Uptake of Triiodothyronine Sulfate and Suppression of Thyrotropin Secretion in Cultured Anterior Pituitary Cells

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To investigate the uptake of triiodothyronine sulfate (T_3S) and its effect on thyrotropin-releasing hormone (TRH)-induced thyrotropin (TSH) secretion, anterior pituitary cells were isolated from euthyroid rats and cultured for 3 days in medium containing 10% fetal calf serum. Incubation was performed at 37°C in medium containing 0.5% bovine serum albumin (BSA). Exposure of the pituitary cells to TRH (0.1 μmol/L) for 2 hours stimulated TSH secretion by 176%. This effect was reduced by approximately 45% after a 2-hour preincubation with T_3 (0.001 to 1 μmol/L). A significant inhibitory effect of T_3S on TRH-induced TSH release was only observed at a concentration of 1 μmol/L. The uptake of [^{125}I]T_3 after 1 hour of incubation was reduced by 40% ± 4% ($P < .001$) by simultaneous addition of 10 nmol/L unlabeled T_3, whereas 1 μmol/L T_3S was required to obtain a reduction of the [^{125}I]T_3 uptake by 34% ± 2% ($P < .001$). The amount of T_3 present in the unlabeled T_3S preparation was 0.25% as determined by radioimmunoassay. When pituitary cells were incubated for 1 hour with [^{125}I]T_3S or [^{125}I]T_3 (both 50,000 cpm/0.25 mL), the uptake of [^{125}I]T_3S expressed as a percentage of the dose was 0.04% ± 0.02% (mean ± SE, n = 4), whereas that of [^{125}I]T_3 amounted to 3.0% ± 0.4% (n = 4). In contrast, when hepatocytes were incubated for 1 hour with [^{125}I]T_3S, the uptake amounted to 5.1% ± 0.8% (n = 9), whereas that of [^{125}I]T_3 was 22.1% ± 1.7% (n = 9). Furthermore, [^{125}I]T_3S was as rapidly deiodinated (iodide production, 14.9% ± 2.6%; n = 9) as [^{125}I]T_3 (12.1% ± 0.8%, n = 9) by hepatocytes. It is concluded that (1) T_3S is poorly taken up by pituitary cells, and (2) the suppressive effect of high concentrations of T_3S on TRH-induced TSH secretion and on [^{125}I]T_3 uptake can be explained by slight contamination with T_3. Thus, it appears that T_3S has only a minor biological effect, if any, on the pituitary.

SULFATION plays a significant role in the metabolism of iodothyronines in both man and animals, although it is probably more important for triiodothyronine (T_3) than for thyroxine, at least in rats and dogs.[1-3] However, during nonthyroidal illness (NTI) the contribution of the different pathways, ie, deiodination, glucuronidation, and sulfation, to thyroid hormone metabolism might change considerably.[4,5] Measurements of the hormone levels in sera of NTI patients showed that the reduction in serum T_3 and thyroxine was accompanied by a threefold increase in serum T_3 sulfate (T_3S) apart from the known increase in serum reverse T_3.[6] Despite these changes, serum thyrotropin (TSH) was in the normal range.[6]

In the study by LoPresti et al,[7] the question was raised as to whether the increased T_3S production might contribute to the suppression of TSH secretion during NTI. In their experiments, infusion of T_3S in healthy volunteers, calculated to result in a relatively low serum level of T_3S, did not reduce the serum TSH level.[7] In another study with the growth hormone–producing pituitary tumor cell line GH_4C_1, it was shown that T_3S failed to displace [^{125}I]T_3 from the nuclear binding sites.[8] However, the possibility that T_3S could have thyromimetic activity after desulfation was not excluded.[8,9] More recently, it was reported that administration of T_3S to hypothyroid rats produced thyromimetic effects with a potency of approximately 20% that of T_3.[10] In the same study, it was shown that treatment of hypothyroid rats with a relatively high dose of T_3S resulted in a reduction of serum TSH and a normalization of the serum T_3 level, indicating desulfation of T_3S in vivo.[10]

