0013-7227/94/1346-2490$03.00/0
Endocrinology

Vol. 134, No. 6
Printed in U.S.A.

Uptake of Thyroxine in Cultured Anterior Pituitary Cells of Euthyroid Rats*

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ABSTRACT

The uptake of [^{125}I]T_4 was investigated in cultured anterior pituitary cells isolated from adult fed Wistar rats and cultured for 3 days in medium containing 10% fetal calf serum. Experiments were performed with [^{125}I]T_4 (10^5 to 2×10^6 cpm; 0.35–7 nM) in medium containing 0.5% or 0.1% BSA. The uptake of [^{125}I]T_4 increased with time and showed equilibrium after around 1 h of incubation. The presence of 10 μM unlabeled T_4 during incubation decreased the uptake of [^{125}I]T_4 by 65–70% at all time intervals. After 24 h of incubation, 1.5% iodide and 3.2% conjugates were detected in the medium, whereas around 20% of cellular radioactivity represented [^{125}I]T_3. The 15-min uptake of [^{125}I]T_4 was significantly reduced by simultaneous incubation with 100 nM T_4 (by 24%; $P < 0.05$), 100 nM T_3 (by 38%; $P < 0.001$), or 10 μM rT_3 (by 32%; $P < 0.001$), whereas 10 μM tetraiodothyroacetic acid (Tetrac) had no effect. Furthermore, preincubation (30 min) and incubation (15 min) with 10 μM monodansylcadaverine, oligomycin, or monensin reduced the uptake of [^{125}I]T_4 by 30%, 50%, and 40%, respectively (all $P < 0.001$). Substitution of Na^+ in the buffer by K^+ diminished the uptake of [^{125}I]T_4 by 39% ($P < 0.005$); 2 mM phenylalanine, tyrosine, or tryptophan reduced [^{125}I]T_4 uptake by 18% ($P < 0.05$), 18% (P = NS), and 33% ($P < 0.005$), respectively. Our data suggest that the pituitary contains a specific carrier-mediated energy-requiring mechanism for [^{125}I]T_4 uptake that is partly dependent on the Na^+ gradient. In addition, part of [^{125}I]T_4 uptake in the pituitary might occur through an amino acid transport system. When expressed per pM of free hormone, the 15-min uptake of [^{125}I]T_4 was approximately as high as that of [^{125}I]T_3. Because the reduction of [^{125}I]T_4 uptake by T_4, T_3, monodansylcadaverine, oligomycin, and monensin was roughly the same as the previously reported reduction of [^{125}I]T_3 uptake by the same compounds, it is further suggested that T_4 and T_3 share a common carrier in cultured anterior pituitary cells. (*Endocrinology* **134:** 2490–2497, 1994)

AROUND 50% of the intracellular available T_3 in the pituitary is derived from plasma T_3, and around 50% is from intracellular T_4 to T_3 conversion (1, 2). During starvation in rats, plasma T_4 and T_3 are decreased, and the T_4 to T_3 converting activity in the pituitary is reduced (3). Nevertheless, nuclear T_3 is maintained at a normal level in the pituitary of the fasted animal (3), and the plasma level of TSH is not increased (4) or may even decline (3). Although diminished release of TRH at least partly may explain the low TSH level during food deprivation in rats (4, 5), the observations of St. Germain and Galton (3) suggest that the suppression of TSH secretion during starvation could also be mediated by local pituitary mechanisms, *i.e.* active uptake of T_3 and/or T_4 to maintain normal nuclear receptor occupancy.

The early studies of Horiuchi *et al.* (6) and Halpern and Hinkle (7) and the recent study of Yan and Hinkle (8) demonstrated that T_3 is taken up by a carrier-mediated process in pituitary tumor cells. In cultured anterior pituitary cells of euthyroid rats, the uptake of [^{125}I]T_3 was found to be carrier mediated and dependent on the cellular energy status and the transmembrane Na^+ gradient (9).

The purpose of the present study was to investigate whether a comparable transport system exists for T_4 in the normal anterior pituitary gland. Apart from hepatocytes isolated from rats (10) or trout (11), the uptake of T_4 was previously shown to be saturable and energy dependent in rat glial cells in primary culture (12), in a mouse neuroblastoma cell line (13), as well as in pituitary tumor cells (8). Furthermore, in the mouse neuroblastoma cell line (13), cultured astrocytes (14), as well as pituitary tumor cells (8), the transport of T_3 and T_4 was competitively inhibited by amino acids transported by system L. On the other hand, in erythrocytes, the uptake of T_3 was competitively inhibited by amino acids transported by system T (15). Both systems L and T are independent of the Na^+ gradient, and both systems transport the aromatic amino acids (16).

Experiments were performed with primary cultures of anterior pituitary cells of euthyroid rats (9). The uptake of T_4 was characterized by testing the time and energy dependence; the specificity of inhibition by T_4, T_3, rT_3, and Tetrac; the dependence on the Na^+ gradient; and the effect of the aromatic amino acids phenylalanine, tyrosine, and tryptophan.

Materials and Methods

Materials

All solutions used for cell isolation and cell culture were obtained from GIBCO Europe (Breda, The Netherlands), with the exception of human serum albumin (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), dispase (grade II; Boehringer, Mannheim, Germany), and Fungizone (Bristol-Myers

Received November 17, 1993.
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* This work was supported by the Trustfonds of the Erasmus University Rotterdam (The Netherlands).
† Recipient of a fellowship from the Royal Netherlands Academy of Arts and Sciences.

