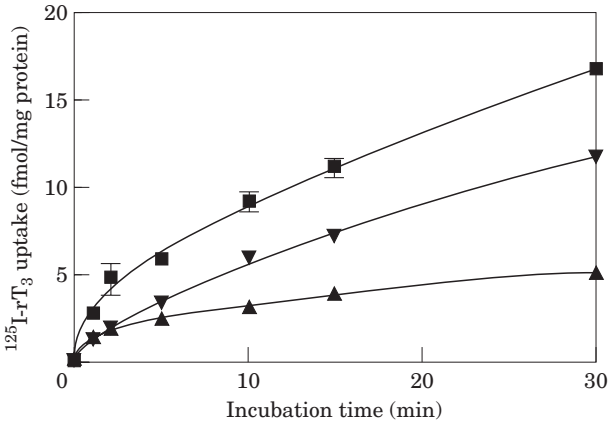

Figure 1. Time dependence of ^{125}I-rT_3 uptake in the human choriocarcinoma cell line, JAr. Cells were incubated at 37°C with 100pM ^{125}I-rT_3 in the absence (■) or in the presence (▲) of 10 μM unlabelled rT_3. Saturable uptake (▼) was obtained by subtracting non-saturable uptake in the presence of 10 μM unlabelled rT_3 from the total uptake in the absence of the unlabelled rT_3. Values shown are means ± SEM (n=9). Absence of error bars indicates that they are smaller than the symbol.

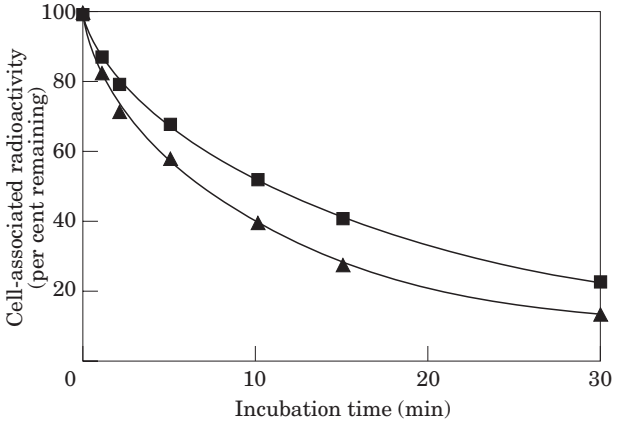

Figure 2. Washout of ^{125}I-rT_3 from the human choriocarcinoma cell line, JAr. Cells were labelled by incubation for 30 min at 37°C with 100 pM ^{125}I-rT_3, washed and incubated in fresh, non-radioactive medium with (▲) or without (■) 10 μM unlabelled rT_3 for the indicated times. The values are means ± SEM (n=9). Absence of error bars indicates that they are smaller than the symbol.

uptake than L-rT_3 indicating that the uptake process was not specific for rT_3. Unlabelled L-T_3 and L-T_4 (both 10 μM) inhibited uptake of ^{125}I-rT_3 by 82.2 ± 1.3 per cent (n=9) and 73.6 ± 2.7 per cent (n=9) respectively. Ten micromolar phenylalanine also inhibited 37.0 ± 3.7 per cent (n=3) of ^{125}I-rT_3 uptake, while 10 μM tryptophan had no significant effect.

Increasing concentrations of unlabelled rT_3 (0–10 μM) progressively inhibited the initial rate of specific uptake of ^{125}I-rT_3 (Figure 3). The initial rate of specific uptake of ^{125}I-rT_3 was reduced by around 60 per cent at the maximal concentration of rT_3. Kinetic analysis of the initial specific uptake rates revealed an uptake process with a Michaelis constant (K_m) value of 3.04 ± 0.53 μM (n=15) and a corresponding maximum velocity (V_{max}) of 9.65 ± 2.49 pmol/min/mg protein (n=15).