Together, these results could be explained by the assumption that T_3S is not taken up by the pituitary, and that the effects of T_3S in vivo might be due to an effect of T_3 (after desulfation or due to contamination of T_3S). To test this hypothesis, the present study was undertaken to examine in vitro the effects of T_3S on TSH secretion and the uptake of [^{125}I]T_3S by the pituitary. We used primary cultures of anterior pituitary cells of euthyroid rats. This preparation contains active thyrotrophs that release TSH in response to (TSH-releasing hormone [TRH]) and transport T_3 by a carrier-mediated mechanism.[11] Since it is not known whether T_3S is metabolized in the pituitary like it is in the liver[12] and whether possible effects of T_3S on pituitary function should be ascribed to T_3S itself or to any metabolic product, we have also compared pituitary cells and hepatocytes with respect to the uptake and deiodination of [^{125}I]T_3S and [^{125}I]T_3.

MATERIALS AND METHODS

Animals

All experiments were performed using male Wistar rats weighing 220 to 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 hours).

Pituitary Cell Culture

Animals (12 for each experiment) were killed between 9:00 and 9:30 AM by decapitation. The pituitary glands were removed within 5 minutes, the neurointermediate lobe was discarded, and the anterior lobes were collected in calcium- and magnesium-free Hanks balanced salt solution supplemented with 10 g/L human serum albumin, penicillin (10^5 U/L), amphotericin B (Fungizone,

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Bristol-Myers Squibb, Wœrden, The Netherlands; 0.5 mg/L), and sodium bicarbonate (0.4 g/L). The anterior pituitary lobes were dissociated with dispase (2.4×10^3 U/L) as described in detail elsewhere.[13] From each pituitary, 1 to 1.5×10^6 cells were obtained, and the viability of the cells as determined by trypan blue exclusion was greater than 90%.

The cells were cultured at 37°C in a water-jacketed incubator in humidified air with 5% CO_2 at a density of 3 to 5×10^5 cells per well in 48-well culture dishes. The culture medium consisted of Minimal Essential Medium with Earle's salts supplemented with nonessential amino acids, sodium pyruvate (1 mmol/L), 10% fetal calf serum, penicillin (10^5 U/L), amphotericin B (0.5 mg/L), L-glutamine (2 mmol/L), and sodium bicarbonate (2.2 g/L, pH 7.4).[13,14] The cells had attached to the wells after 2 days of culture; on day 3, the cells were used for experiments. In a previous study it was shown that the TSH content of the pituitary cell preparation was highest after this short period of culture.[11]

TSH Secretion

The culture medium was removed from the cells, and the cells were washed once with incubation medium. This medium was identical to the culture medium except that the fetal calf serum was replaced by 0.5% bovine serum albumin (BSA). The pituitary cells were preincubated for 2 hours at 37°C in the absence or presence of variable concentrations of T_3 or T_3S (0.001 to 1 µmol/L). The medium was then discarded, and fresh medium was added containing TRH (0.1 µmol/L) with or without T_3 or T_3S. Incubation was continued for 2 hours at 37°C and was followed by removal of the medium. The cells were washed once with 1 mL ice-cold saline (0.9% NaCl). Incubation media were centrifuged ($2,000 \times g$), and the supernatants were frozen for later determination of TSH. Incubations were performed in triplicate.

Hepatocytes

Hepatocytes were isolated by collagenase perfusion as previously described.[15] Cells were cultured at 37°C at a density of 2×10^6 cells per well in 6-well culture dishes in 2 mL Ham's F10, HEPES (8.9 mmol/L), PIPES (10.6 mmol/L), and *N,N*-bis[2-hydroxyethyl]-2-aminoethane sulfonic acid ([BES] 11.2 mmol/L) supplemented with 10% fetal calf serum, $CaCl_2$ (2 mmol/L), glucose (6.7 mmol/L), insulin (12 U/L), and penicillin/streptomycin (5×10^4 U/L, pH 7.4). After 4 hours of culture, the cells were washed once with rinsing medium and used for experiments.[16]

Uptake of [^{125}I]T_3 and [^{125}I]T_3S

For these studies, cells were cultured as described above. On the same culture dishes, an equal number of wells contained only culture medium (blanks). All incubations with and without cells were performed in triplicate.