Squibb, Woerden, The Netherlands). Culture dishes (48 wells) were purchased from Costar (Cambridge, MA). L-T_3, L-T_4, rT_3, tetraiodothyroacetic acid (Tetrac), piperazine-*N,N'*-bis-[2-ethane sulfonic acid] (PIPES), HEPES, *N,N*-bis-[2-hydroxyethyl]2-aminoethane sulfonic acid (Bes), BSA (fraction V), monensin, monodansylcadaverine (MDC), oligomycin, L-phenylalanine (L-Phe), L-tyrosine (L-Tyr), L-tryptophan (L-Trp), and bromodeoxyuridine (BrdU) were purchased from Sigma (St. Louis, MO). [3',5'-^{125}I]T_4 (1500 μCi/μg) and [3'-^{125}I]T_3 (3070 μCi/μg) were purchased from Amersham International (Aylesbury, United Kingdom). Sephadex LH-20 was obtained from Pharmacia (Uppsala, Sweden). All other reagents were of the highest purity available.

Animals

All experiments were performed using male Wistar rats, weighing 220–250 g. The animals had free access to food and water and were kept in a controlled environment (21 C) with constant day length (12 h).

Cell culture

Animals (12 for each experiment) were killed between 0900–0930 h by decapitation. The pituitary glands were removed within 5 min, the neurointermediate lobe was discarded, and the anterior lobes were collected in calcium- and magnesium-free Hanks' Balanced Salt Solution, supplemented with 10 g/liter human serum albumin, penicillin (10^5 U/liter), Fungizone (0.5 mg/liter), and sodium bicarbonate (0.4 g/liter). Anterior pituitary cells were dissociated with dispase (final concentration, 2.4×10^3 U/liter), as described in detail previously (17). From each pituitary, $1–2 \times 10^6$ cells were obtained, and the viability of the cells, determined by trypan blue exclusion, was greater than 90%.

The cells were cultured at 37 C in a water-jacketed incubator with 5% CO_2 at a density of 500,000 cells/well in 48-well culture dishes. The cells had attached to the wells after 2 days of culture. On day 3, the cells were used for experiments. The culture medium consisted of Minimum Essential Medium with Earle's salts (MEM) supplemented with nonessential amino acids, sodium pyruvate (1 mmol/liter), 10% fetal calf serum, penicillin (10^5 U/liter), Fungizone (0.5 mg/liter), L-glutamine (2 mmol/liter), and sodium bicarbonate (2.2 g/liter; pH 7.4) (17, 18).

In some experiments, the contamination of the pituitary cell preparation with fibroblasts was evaluated by adding BrdU (15 μg/ml) to the cell culture (19) or by replacing L-valine (L-Val) with D-Val in the culture medium (20, 21).

Uptake of [^{125}I]T_4

After removal of culture medium, cells were preincubated with 0.5 ml incubation medium. The incubation medium was identical to the culture medium, except that the fetal calf serum was replaced by 0.1 or 0.5% BSA. Preincubation was carried out at 37 C for 30 min in the presence or absence of monensin (10 μM), MDC (10 or 25 μM), oligomycin (10 μM), L-Phe (2 mM), L-Tyr (2 mM), or L-Trp (2 mM). After preincubation, the medium was removed, and incubation was started with 0.25 ml medium containing the same additions as described above or T_4, T_3, rT_3, or Tetrac (10 nM to 10 μM) and in all cases [^{125}I]T_4 (100,000–200,000 cpm; 350–700 pM). In one experiment lasting 24 h, the cells were incubated with 2×10^6 cpm [^{125}I]T_4 (7 nM) to allow analysis of the cell-associated radioactivity by Sephadex LH-20 chromatography and subsequently by HPLC.

Incubations lasting for more than 1 h were performed at 37 C in humidified air with 5% CO_2. Incubations of shorter duration took place in a 37 C incubation chamber on a rotating device without CO_2. Therefore, the $NaHCO_3$ in the culture medium was replaced by an equimolar amount of HEPES (8.9 mM), PIPES (10.6 mM), and Bes (11.2 mM).

After incubation, the medium was removed, and the cells were washed with 1-ml volumes of ice-cold saline to remove tracer not bound to the cells. Cells were dissolved in 1 ml 0.1 N NaOH and counted for ^{125}I activity in a 16-channel γ-counter (NE 1600, Nuclear Enterprises, Sighthill, Edinburgh, Scotland). The amount of [^{125}I]T_4 taken up was expressed as a percentage of the radioactivity in the incubation medium (percent dose). The same procedure was applied to the incubations without cells. All results are corrected for the amount of radioactivity retained in the wells without cells.

To allow evaluation of the Na^+ dependence of the uptake of [^{125}I]T_4, the cells were incubated in a Krebs-Ringer (KR) buffer containing 140 mM NaCl, 4.2 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM KH_2PO_4, 1.4 mM $MgSO_4 \cdot 7H_2O$, 5 mM D-glucose, 25 mM Tris-HCl, and 0.1% BSA (pH 7.4) or in KR buffer where Na^+ was replaced by K^+ or choline.

In some experiments, the uptake of [^{125}I]T_3 was measured in medium containing 0.5% BSA, as previously described (9).

Iodide production

Aliquots of the incubation medium were chromatographed on Sephadex LH-20 (22). Iodide was eluted from the column with 4×1 ml 0.1 N HCl. Subsequently, conjugates were eluted with 8×1 ml H_2O; finally, the iodothyronines were removed from the column with 3×1 ml NH_4OH in ethanol.