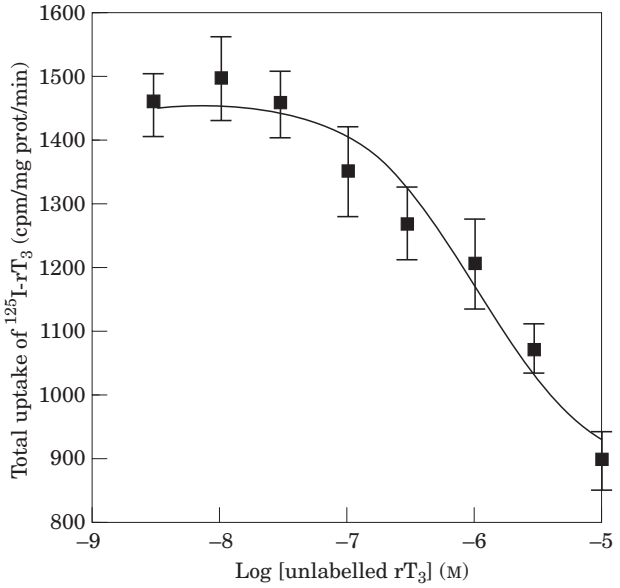

Figure 3. Concentration dependence of the initial uptake rate of ^{125}I-rT_3 in the human choriocarcinoma cell line, JAr. Cells were incubated for 2 min at 37°C with 100 pM ^{125}I-rT_3 in the presence of increasing concentrations of rT_3. Values shown are means ± SEM (n=15).

Unlabelled L-rT_3 (10 μM) significantly (P<0.05) reduced uptake of ^{125}I-T_3 and ^{125}I-T_4 to 66.0 ± 7.0 per cent (n=4) and 77.2 ± 4.0 per cent (n=7) respectively of the control uptake after 30 min of incubation. Similarly, external rT_3 significantly (P<0.05) reduced efflux of ^{125}I-T_3 from the cells. The amount of ^{125}I-T_3 released after 30 min incubation in the presence of 10 μM external unlabelled rT_3 was reduced to 82.9 ± 2.1 per cent (n=9) of control dishes. Efflux of ^{125}I-T_4 was, in contrast to efflux of ^{125}I-T_3, not reduced in the presence of 10 μM excess of external rT_3.

Increasing concentrations of unlabelled T_3 and T_4 (0–10 μM) progressively inhibited the initial rate of specific uptake of ^{125}I-rT_3 (Figure 4). In the presence of maximal concentration of either T_3 or T_4 (10 μM) the initial rate of specific ^{125}I-rT_3 uptake was reduced by around 58 and 51 per cent, respectively. The calculated inhibition constants K_i were 1.23 ± 0.15 μM (n=4) and 0.66 ± 0.10 μM (n=4) for the inhibition of ^{125}I-rT_3 uptake by T_3 and T_4 respectively. Similarly, increasing concentrations of unlabelled rT_3 (0–10 μM) progressively inhibited the initial rate of specific uptake of ^{125}I-T_3 and ^{125}I-T_4 (Figure 4). The calculated inhibition constants K_i were 1.75 ± 0.55 μM (n=8) and 1.08 ± 0.36 μM (n=8) for the inhibition of ^{125}I-T_3 and ^{125}I-T_4 uptake, respectively, by rT_3.

Only minimal evidence of metabolism of ^{125}I-rT_3 by the cells was found after uptake for 30 min. Free iodine, degradation products and intact ^{125}I-rT_3 accounted for 4.6, 3.5 and 91.9 per cent, respectively, of the radioactivity recovered from the cells, and 3.6, 1.0, and 95.8 per cent, respectively, of the radioactivity recovered from the incubation media. The corresponding figures for the radioactivity recovered from the medium incubated without the cells were 3.1, 1.2 and 95.8 per cent.