Pituitary cells were equilibrated in 0.5 mL culture medium containing 0.5% BSA. After equilibration for 30 minutes, the medium was removed and the cells were incubated for 1 hour at 37°C with [^{125}I]T_3 (50,000 cpm, 50 pmol/L) in 0.25 mL medium alone or together with 0.01 to 10 µmol/L T_3 or T_3S. The uptake of [^{125}I]T_3S (50,000 cpm) was measured exactly as for [^{125}I]T_3.

To allow direct comparison with the hepatocyte experiments, two experiments with pituitary cells were performed in incubation medium where $NaHCO_3$ was replaced by an equimolar amount of HEPES (8.9 mmol/L), PIPES (10.6 mmol/L), and BES (11.2 mmol/L). As previously described, this did not affect the uptake of [^{125}I]T_3 by the pituitary cells[11] (Table 2).

After incubation, the medium was removed and the cells were washed with 1 mL ice-cold saline to remove labeled compounds not bound to the cells. Cells were dissolved in 1 mL 0.1N NaOH and were counted for radioactivity in a 16-channel gamma counter (NE 1600, Nuclear Enterprises, Sighthill, Edinburgh, Scotland). The amount of [^{125}I]T_3 or [^{125}I]T_3S taken up by the cells was expressed as a percentage of the 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.

Hepatocytes were incubated with [^{125}I]T_3 or [^{125}I]T_3S (both 100,000 cpm/mL) at 37°C for 1 to 120 minutes in 1 mL hepatocyte culture medium with 0.5% BSA instead of fetal calf serum. Further processing was the same as for pituitary cells.

Iodide Production

Aliquots of the incubation medium from the three uptake experiments with hepatocytes (Fig 2) and the two experiments with pituitary cells (Table 2) were chromatographed on Sephadex LH-20.[16] Iodide was eluted from the column with 3×1 mL 0.1N HCl. Subsequently, glucuronides and sulfates (conjugates) were eluted with 8×1 mL H_2O, and finally the iodothyronines were removed from the column with 3×1 mL 50% ethanol in 0.1N NaOH.

Determination of TSH

TSH levels in the incubation media were measured by radioimmunoassay as previously described.[11]

Analysis of T_3S

The purity of the unlabeled T_3S preparation was tested by conventional radioimmunoassay with two T_3 antisera produced in our laboratory (no. 7157 and 7160). Both antisera were used in a final dilution of 1:200,000.

Free T_3 and T_3S Concentrations

Calculation of the free T_3 and T_3S concentrations in the experiments was based on the determination of the free fractions by equilibrium dialysis.[17] With 0.5% BSA in the incubation medium, the free T_3 fraction amounted to $2.85\% \pm 0.01\%$ ($n = 4$), and the free T_3S fraction to 2.12% ($n = 2$). The free T_3S fraction was corrected for the fact that only 60% of the T_3S precipitated with $MgCl_2$. It was further checked by high-performance liquid chromatography that this was not due to the presence of iodide in the [^{125}I]T_3S preparation.

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 (Rhone-Poulenc, Amstelveen, The Netherlands), dispase (grade II, Boehringer, Mannheim, Germany), and collagenase (type I, Sigma, St Louis, MO). Culture dishes (48- and 6-well) were purchased from Costar (Cambridge, MA) and Nunc (Roskilde, Denmark), respectively. TRH (Relefact) was obtained from Hoechst, Frankfurt am Main, Germany. T_3, PIPES, HEPES, BES, and BSA (fraction V) were purchased from Sigma (St Louis, MO). [3'-^{125}I]T_3 (3,070 µCi/µg) was purchased from Amersham International (Aylesbury, Buckinghamshire, UK). T_3S and [^{125}I]T_3S were prepared from T_3 and [^{125}I]T_3, respectively, using $ClSO_3H$.[18] [^{125}I]T_3S was purified by LH-20 chromatography, and analysis of the fractions by high-performance liquid chromatography[18] showed that the preparation consisted of 98% T_3S and contained less than 2% iodide. Reagents for the rat TSH radioimmunoassay were kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD). Sephadex G25 was obtained from Pharmacia (Uppsala, Sweden). All other reagents were of the highest purity available.