HPLC

Solvent (1% NH_4OH in ethanol) of samples prepurified on Sephadex LH-20 was evaporated under a stream of N_2 at 50 C. The residue was redissolved in the HPLC mobile phase and injected onto Chromspher C_{18} columns (10×0.3 cm; Chrompack International, Middelburg, The Netherlands) fitted in a Waters HPLC system (Waters Associates, Milford, MA). Isocratic elution was performed with a 50:50 mixture of methanol in 0.02 M ammonium acetate (pH 4) at a flow rate of 0.9 ml/min. Fractions of 0.3 min were collected and counted for radioactivity. Retention times of the unlabeled reference compounds were determined by monitoring the absorbance of the eluate at 254 nm (23).

Free T_4 (FT_4) concentration

Calculation of the FT_4 concentration was based on determination of the free fraction by equilibrium dialysis (24). The incubation medium for the [^{125}I]T_4 uptake experiments contained in the first experiments 0.5% BSA. However, as the FT_4 fraction was only $0.41 \pm 0.03\%$ ($n = 5$) in medium with this protein concentration (Fig. 1), cellular uptake was low, and further experiments were performed with a reduced protein concentration in the incubation medium (0.1% BSA), resulting in a FT_4 fraction of $2.7 \pm 0.2\%$ ($n = 8$). With both 0.5% and 0.1% BSA in the incubation medium, the FT_4 fraction increased substantially after the addition of 1 nM to 10 μM unlabeled T_4 (Fig. 1). This was taken into account for calculation of the net cellular [^{125}I]T_4 uptake by expressing the data per pM of FT_4. The addition of monensin and oligomycin did not change the FT_4 fraction, whereas 10 and 25 μM MDC decreased the FT_4 fraction by 12% and 21%, respectively. A concentration of 2 mM of L-Phe, L-Tyr, and L-Trp increased the FT_4 fraction by 10%, 10%, and 16%, respectively.

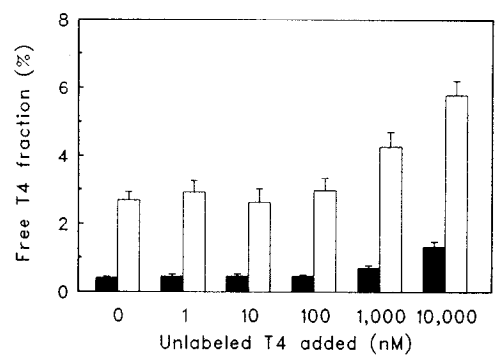

FIG. 1. The effect of addition of increasing concentrations of unlabeled T_4 on the FT_4 fraction in incubation medium with 0.5% (■) or 0.1% (□) BSA. *Bars* show the mean ± SE of three to eight determinations of the FT_4 fraction by equilibrium dialysis.

TSH determination

TSH release was measured in the different culture media after 3 days of culture. TSH was determined by RIA, as previously described (9).

DNA determination

The DNA content of the wells after variation of the culture conditions was determined using a modification (25) of the fluorescence technique described by Downs and Wilfinger (26).

Statistics

The statistical significance of the effects of the various compounds on [^{125}I]T_4 uptake was calculated using Student's t test for unpaired observations. $P < 0.05$ was regarded as statistically significant.

Results

Figure 2 shows the time course of the uptake of [^{125}I]T_4 by cultured anterior pituitary cells in incubation medium containing 0.5% BSA. [^{125}I]T_4 uptake increased with time and reached equilibrium between 1–6 h of incubation (Fig. 2, *upper curve*). The presence of 10 μM unlabeled T_4 during incubation (Fig. 2, *lower curve*) decreased [^{125}I]T_4 uptake at all time intervals (65–70%). By subtracting [^{125}I]T_4 uptake in the presence of 10 μM T_4 from uptake in the absence of unlabeled hormone, the curve for the saturable [^{125}I]T_4 uptake was obtained, which clearly showed equilibrium after 1 h of incubation (Fig. 2, *middle curve*).

To see whether [^{125}I]T_4 was metabolized in the cultured anterior pituitary cells, the incubation media from the experiment shown in Fig. 2 were analyzed by Sephadex LH-20 chromatography. Iodide production from [^{125}I]T_4 increased roughly linearly with time and was at all time intervals completely blocked by the addition of 10 μM unlabeled T_4 (Table 1). The formation of conjugates also increased with time and was inhibited by the addition of unlabeled T_4, although to a smaller extent than the production of iodide (Table 1).

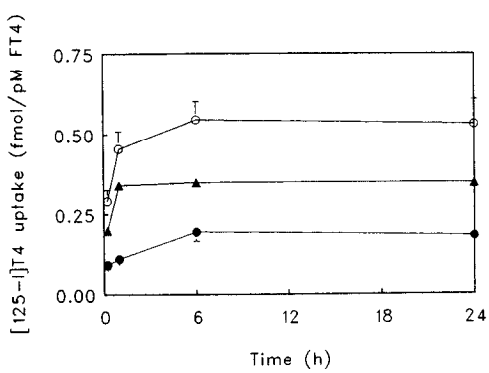

FIG. 2. Time course of [^{125}I]T_4 uptake in the absence (○) or presence (●) of 10 μM unlabeled T_4 in cultured anterior pituitary cells. The saturable component of [^{125}I]T_4 uptake (▲) was calculated as the difference in the values in the absence and presence of 10 μM T_4. Cells were cultured for 3 days at a density of 500,000 cells/well. After removal of the culture medium, the cells were preincubated for 30 min in incubation medium containing 0.5% BSA. Thereafter, they were incubated in the same medium for periods varying between 15 min and 24 h with [^{125}I]T_4 (200,000 cpm) without or with 10 μM unlabeled T_4, as described in *Materials and Methods*. Data represent the mean ± SE of triplicate observations obtained in a single experiment.