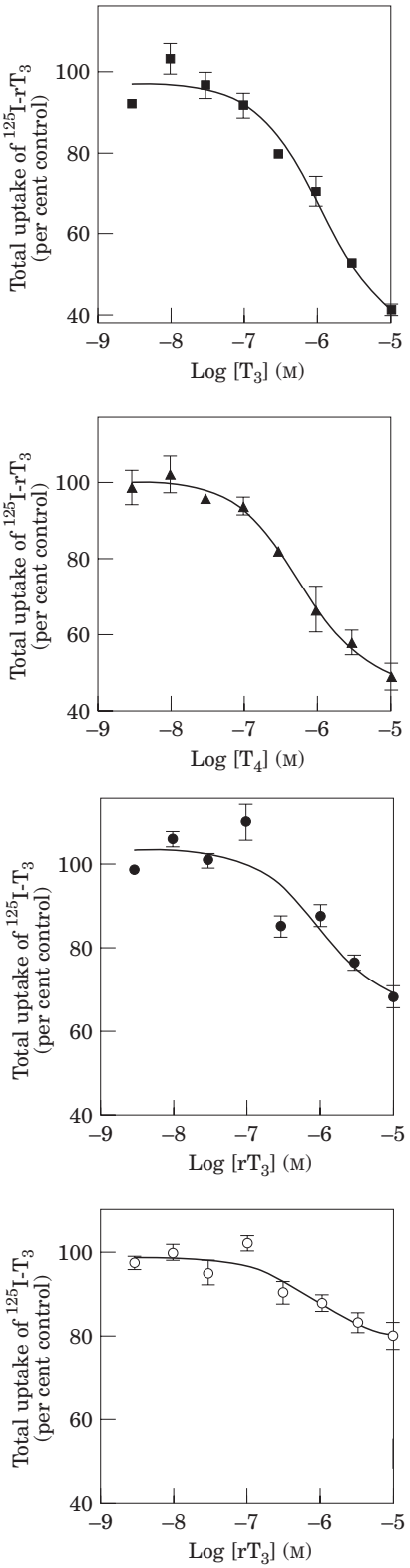

Figure 4. Inhibition of ^{125}I-rT$_3$ uptake by T$_3$ (■) and T$_4$ (▲) and ^{125}I-T$_3$ (●) and ^{125}I-T$_4$ (○) uptake by rT$_3$ in the choriocarcinoma cell line JAr. Cells were incubated for 2 min at 37°C either with 100 pM ^{125}I-rT$_3$ in the presence of increasing concentrations of either T$_3$ (n=4) or T$_4$ (n=4), or with 30 pM ^{125}I-T$_3$ (n=8) or 50 pM ^{125}I-T$_4$ (n=8) in the presence of increasing concentrations of rT$_3$. Values are means ± SEM.

DISCUSSION

Studies of thyroid hormone levels in parallel samples of human arterial and venous cord blood provided evidence that in the placental inner ring deiodination of maternal T$_4$ contributes significantly to the pool of fetal rT$_3$ (Penny et al., 1986). The concentration of total rT$_3$ in umbilical cord serum (1.5–4.5 nM) is much higher than in maternal serum (300–600 pM) and reflects the high concentrations of rT$_3$ in the fetus during the third trimester (Chopra et al., 1975). Reverse T$_3$ circulates almost entirely bound to proteins, with the free fraction (9–18 pM) being about 3 per cent of total rT$_3$ (Chopra, 1974).

Membrane transport of rT$_3$ has been described only in a small number of cells, including rat (Krenning et al., 1982) and human hepatocytes (de Jong et al., 1993), human liver-derived cell line HepG2 (van Stralen et al., 1996), rat anterior pituitary cells (Everts et al., 1995) and human monocytic leukemia cells (Yap and Schussler, 1997). In hepatocytes, rT$_3$ is taken up at least in part by saturable, high-affinity systems (K_m value in the nanomolar range in rat hepatocytes) dependent on intracellular energy (Krenning et al., 1982; de Jong et al., 1993), and Na^+ gradient (de Jong et al., 1993; van Stralen et al., 1996), and partially inhibited by a monoclonal antibody against the rat hepatocyte membrane (Hennemann et al., 1986). In anterior pituitary cells rT$_3$, T$_4$, and T$_3$ share a common transporter (Everts et al., 1994), while in rat hepatocytes rT$_3$ and T$_4$ share a transporter, which is different from that of T$_3$ (Krenning et al., 1982). Although the interactions between the transport of T$_3$, T$_4$ and rT$_3$ were not examined in human hepatocytes, recent evidence from serum tracer kinetic studies in humans indicates the presence of separate transport processes for T$_4$, T$_3$ and for rT$_3$ from serum to rapidly equilibrating tissues, including the liver (Kaptein, 1997). Reverse T$_3$ uptake in human monocytic leukaemia cells is mediated by transthyretin, a plasma transport protein for T$_4$ and rT$_3$ (Yap and Schussler, 1997).