Statistics

Significances of differences were calculated with Student's *t* test for unpaired observations. A *P* value less than .05 was regarded as statistically significant.

RESULTS

Effects of T_3S on TSH Secretion

Table 1 shows a comparison of the effects of various concentrations of T_3 and T_3S on the TRH-induced TSH secretion by anterior pituitary cells. When TRH was added at a concentration of 0.1 μmol/L, TSH release was stimulated by 176% ($P < .001$). This effect was reduced by approximately 45% after preincubation with 1 μmol/L T_3 or T_3S ($P < .001$ for both). The inhibitory effect of T_3 on TRH-induced TSH secretion was the same after preincubation with lower concentrations of T_3 (0.1 and 0.01 μmol/L). In contrast, the effect of preincubation with 0.1 μmol/L T_3S was much smaller (14%, NS), and at 0.01 μmol/L T_3S the effect was completely absent. An additional experiment was performed where the effects of even lower concentrations of T_3 and T_3S on TRH-induced TSH secretion were compared. TRH-induced TSH release was inhibited by 62% ($P < .025$ or less) at T_3 concentrations of 0.01, 0.1, and 1.0 μmol/L, and by 41% ($.05 < P < .10$) at a T_3 concentration of 0.001 μmol/L. The effect of T_3S was absent at 0.001 and 0.01 μmol/L, and the maximum effect was obtained at a concentration of 1 μmol/L T_3S and amounted to 53% ($P < .025$).

Effects of T_3S on $[^{125}I]T_3$ Uptake

A comparison between the effects of T_3 and T_3S was also made with respect to the uptake of $[^{125}I]T_3$ by the pituitary cells. Figure 1 shows the combined results of three experiments. In the first experiment with 300,000 cells per well the control uptake of $[^{125}I]T_3$ was 1.62% ± 0.03% (n = 3), and in the second and third experiments with 500,000 cells per well it was 3.96% ± 0.15% (n = 3) and 2.39% ± 0.08% (n = 5). Simultaneous incubation of the cells with 0.01, 0.1, 1, and 10 μmol/L unlabeled T_3 reduced the uptake of $[^{125}I]T_3$ by 40% ± 4% ($P < .001$), 40% ± 5% ($P < .001$), 52% ± 2% ($P < .001$), and 56% ± 1% ($P < .001$), respectively. When T_3S was added at concentrations of 0.01, 0.1, 1, or 10 μmol/L, $[^{125}I]T_3$ uptake was reduced by 6% ± 4% (NS), 11% ± 4% ($P < .025$), 34% ± 2% ($P < .001$), and 52% ± 5% ($P < .001$), respectively.

Table 1. Effects of T_3 and T_3S on the TRH-Induced Stimulation of TSH Release From Anterior Pituitary Cells

Experimental Conditions	TSH Release (ng)	Effect (%)	*P*
Controls (14)	2.1 ± 0.1		
+TRH 0.1 μmol/L (14)	5.8 ± 0.2	+176	<.001
TRH + T_3 1 μmol/L (8)	4.1 ± 0.3	+95	<.001*
TRH + T_3S 1 μmol/L (9)	3.9 ± 0.3	+86	<.001*
TRH + T_3 0.1 μmol/L (3)	4.3 ± 0.1	+104	<.025*
TRH + T_3S 0.1 μmol/L (6)	5.3 ± 0.2	+152	NS*
TRH + T_3 0.01 μmol/L (6)	4.2 ± 0.2	+100	<.001*
TRH + T_3S 0.01 μmol/L (6)	5.7 ± 0.1	+171	NS*

NOTE. Data are the mean ± SE of three independent experiments, with the number of observations in parentheses. Anterior pituitary cells were cultured for 3 days at a density of 5 × 10^5 cells/well. The cells were preincubated for 2 hours in the absence or presence of T_3 (0.01 to 1 μmol/L) or T_3S (0.01 to 1 μmol/L). Then they were incubated with TRH (0.1 μmol/L) for 2 hours with or without the additions indicated.