TABLE 1. Time course of iodide production and conjugate formation from [^{125}I]T_4 in cultured anterior pituitary cells

Experimental conditions	Iodide production (%)	Conjugate formation (%)
15 min		
No additions	0.06 ± 0.10	1.00 ± 0.05
+10 μM T_4	0.00 ± 0.08	0.22 ± 0.05[a]
1 h		
No additions	0.11 ± 0.19	1.04 ± 0.16
+10 μM T_4	0.00 ± 0.09	0.21 ± 0.04[a]
6 h		
No additions	0.61 ± 0.04	1.67 ± 0.36
+10 μM T_4	0.06 ± 0.04[a]	0.61 ± 0.02[a]
24 h		
No additions	1.48 ± 0.13	3.18 ± 0.31
+10 μM T_4	0.13 ± 0.08[a]	2.09 ± 0.08[a]

Data represent the mean ± SE of three observations in a single experiment (same as Fig. 2). After incubation, the medium was removed and analyzed by Sephadex LH-20 chromatography.

[a] $P < 0.05$ or less, 10 μM T_4 *vs.* no additions.

TABLE 2. Effects of 10 nM unlabeled T_3 and T_4 on the uptake and metabolism of [^{125}I]T_4 in cultured anterior pituitary cells

Experimental conditions	[^{125}I]T_4 uptake (% dose)	Cellular content	
		[^{125}I]T_3 (%)	[^{125}I]T_4 (%)
No additions	0.23 ± 0.01	21.5 ± 1.5	71.8 ± 1.0
+10 nM T_3	0.12	8.4	81.9
+10 nM T_4	0.12 ± 0.01[a]	11.2 ± 0.1[a]	81.6 ± 0.6[a]

Data indicate mean of two or mean ± SE of three observations in a single experiment. Anterior pituitary cells were cultured for 3 days at a density of 500,000 cells/well. The cells were preincubated for 30 min and then incubated for 24 h with [^{125}I]T_4 (2×10^6 cpm) in buffer containing 0.5% BSA in the absence or presence of 10 nM T_3 or T_4. Cell-associated radioactivity was analyzed by successive chromatography on Sephadex LH-20 and HPLC.

[a] $P < 0.001$ *vs.* no additions.

In a second experiment of this type, it was further investigated whether the uptake of [^{125}I]T_4 into pituitary cells resulted in detectable [^{125}I]T_3 formation. The results shown in Table 2 indicate that after 24 h of incubation, around 20% of the cell-associated radioactivity represented [^{125}I]T_3. Furthermore, it can be seen from Table 2 that both the uptake and conversion of [^{125}I]T_4 were reduced to the same extent (~50%) by simultaneous incubation with 10 nM T_3 or T_4.

To study the uptake of [^{125}I]T_4 at the level of the pituitary plasma membrane, further experiments were performed with a short incubation time (15 min) (9). To increase the FT_4 fraction and, thus, T_4 availability to the cells, the protein concentration of the incubation medium was reduced to 0.1% BSA. In two experiments, the effect of decreasing the BSA concentration on 15-min [^{125}I]T_4 uptake was tested. With 0.5% BSA in the medium, [^{125}I]T_4 uptake, expressed as a percentage of the dose, was 0.15 ± 0.03 (n = 9); with 0.1% BSA, [^{125}I]T_4 uptake was 0.50 ± 0.04 (n = 9; $P < 0.001$). [^{125}I]T_4 uptake, expressed as femtomoles per pM FT_4, amounted to 0.087 ± 0.017 (n = 9) with 0.5% BSA and 0.045 ± 0.004 (n = 9) ($P < 0.05$) with 0.1% BSA in the medium.

Figure 3 shows the effect of increasing concentrations unlabeled T_4 (10 nM to 10 μM) on the 15-min uptake of [^{125}I]

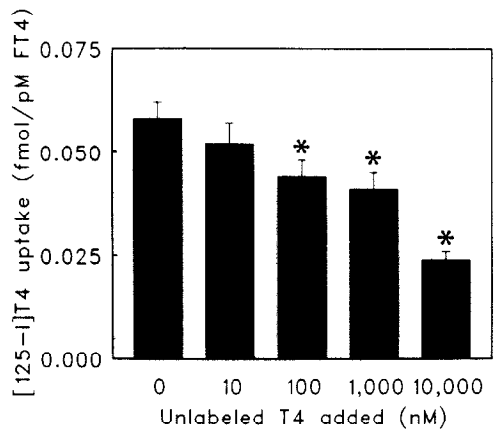

FIG. 3. Effects of increasing concentrations unlabeled T_4 on [^{125}I]T_4 uptake in cultured anterior pituitary cells. Cells were cultured for 3 days at a density of 500,000 cells/well, and the experiment was performed for 15 min in buffer containing 0.1% BSA, as described in *Materials and Methods*. Unlabeled T_4 was added simultaneously with [^{125}I]T_4 (100,000 cpm). Data represent the mean ± SE of six to nine observations obtained in two experiments. *, $P < 0.05$ or less, unlabeled T_4 *vs.* controls (*left bar*).