The aim of the present study was to examine whether the JAr cells possess a membrane transport mechanism mediating uptake and efflux of rT$_3$, and whether transport of rT$_3$, T$_3$ and T$_4$ interact in these cells. We chose JAr cells as a model of human trophoblast. Although these cells are transformed, they do have taurine and T$_3$ transporters that are kinetically identical to those of normal trophoblast that have been previously described in these cells (Kulanthaivel et al., 1991; Mitchell, Manley and Mortimer, 1992a). In the present study uptake of ^{125}I-rT$_3$ was time dependent, reversible and saturable suggesting that a carrier-mediated process was involved. We have previously described carrier mediated transport of T$_4$ and T$_3$ in these cells (Mitchell, Manley and Mortimer, 1992b; Mitchell et al., 1995). The saturation kinetics of ^{125}I-rT$_3$ uptake indicated a mechanism with a single K_m value of 3 μM which was similar to the K_m value of 1 μM previously found for T$_3$ uptake in these cells, but higher than the K_m value of 60 nM previously found for T$_4$ uptake. The K_m for rT$_3$ uptake in JAr cells was around 500 times higher than K_m for rT$_3$ uptake found in rat hepatocytes (6 nM;

Krenning et al., 1982). As we previously found with T_3 uptake in JAr cells (Mitchell, Manley and Mortimer, 1992b), the saturation data suggested the presence of a second saturable uptake site with a K_m value in the picomolar range, but again the precision of the fit was not improved by use of a two-site model.

We have previously shown that uptake and efflux of T_3 and Trp in JAr cells interact (Mitchell, Manley and Mortimer, 1994). In the present study Trp did not inhibit uptake of rT_3 and Phe inhibited only 37 per cent of rT_3 uptake. These results suggest that in JAr cells rT_3 was not taken up via an amino acid carrier.

We found no significant deiodination of rT_3 by JAr cells under the experimental conditions used in the present study. These are similar results to those of Roti et al. (1982), who found no conversion of rT_3 to other labelled compounds by rat placental homogenates.

We examined the efflux of ^{125}I-rT_3 from the cells in the presence of an excess of unlabelled rT_3 in the external medium. This was done to determine whether rT_3 was released from the cells via a saturable process, similarly to the saturable release of T_3 from these cells described by us previously, and to determine whether both uptake and efflux of rT_3 in JAr cells were mediated by the same or different transporters. Efflux of rT_3, in contrast to rT_3 uptake and efflux of T_3 (Mitchell, Manley and Mortimer, 1994) in these cells, was not saturable.

We postulated that rT_3, which is present at a higher concentration in fetal than in maternal plasma, may play a role in regulation of uptake and efflux of thyroid hormones in the trophoblast. Although external rT_3 (10 μM) inhibited efflux of T_3 from the cells, it did not inhibit T_4 efflux. While it is not known whether similar interactions occur in the trophoblast in vivo, the results of the present study suggest that rT_3 is unlikely to control the transfer of maternal T_4 to the fetus by inhibiting efflux of thyroid hormone on the fetal side.

T_3 and T_4 both inhibited uptake of rT_3. Inhibition of initial saturable ^{125}I-rT_3 uptake by unlabelled T_3 and T_4 was dose dependent with calculated inhibition constants (K_i) of 1.23 ± 0.15 μM ($n=4$) and 0.66 ± 0.10 μM ($n=4$), respectively. Similarly, rT_3 inhibited uptake of both T_3 and T_4 in a dose dependent way, with K_i values of 1.75 ± 0.55 μM ($n=8$) and 1.08 ± 0.36 μM ($n=8$).