*P values calculated compared with the addition of TRH alone.

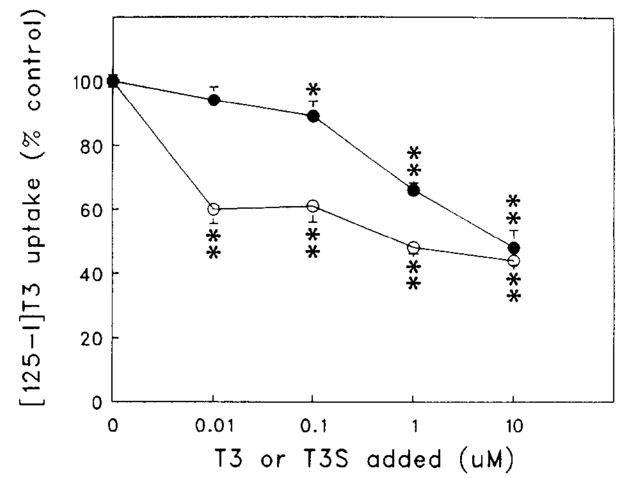

Fig 1. Effects of unlabeled (○) T_3 and (●) T_3S on the uptake of $[^{125}I]T_3$ by anterior pituitary cells. The cells were cultured for 3 days at a density of 3 to 5 × 10^5 cells/well. After equilibration, the cells were incubated for 1 hour with $[^{125}I]T_3$ (50,000 cpm) with various concentrations of unlabeled T_3 or T_3S. Data represent the mean ± SE of three to nine observations from three independent experiments. Significances of differences were calculated as compared with the controls (100%): *$P < .025$, **$P < .001$.

Uptake of $[^{125}I]T_3S$ by Pituitary Cells and Hepatocytes

In four experiments with 500,000 cells per well, the uptake of $[^{125}I]T_3S$ by pituitary cells was compared with that of $[^{125}I]T_3$ (Table 2). After 1 hour of incubation the uptake of $[^{125}I]T_3S$ was less than 0.1%, whereas the mean uptake of $[^{125}I]T_3$ amounted to 3.0% ± 0.4% (Table 2). In contrast, both $[^{125}I]T_3S$ and $[^{125}I]T_3$ were taken up by hepatocytes (Fig 2A), although the uptake of $[^{125}I]T_3$ was roughly fourfold greater than that of $[^{125}I]T_3S$. The uptake of both compounds reached a maximum value between 0.5 and 1 hour of incubation, and then declined slightly. As can be seen from Fig 2B, both T_3 and T_3S were deiodinated in hepatocytes, and iodide production from $[^{125}I]T_3S$ was at

Table 2. Comparison of the Uptake of $[^{125}I]T_3$ and $[^{125}I]T_3S$ in Anterior Pituitary Cells

Experiment No.	$[^{125}I]T_3$ Uptake (% dose)	$[^{125}I]T_3S$ Uptake (% dose)
1	2.96 ± 0.02	0.01 ± 0.04
2	2.64 ± 0.09	0.07 ± 0.02
3	2.34 ± 0.13	0.00 ± 0.05
4	3.96 ± 0.15	0.09 ± 0.06

NOTE. Data represent the mean ± SE of triplicate determinations. Anterior pituitary cells were cultured for 3 days at a density of 5 × 10^5 cells/well. Experiments 1 and 2 were performed in incubation medium containing $NaHCO_3$, and experiments 3 and 4 in incubation medium containing HEPES, BES, and PIPES. The cells were equilibrated for 30 minutes in incubation medium and then incubated for 1 hour with $[^{125}I]T_3$ or $[^{125}I]T_3S$ (both 50,000 cpm/0.25 mL).