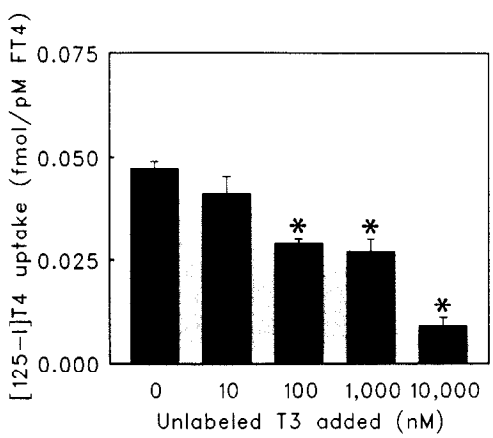

FIG. 4. Effects of increasing concentrations of unlabeled T_3 on [^{125}I]T_4 uptake in cultured anterior pituitary cells. Cells were cultured for 3 days at a density of 500,000 cells/well, and the experiment was performed for 15 min in buffer containing 0.1% BSA, as described in *Materials and Methods*. Unlabeled T_3 was added simultaneously with [^{125}I]T_4 (100,000 cpm). Data represent the mean ± SE of six observations obtained in two experiments. *, $P < 0.001$, unlabeled T_3 *vs.* controls (*left bar*).

T_4 in medium containing 0.1% BSA. Reduction of the uptake of [^{125}I]T_4 was already observed after the addition of 10 nM unlabeled T_4 (10%), and there was a significant reduction at 100 nM T_4 (24%; $P < 0.05$). The maximum effect (59%; $P < 0.001$) was observed with 10 μM T_4. When [^{125}I]T_4 uptake was measured in the presence of unlabeled T_3 (10 nM to 10 μM), a significant inhibition (38%; $P < 0.001$) was observed with 100 nM T_3, whereas the maximum effect (63%; $P < 0.001$) was obtained with a T_3 concentration of 10 μM (Fig. 4).

The ability of rT_3 and Tetrac to compete with the uptake of [^{125}I]T_4 in cultured anterior pituitary cells was examined in a separate series of experiments. The results are shown in Table 3. rT_3 at a concentration of 10 μM reduced the uptake

TABLE 3. The effects of rT_3 and Tetrac on the 15-min uptake of [^{125}I]T_4 by cultured anterior pituitary cells

Experimental conditions	[^{125}I]T_4 uptake (% dose)	FT_4 fraction (%)	[^{125}I]T_4 uptake (fmol/pM FT_4)
No additions	0.54 ± 0.02 (9)	3.4	0.040 ± 0.001
+10 μM rT_3	0.65 ± 0.03 (6)	6.1	0.027 ± 0.001[a]
+10 nM Tetrac	0.58 ± 0.05 (3)	3.5	0.041 ± 0.003
+10 μM Tetrac	1.20 ± 0.04 (6)	7.8	0.038 ± 0.001

Data represent the mean ± SE of three to nine observations obtained in two independent experiments. Anterior pituitary cells were cultured for 3 days at a density of 500,000 cells/well. After preincubation for 30 min in buffer containing 0.1% BSA, [^{125}I]T_4 uptake was measured in the same buffer with [^{125}I]T_4 (100,000 cpm) without or with the additions indicated.

[a] $P < 0.001$ *vs.* no additions.

TABLE 4. Effects of T_4, T_3, and various compounds on the 15-min uptake of [^{125}I]T_4 in cultured anterior pituitary cells

Experimental conditions	[^{125}I]T_4 uptake (fmol/pM FT_4)	Effect (%)	*P*
No additions	0.060 ± 0.002 (21)		
+10 μM T_4	0.029 ± 0.003 (9)	−52	<0.001
+10 μM T_3	0.022 ± 0.001 (6)	−63	<0.001
+10 μM MDC	0.042 ± 0.003 (9)	−30	<0.001
+25 μM MDC	0.039 ± 0.001 (9)	−35	<0.001
+10 μM oligomycin	0.030 ± 0.005 (9)	−50	<0.001
+10 μM monensin	0.036 ± 0.003 (12)	−40	<0.001

Data indicate the mean ± SE of 6-21 observations. Each experiment was performed in triplicate and included at least one set of controls and two to four of the other additions. Anterior pituitary cells were cultured for 3 days at a density of around 500,000 cell/well. Experiments were performed in buffer containing 0.1% BSA. The cells were preincubated for 30 min in the absence or presence of MDC (10 or 25 μM), oligomycin (10 μM), or monensin (10 μM). Thereafter, they were incubated for 15 min with [^{125}I]T_4 (100,000 cpm), as described in *Materials and Methods*, without or with the additions indicated.

of [^{125}I]T_4 by 32% ($P < 0.001$). Up to 10 μM Tetrac had no significant inhibitory effect on the uptake of [^{125}I]T_4.

In a previous study it was found that the uptake of [^{125}I]T_3 by cultured anterior pituitary cells was reduced by MDC, oligomycin, and monensin (9). These substances were also tested with respect to their effects on [^{125}I]T_4 uptake. The results are shown in Table 4. The 15-min uptake of [^{125}I]T_4 amounted to 0.060 fmol/pM FT_4. When MDC, an inhibitor of receptor-mediated endocytosis, was present at concentrations of 10 or 25 μM during preincubation and incubation, the uptake of [^{125}I]T_4 was reduced by 30% ($P < 0.001$) and 35% ($P < 0.001$), respectively. Preincubation and incubation with oligomycin (10 μM) reduced the uptake of [^{125}I]T_4 by 50% ($P < 0.001$), indicating energy dependence of the uptake process. Finally, 10 μM monensin reduced the uptake of [^{125}I]T_4 by 40% ($P < 0.001$), indicating the importance of the Na^+ gradient.