The K_i for the inhibition of T_3 uptake by rT_3 (1.75 ± 0.55 μM, $n=8$) was similar to the K_m for rT_3 uptake (3.04 ± 0.53 μM), and, similarly, the K_i for the inhibition of rT_3 uptake by T_3 was similar to the K_m for T_3 uptake (1.06 ± 0.15 μM, $n=15$, Mitchell, Manley and Mortimer, 1994), suggesting that rT_3 and T_3 shared a common transporter in JAr cells. Similarly, the K_i for the inhibition of T_4 uptake by rT_3 (1.08 ± 0.36 μM, $n=8$) was similar to the K_m for rT_3 uptake (3.04 ± 0.53 μM, $n=15$), however, the K_i for the inhibition of rT_3 uptake by T_4 (0.66 ± 0.10 μM, $n=4$) was significantly ($P<0.001$) higher than the K_m for T_4 uptake (59.4 ± 13.9 nM, $n=12$; Mitchell et al., 1995). These results suggested that while uptake of rT_3 and T_4 in JAr cells interacted, T_4 was probably also taken up by a specific, high-affinity carrier which did not interact with rT_3, in addition to the non-specific carrier which interacted with T_3, rT_3 and T_4. Taken together, these results suggest that rT_3, T_4 and T_3 share a common transporter in JAr cells similarly to rat anterior pituitary cells (Everts et al., 1995). In addition to this non-specific transporter, JAr cells also posses a specific carrier which interacts with T_4, but not with rT_3.

In summary, we demonstrated the presence of a saturable uptake mechanism for rT_3 in the human choriocarcinoma cell line JAr. This uptake process was similar to the uptake of T_3 and T_4 previously described by us in these cells. The kinetic parameters of inhibition of rT_3 uptake by T_3 and T_4 and of T_3 and T_4 uptake by rT_3 suggested that rT_3, T_3 and T_4 may share the same transporter in JAr cells. Efflux of rT_3 from the cells was not saturable indicating that the release of rT_3 from the JAr cells proceeds via a separate mechanism from that mediating uptake of rT_3 and is likely to occur by passive diffusion. Reverse T_3 did not inhibit T_4 efflux and therefore did not provide a mechanism for control of maternofetal T_4 transport.

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REFERENCES

Chopra IJ (1974) A radioimmunoassay for measurement of 3,3′,5′-triiodothyronine (reverse T_3). *J Clin Invest*, **54**, 583–592.

Chopra IJ, Chopra U, Smith SR, Reza M & Solomon DH (1975) Reciprocal changes in serum secretion of reverse T_3 (rT_3) and T_3 in systemic illnesses: evidence for independent pathways of T_4 metabolism in adults. *J Clin Endocrinol Metab*, **41**, 1043–1049.

Christensen HN (1990) Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev*, **70**, 43–77.

de Jong M, Visser TJ, Bernard BF, Docter R, Vos RA, Hennemann G & Krenning EP (1993) Transport and metabolism of iodothyronines in cultured human hepatocytes. *J Clin Endocrinol Metab*, **77**, 139–143.

Everts ME, Docter R, Moerings EPCM, van Koetsveld PM, Visser TJ, de Jong M, Krenning EP & Hennemann G (1994) Uptake of thyroxine in cultured anterior pituitary cells of euthyroid rats. *Endocrinology*, **134**, 2490–2497.

Everts ME, Visser TJ, Moerings EPCM, Tempelaars AMP, van Toor H, Docter R, de Jong M, Krenning EP & Hennemann G (1995) Uptake of 3,3′,5,5′-tetraiodothyroacetic acid and 3,3′5′-triiodothyronine in cultured anterior pituitary cells and their effects on thyrotropin secretion. *Endocrinology*, **136**, 4454–4461.

Hennemann G, Krenning EP, Polhuys M, Mol JA, Bernard BF, Visser TJ & Docter R (1986) Carrier-mediated transport of thyroid hormone into rat hepatocytes is rate-limiting in total cellular uptake and metabolism. *Endocrinology*, **119**, 1870–1872.