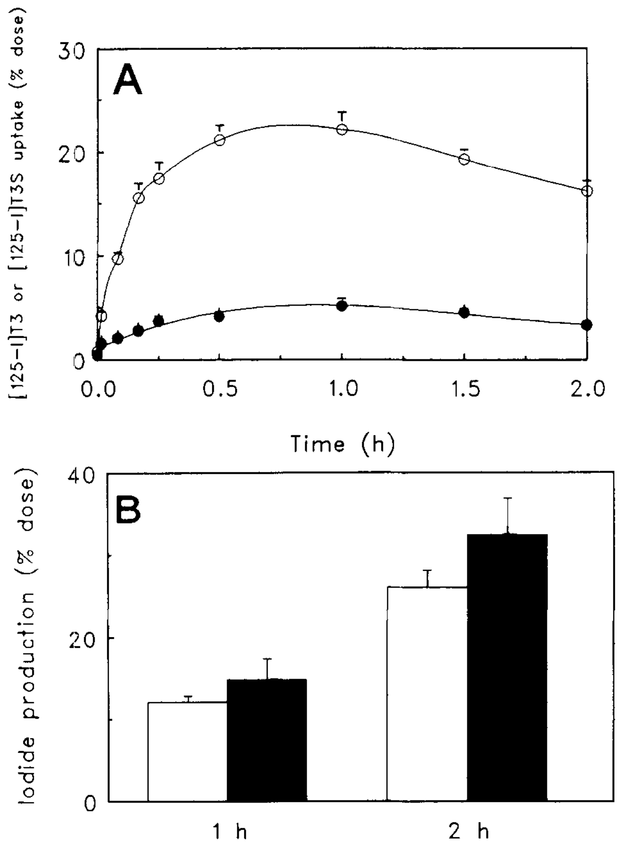

Fig 2. Time course of the (A) uptake of (○) [^{125}I]T_3 and (●) [^{125}I]T_3S and (B) iodide production by primary cultures of hepatocytes. Hepatocytes were incubated for periods varying from 1 minute to 2 hours with [^{125}I]T_3 or [^{125}I]T_3S (both 100,000 cpm/mL). Iodide production after 1 and 2 hours of incubation was measured in the media by LH-20 chromatography. Data represent the mean ± SE of nine observations from three independent experiments. (□) Iodide production from [^{125}I]T_3; (■) iodide production from [^{125}I]T_3S.

least as high as that from [^{125}I]T_3. Furthermore, iodide production from T_3 and T_3S doubled when the incubation time was prolonged from 1 to 2 hours. In the same 1-hour period, the mean conjugate formation from [^{125}I]T_3 was 4.7% ± 0.8% (not shown). There was no production of unconjugated T_3 from [^{125}I]T_3S by hepatocytes during the course of the experiment (not shown).

To determine if pituitary cells also metabolized T_3 and T_3S, incubation media from the last two experiments shown in Table 2 were analyzed for iodide production. Neither in the media from the pituitary cells incubated with [^{125}I]T_3 nor in those from [^{125}I]T_3S incubations was iodide detectable. After incubation with [^{125}I]T_3, no conjugates were detected, and 95% of the radioactivity appeared in the iodothyronine fraction. In the case of [^{125}I]T_3S, 90% to 95% of the radioactivity was found in the conjugate fraction, and no radioactivity was found in the iodothyronine fraction (not shown).

Purity of Unlabeled T_3S

Finally, the amount of T_3 present in the unlabeled T_3S preparation was determined by radioimmunoassay with two different antisera, showing that the T_3S preparation contained 0.25% T_3. Incubation medium with 1 μmol/L T_3S contained 2.49 nmol/L T_3 before incubation with cells and 2.08 ± 0.02 (n = 3) nmol/L T_3 after incubation with cells.

DISCUSSION

The results of the present study indicate that T_3S does not play a significant role in the suppression of TSH secretion in pituitary cells in vitro. Furthermore, our results suggest essential differences in the metabolic handling of T_3 and T_3S by pituitary cells and hepatocytes.

LoPresti et al[7] suggested that T_3S might play a role in the suppression of TSH secretion during NTI. This hypothesis seemed already somewhat weakened by their own observation that infusion of T_3S in healthy volunteers did not result in decreased levels of serum TSH. On the other hand, the serum level of T_3S achieved during the infusion was not measured, and the lack of effect of T_3S could be due to the low calculated T_3S/T_3 ratio (46%).