Another approach to evaluate the Na^+ dependence of the [^{125}I]T_4 uptake was to incubate the cultured anterior pituitary cells in buffer where Na^+ could be replaced by choline or K^+. These effects could then be compared with the effects of 10 μM monensin. The results of these experiments are shown in Table 5. It can be seen from Table 5, that the effect of 10 μM monensin in this type of buffer (38%; $P < 0.001$) was the

TABLE 5. Na^+ dependence of the 15-min uptake of [^{125}I]T_4 by cultured anterior pituitary cells

Experimental conditions	[^{125}I]T_4 uptake (% dose)	Effect (%)	P
KR buffer	1.15 ± 0.08 (9)		
+10 μM monensin	0.71 ± 0.04 (6)	−38	<0.001
K^+-KR buffer	0.70 ± 0.08 (6)	−39	<0.005
Ch-KR buffer	0.89 ± 0.10 (9)	−23	<0.1

Data indicate the mean ± SE of six to nine observations obtained in two independent experiments. Anterior pituitary cells were cultured for 3 days at a density of around 500,000 cells/well. The cells were preincubated for 30 min in the absence or presence of 10 μM monensin in Krebs-Ringer (KR) buffer with 0.1% BSA (see *Materials and Methods*). Thereafter, they were incubated for 15 min in the same buffer without or with monensin and [^{125}I]T_4 (100,000 cpm). Cells were also preincubated and incubated in KR buffer, where Na^+ was replaced by K^+ (K-KR) or by choline (Ch-KR).

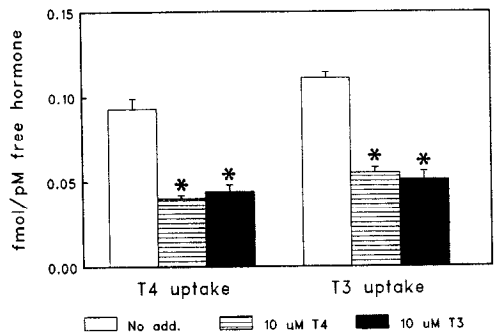

FIG. 5. The uptake of [^{125}I]T_4 and [^{125}I]T_3 and the effects of unlabeled T_4 and T_3 in cultured anterior pituitary cells of euthyroid rats. Cells were cultured for 3 days at a density of 500,000 cell/well. [^{125}I]T_4 uptake was measured during 15 min in buffer containing 0.5% BSA, as described in *Materials and Methods*. Data for [^{125}I]T_3 uptake are derived from Ref. 9. *, $P < 0.001$, unlabeled T_4 or T_3 *vs.* no additions.

same as that observed in the usual buffer (40%; see Table 3). Furthermore, replacing Na^+ in the buffer with K^+ or choline reduced the uptake of [^{125}I]T_4 by 39% ($P < 0.005$) and 23% ($0.05 < P < 0.1$), respectively.

To directly compare [^{125}I]T_4 and [^{125}I]T_3 uptake by cultured anterior pituitary cells and the specificity of inhibition by unlabeled T_4 and T_3, [^{125}I]T_4 uptake was measured over 15 min in medium containing 0.5% BSA. The results are shown in Fig. 5, which also includes data from our previous study on [^{125}I]T_3 uptake (9). For both iodothyronines the uptake data were expressed per pM free hormone. It can be seen from Fig. 5 that the amount of hormone taken up in 15 min is the same for [^{125}I]T_4 and [^{125}I]T_3. The presence of equimolar concentrations of unlabeled T_4 and T_3 reduced [^{125}I]T_4 uptake to the same extent (57% and 53%, respectively; both $P <$ 0.001), and *vice versa*, the uptake of [^{125}I]T_3 was reduced to the same extent by T_4 (50%; $P < 0.001$) and T_3 (54%; $P <$ 0.001).

The possibility that thyroid hormones and amino acids might be transported into the cultured anterior pituitary cells through the same mechanism was also investigated. Therefore, the cells were preincubated and incubated with 2 mM L-Phe, L-Tyr, or L-Trp. The results are shown in Fig. 6. L-Phe had a small, but significant, inhibitory effect (18%; $P < 0.05$) on the uptake of [^{125}I]T_4, but not on the uptake of [^{125}I]T_3.

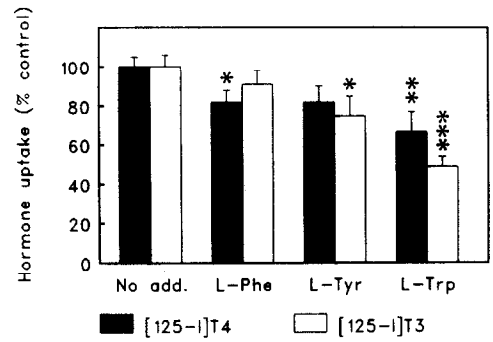

FIG. 6. Effects of aromatic amino acids on the 15-min uptake of [^{125}I]T_4 and [^{125}I]T_3 in cultured anterior pituitary cells. Anterior pituitary cells were cultured for 3 days at a density of around 500,000 cells/well. [^{125}I]T_4 uptake was measured in buffer containing 0.1% BSA, and [^{125}I]T_3 uptake was determined in buffer containing 0.5% BSA. The cells were preincubated for 30 min in the absence or presence of 2 mM L-Phe, L-Tyr, or L-Trp, respectively. Thereafter, they were incubated for 15 min with [^{125}I]T_4 (100,000 cpm) or [^{125}I]T_3 (50,000 cpm), as described in *Materials and Methods*, without or with the additions indicated. Data represent the mean ± SE of 6–18 observations obtained in 4 independent experiments. The results are expressed as a percentage of the control value (no additions). *, $P < 0.05$; **, $P < 0.005$; ***, $P <$ 0.001 (*vs.* no additions).