Kaptein EM (1997) Hormone-specific alterations of T4, T3 and reverse T3 metabolism with recent ethanol abstinence in humans. *Am J Physiol*, **272**, E191–E200.

Krenning EP, Docter R, Bernard B, Visser T & Hennemann G (1982) Decreased transport of thyroxine (T_4), 3,3′,5-triiodothyronine (T_3) and 3,3′,5′-triiodothyronine (rT_3) into rat hepatocytes in primary culture due to a decrease of cellular ATP content and various drugs. *FEBS Lett*, **140**, 229–233.

Kulanthaivel P, Cool DR, Ramamoorthy S, Mahesh VB, Leibach FH & Ganapathy V (1991) Transport of taurine and its regulation by protein kinase C in the JAR human placental choriocarcinoma cell line. *Biochem J*, **277**, 53–58.

Mitchell AM, Manley SW & Mortimer RH (1992a) Uptake of L-tri-iodothyronine by human cultured trophoblast cells. *J Endocrinol*, **133**, 483–486.

Mitchell AM, Manley SW & Mortimer RH (1992b) Membrane transport of thyroid hormone in the human choriocarcinoma cell line, JAR. *Mol Cell Endocrinol*, **87**, 139–145.

Mitchell AM, Manley SW & Mortimer RH (1994) Interactions between transport of iodothyronine and tryptophan in JAR cells. *Mol Cell Endocrinol*, **101**, 203–210.

Mitchell AM, Manley SW, Payne EJ & Mortimer RH (1995) Uptake of thyroxine in the human choriocarcinoma cell line JAR. *J Endocrinol*, **146**, 233–238.

Mortimer RH, Galligan JP, Cannell GR, Addison RS & Roberts MS (1996) Maternal to fetal thyroxine transmission in the human term placenta is limited by inner ring deiodination. *J Clin Endocrinol Metab*, **81**, 2247–2249.

Otten MH, Mol JA & Visser TJ (1983) Sulfation preceding deiodination of iodothyronines in rat hepatocytes. *Science*, **221**, 81–83.

Penny R, Sims ME, Campbell WG, Spencer CA & Nicoloff JT (1986) Thyroid indices in arterial and venous cord blood: significantly greater levels of reverse triiodothyronine in venous blood than in arterial blood. *Metabolism*, **35**, 645–648.

Roti E, Fang SL, Braverman LE & Emerson C (1982) Rat placenta is an active site of inner ring deiodination of thyroxine and 3,3′,5-triiodothyronine. *Endocrinology*, **110**, 34–37.

Salvatore D, Low SC, Berry M, Maia AL, Harney JW, Croteau W, St. Germain DL & Larsen PR (1995) Type 3 iodothyronine deiodinase: cloning, in vitro expression, and functional analysis of the placental selenoenzyme. *J Clin Invest*, **96**, 2421–2430.

van Stralen PGJ, van der Hoek HJ, Docter R, de Jong M, Krenning EP, Everts ME & Hennemann G (1996) Uptake and metabolism of 3,5,3′-triiodothyronine and 3,3′,5;-triiodothyronine by human liver-derived cells: HepG2 cells as a model for thyroid handling by human liver. *J Clin Endocrinol Metab*, **81**, 244–248.

Yap MG & Schussler GC (1997) Transthyretin (TTR) mediates cellular uptake of rT_3 as well as T_4. In *Proceedings of the 79th Annual Meeting of the Endocrine Society*, Minneapolis, Minnesota, 11–14 June 1997, abstract P2-23.

Yoshida K, Suzuki M, Sakurada T, Takahashi T, Furuhashi N, Yamamoto M, Saito S & Yoshinaga K (1987) Measurement of thyroid hormone concentrations in human placenta. *Hormone Metab Res*, **19**, 130–133.

Yudilevich DJ & Sweiry JH (1985) Transport of amino acids in the placenta. *Biochim Biophys Acta*, **822**, 169–201.