Although 1 μmol/L T_3S was as effective as 1 μmol/L T_3 in suppressing the TRH-induced TSH secretion in our cell culture, this effect can simply be explained on the basis of a slight contamination of the T_3S preparation with T_3. The amount of T_3 in the unlabeled T_3S preparation was 0.25%, and hence, 1 μmol/L T_3S will roughly contain 2.5 nmol/L T_3. This idea was confirmed by the experiment where the effect of 1 μmol/L T_3S on TRH-induced TSH secretion was found to correspond to a value (53%) just between 1 nmol/L T_3 (41%) and 10 nmol/L T_3 (62%).

The effect of unlabeled T_3S on the uptake of [^{125}I]T_3 by pituitary cells was dose-dependent, and was still lower (34% inhibition) when T_3S was added at a concentration of 1 μmol/L as compared with the effect of 10 nmol/L T_3 (40% inhibition). In our previous study,[11] it was observed that addition of 1 nmol/L T_3 reduced the uptake of [^{125}I]T_3 by approximately 29%. Thus, the effects of T_3S on [^{125}I]T_3 uptake are also compatible with a contamination of the T_3S preparation with T_3 (<1%). Our results are also in line with those of Spaulding et al,[8] who apparently used more purified T_3S and found no displacement of [^{125}I]T_3 from the nuclear receptors in the growth hormone–producing cell line GH_4C_1 in the concentration range of 0.001 to 1 μmol/L T_3S.

The explanation that the effect of high concentrations of T_3S on TRH-induced TSH secretion or [^{125}I]T_3 uptake is due to contamination of the T_3S preparation with T_3 is also supported by our observation that [^{125}I]T_3S itself was only poorly taken up by the pituitary cells. This was concluded from a series of experiments where a significant amount of [^{125}I]T_3, added at the same radioactive and molar concentration as [^{125}I]T_3S, was taken up by the pituitary cells. In contrast, both [^{125}I]T_3 and [^{125}I]T_3S were taken up by hepatocytes, although the uptake of [^{125}I]T_3 was roughly fourfold higher than that of [^{125}I]T_3S. On the other hand, despite this difference in uptake, iodide production from [^{125}I]T_3S was at least as high as that from [^{125}I]T_3. This indicated that T_3S and T_3 (after preceding sulfation[19]) were deiodinated followed by efflux of $^{125}I^-$ from the liver cells. These results also confirmed the previous observation that

T_3S is a preferred substrate for the liver type I deiodinase, being deiodinated successively in the inner and outer ring.[12,19] During the 2 hours of the experiments, no unconjugated T_3 was detectable in the media from hepatocytes incubated with $[^{125}I]T_3S$. This seems to be in contrast to the observations of Kung et al,[20] who reported 1% hydrolysis of T_3S per hour in isolated hepatocytes. The reason for this discrepancy might be that hepatocytes were incubated in the presence of T_3S in the micromolar range (7 to 50 μmol/L) in the study by Kung et al,[20] which is far above the K_m of the type I deiodinase for T_3S (4.6 μmol/L[21]), resulting in saturation of the deiodination pathway. In our experiments, the T_3S concentration was in the picomolar range.

Although the anterior pituitary is generally regarded as an organ with a low type I deiodinase activity,[22] extremely rapid deiodination of $[^{125}I]T_3S$ and efflux of $^{125}I^-$ could theoretically account for the observation that cellular uptake of $[^{125}I]T_3S$ was hardly detectable. It was recently reported that the posterior pituitary,[23] like the brain,[24] contains type III deiodinase that would deiodinate T_3. This reaction would only be detectable by $^{125}I^-$ production, if the inner-ring deiodination by type III deiodinase is followed by outer-ring deiodination. Furthermore, T_3S is not a substrate for the type III deiodinase.[25] The LH-20 chromatography performed in the same way as for the hepatocyte experiments showed no $^{125}I^-$ production from $[^{125}I]T_3S$ or from $[^{125}I]T_3$ during the hour the experiment lasted.

Taken together, our results strongly suggest that relative to T_3, T_3S is poorly taken up by the pituitary gland. In addition, it seems that T_3S is not hydrolyzed in this tissue, and consequently the biological effects of T_3S on the euthyroid pituitary gland are minimal.

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