The effect of L-Tyr on the uptake of [^{125}I]T_4 was as large as that of L-Phe, but not significant. On the other hand, L-Tyr reduced the uptake of [^{125}I]T_3 by 25% ($P < 0.05$). The presence of L-Trp reduced the uptake of both [^{125}I]T_4 (33%; $P < 0.005$) and [^{125}I]T_3 (51%; $P < 0.001$). The effect of L-Trp was further examined in experiments in which L-Trp was present during preincubation and incubation or only present during incubation. In two experiments of this type, it was observed that the effect of L-Trp on the uptake of [^{125}I]T_3 was just as large when the amino acid was present during incubation alone (41%; $P < 0.001$) compared to its presence during both preincubation and incubation (34%; $P < 0.005$; not shown).

As primary tissue cultures may be contaminated with fibroblasts, and fibroblasts also show active transport of iodothyronines (27), we finally evaluated to what extent the presence of fibroblasts might contribute to the uptake of [^{125}I]T_3 and [^{125}I]T_4 by anterior pituitary cells in primary culture. For this purpose the cells were cultured in the normal culture medium in the absence or presence of BrdU (15 μg/ml) or in a medium in which L-Val was replaced with D-Val. The results are shown in Table 6. To assess the biological effects of this variation in culture conditions, TSH release was measured, whereas the effects on cell proliferation were examined by measuring the DNA content of the wells.

Both addition of BrdU and replacement of L-Val with D-Val reduced the uptake of [^{125}I]T_3 and [^{125}I]T_4 between 5–20%, suggesting that fibroblasts contribute little to the uptake of labeled iodothyronines by the cultured anterior pituitary cells. TSH release was not altered by the various culture conditions, suggesting an equal number of active thyrotrophs under the different conditions. Furthermore, the DNA content of the cells cultured with BrdU or D-Val was not reduced compared to that of cells cultured as usual. This indicated

TABLE 6. Effects of culture conditions on the uptake of [^{125}I]T_3, [^{125}I]T_4, TSH release, and DNA content of anterior pituitary cells

Parameter measured	Culture conditions MEM-L-Val-FCS	MEM-L-Val-FCS + BrdU (15 μg/ml)	MEM-D-Val-FCS
[^{125}I]T_3 uptake (% dose)	1.34 ± 0.08 (12)	1.25 ± 0.05 (9)	1.06 ± 0.04 (12)[a]
[^{125}I]T_4 uptake (% dose)	0.37 ± 0.02 (9)	0.36 ± 0.02 (9)	0.31 ± 0.03 (11)
TSH secretion (ng/3 days)	33.3 ± 1.5 (5)	31.0 ± 0.7 (5)	31.4 ± 0.7 (10)
DNA content (μg/well)			
d0	3.00 ± 0.11 (4)		3.19 ± 0.05 (4)
d3	2.26 ± 0.06 (3)	2.39 ± 0.12 (3)	2.55 ± 0.05 (6)[b]

Data on the uptake of [^{125}I]T_3 and [^{125}I]T_4 indicate the mean ± SE of four experiments, with the number of observations in *parentheses*. In one of these experiments, TSH was determined in the medium removed from the cells after 3 days of culture, whereas some of the plates were used for determination of the DNA content. Anterior pituitary cells were cultured for 3 days at a density of about 500,000 cells/well. After separation of the cells by dispase, two thirds of the cells were suspended in the normal culture medium (MEM-L-Val-FCS), and one third in culture medium in which L-Val was replaced by D-Val (MEM-D-Val-FCS). To half of the cells in normal culture medium, BrdU (15 μg/ml) was added. The 15-min uptake of [^{125}I]T_3 (50,000 cpm) was measured in buffer with 0.5% BSA, and that of [^{125}I]T_4 (100,000 cpm) was measured in buffer with 0.1% BSA. d0, Day 0; d3, after 3 days of culture.

[a] $P < 0.005$ *vs.* MEM-L-Val-FCS.

[b] $P < 0.01$ *vs.* MEM-L-Val-FCS.

that the presence of fibroblasts is only of minor importance in the short term primary culture of pituitary cells.

Discussion

The present study shows that the uptake of T_4 into cultured anterior pituitary cells of euthyroid rats occurs by a transport system that is energy dependent and partly dependent on the Na^+ gradient. Our results are consistent with the possibility that T_4 is taken up by cultured anterior pituitary cells by the same transport system as T_3. In addition, our results suggest a relationship between the transport systems of iodothyronines and aromatic amino acids.

The peripheral effects of the thyroid hormones are primarily ascribed to the action of T_3, but T_4 is effective in suppressing pituitary TSH secretion *in vivo*, although to what extent this is due to a direct action of T_4 or to intrapituitary conversion of T_4 has not been decided (28–31). We have recently shown that both T_3 and T_4 were capable of reducing TRH-induced TSH secretion *in vitro*, and that the uptake of T_3 in cultured anterior pituitary cells occurred by a carrier-mediated mechanism (9). Therefore, it was important to investigate whether a comparable uptake system existed for T_4 in these cells.

Whereas carrier-mediated uptake of T_3 has been demonstrated in a variety of tissues (32, 33), the evidence for an active transport system for T_4 is not as abundant (32). Apart from hepatocytes (10, 11), it has been described for a mouse neuroblastoma cell line (13), rat glial cells in primary culture (12), and, very recently, pituitary tumor cells (8). The three last-mentioned cell types have in common that at least 50% of the nuclear bound T_3 is derived from intracellular T_4 to T_3 conversion (2).

The uptake of [^{125}I]T_4 in cultured anterior pituitary cells showed roughly the same time dependence as the uptake of [^{125}I]T_3 (9). That [^{125}I]T_4 had indeed entered the pituitary cells was evident from significant iodide production, conjugate formation, and intracellular conversion of T_4 to T_3. Both the uptake and metabolism of [^{125}I]T_4 were reduced to the same extent by incubation with a low concentration of unlabeled T_3 or T_4. Although the 50% reduction of [^{125}I]T_3 formation in the cells in the presence of 10 nM T_4 could be due to saturation of the type II deiodinase (34), the conversion of [^{125}I]T_4 to [^{125}I]T_3 was also reduced by 50% in the presence of 10 nM T_3. As T_3 is not a substrate for the type II deiodinase, this could be due to reduced entry of [^{125}I]T_4 or to a down-regulation of the enzyme (35). At any rate, these results show that uptake and conversion of T_4 in the cultured anterior pituitary cells are subject to regulation by thyroid hormone.

In line with our previous study on the uptake of [^{125}I]T_3 in cultured anterior pituitary cells, the plasma membrane uptake of [^{125}I]T_4 was examined in experiments with 15 min of incubation. The 15-min uptake of [^{125}I]T_4 expressed per pM FT_4 increased 2-fold when the albumin concentration in the medium was increased from 0.1% to 0.5% BSA. This was also observed with respect to T_3 uptake in cultured hepatocytes (36). These researchers suggested that albumin is necessary for optimal diffusion through the unstirred water layer around the cells, and that increasing the concentration of albumin leads to augmentation of the processes involved in the transport and binding of T_3 (36).

The 15-min uptake of [^{125}I]T_4 was significantly reduced by unlabeled T_4 at concentrations of 100 nM or higher. Unlabeled T_3 reduced [^{125}I]T_4 uptake to at least the same extent as unlabeled T_4. On the other hand, rT_3 at a concentration of 10 μM had only a small effect, and Tetrac did not affect [^{125}I]T_4 uptake. Substances that previously were found to reduce [^{125}I]T_3 uptake by cultured anterior pituitary cells were in the present study tested on [^{125}I]T_4 uptake. MDC concentrations of 10 and 25 μM reduced the 15-min uptake of [^{125}I]T_4 by 30% and 35%, respectively, indicating that receptor-mediated endocytosis is involved in the uptake process. That the uptake of [^{125}I]T_4 was dependent on the cellular energy status was indicated by the experiments with oligomycin (10 μM), a compound that reduced T_4 uptake by around 50%. Finally, after Na^+ loading of the cells with monensin (10 μM) or reduction of the Na^+ gradient by replacing the Na^+ in the buffer by K^+, [^{125}I]T_4 uptake was reduced by 40%, indicating that the uptake process is at least partly dependent on the Na^+ gradient. In addition, the experiments with the aromatic amino acids indicate that part of the [^{125}I]T_4 uptake into pituitary cells might occur through the system L and system T amino acid transporters, which are both Na^+ independent.

Surprisingly, all of these effects were similar to the effects on the uptake of [^{125}I]T_3 by pituitary cells. The same concentrations of MDC (25 μM), oligomycin (10 μM), and monensin (10 μM) reduced T_3 uptake by 26%, 68%, and 37%, respec-

tively (9). Also, the effects of L-Phe, L-Tyr, and L-Trp on the uptake of [^{125}I]T_3 described in the present study were similar to those on [^{125}I]T_4 uptake. Together, these results suggest that the putative iodothyronine-transporting membrane protein may transport both T_3 and T_4 across the plasma membrane of pituitary cells. This is different from the situation in liver, where separate carriers presumably are involved in T_3 and T_4 uptake (10).

Finally, by expressing data on the 15-min uptake of [^{125}I]T_4 and [^{125}I]T_3 per pM free hormone, it was found that the rates of T_3 and T_4 uptake were very similar. Moreover, the simultaneous addition of unlabeled T_4 or T_3 reduced the uptake of [^{125}I]T_4 to roughly the same extent; *vice versa*, the uptake of [^{125}I]T_3 was reduced by about 50% by unlabeled T_4 and T_3. In agreement with earlier findings in rat glial cells in primary culture (12) and in pituitary tumor cells (8), our results are consistent with the view that T_3 and T_4 share a common carrier in the anterior pituitary.

In conclusion, the present results indicate that T_4 is transported into cultured anterior pituitary cells by a carrier-mediated mechanism that is dependent on the cellular energy status and partly on the Na^+ gradient. Furthermore, the present results in combination with our previous data on [^{125}I]T_3 uptake are consistent with the possibility that T_3 and T_4 share the same transporter in the anterior pituitary gland. Experiments to investigate whether adaptation of this transport system plays a role in the suppression of TSH secretion during starvation and nonthyroidal illness are in progress.

Acknowledgments

We thank Dr. L. J. Hofland for valuable discussions, and Mr. H. van Toor and Ms. A. M. P. Tempelaars for skilled technical assistance.

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