Organ-specific effects of 3,5,3' -triiodothyroacetic acid in rats

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Abstract

In order to compare the effect of 3,5,3' -triiodothyroacetic acid (TRIAC) with those of triiodothyronine (T₃) and thyroxine (T₄), severely hypothyroid rats (n = 56) were infused over 13 days with 1, 2 or 4 nmol/100 g body weight (BW) per day of T₃ or 2, 4 or 8 nmol/100 g BW per day of T₄ or TRIAC. The 8 nmol/100 g BW per day of T₄ or TRIAC induced the same increase in resting metabolic rate, yet 4 nmol/100 g BW per day of T₃ was more potent (P < 0.05). For inhibiting serum TSH levels, 2 nmol/100 g BW per day of TRIAC were significantly less active than 2 nmol/100 g BW per day of T₄ or 1 nmol/100 g BW per day of T₃ (TRIAC, serum TSH 35.5 ± 5.7; T₃ 2.58 ± 0.91; T₄ 2.12 ± 0.59 ng/ml). At higher doses serum TSH and β-TSH mRNA were unmeasurable.

Using serum T₃ levels as covariate, the action of T₃ and T₄ was identical on cardiac monodeiodinase type 1 (5₀D1) activity and hepatic malic enzyme (Me) mRNA levels and similar for hepatic 5₀D₁ activity. The effect of TRIAC was compared with T₃ by using increasing doses of 1, 2 and 4 nmol/100 g BW per day of T₃ and 2, 4 and 8 nmol/100 g BW per day of TRIAC. ANOVA indicated that there was no major difference between the effects of the hormones since with increasing doses the response of hepatic 5₀D₁ mRNA levels and enzyme activity and Me mRNA remained parallel. However, when studying the effect on cardiac 5₀D₁ activity there was not only a difference for type of treatment (T₃ > TRIAC) but this difference became greater with each increment in dose. Interestingly there was also only a small effect of TRIAC on increase in heart weight compared with T₃ and T₄. Brain cortex monodeiodinase type 2 (5₀D₂) was mainly inhibited by T₄ infusions. Monodeiodinase type 3 (5₀D₃) was stimulated by T₄, less so by TRIAC and least by T₃, expressing probably the local T₃ and TRIAC concentrations.

In conclusion, despite apparently similar effects of TRIAC and T₃ and T₄ on hepatic parameters of thyroid hormone action, TRIAC differs considerably in terms of its effects on cardiac 5₀D₁ activity and possibly on other fundamental effects of thyroid hormones on the heart since heart weight increased significantly less with TRIAC than with T₃ or T₄.

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Introduction

3,5,3’-Triiodothyroacetic acid (TRIAC) is assumed to be of therapeutic interest, since it has been postulated to have less peripheral effects than thyroxine (T₄) and triiodothyronine (T₃) and may therefore be used advantageously for thyrotropin (TSH) suppressive therapy in goitrous patients and in some cases with thyroid cancer (1, 2). It has also been used in thyroid hormone resistance (1) and thyroid hormone cancer (1, 2). There is controversy as to its therapeutic advantage for this indication (3, 4).

Recently two clinical investigations have been published with differing conclusions. Sherman & Ladenson (5) showed that TRIAC has a more pronounced effect on hepatic parameters such as sex hormone binding globulin and serum cholesterol levels than T₄ and we showed that TRIAC was more effective in inhibiting TSH secretion than stimulating basal and sleeping metabolic rate (6). We also found a slightly better inhibition of serum TSH with TRIAC than with T₄. However, the effect on serum TSH was short-lived and therefore three or four daily doses were necessary to extend the effect over 24 h. This represents a limitation for its therapeutic use. These studies were complemented recently in rats where the decay of the effects of TRIAC and T₄ after stopping treatment was studied (7).

It became clear that conclusive comparisons between TRIAC and T₄ and T₃ could only be obtained if half-lives would not influence the experiments. Therefore we chose to study these hormones under constant and long-lasting infusion where one can assume not only that the hormones had achieved a kinetic equilibrium but that their effects had also reached a maximum.

Materials and methods

3,3’-Diiodothyronine (3,3’-T₂), 3,5-diiodothyroacetic acid (3,5-Diac), 3,5,3’-triiodothyroacetic acid (TRIAC),

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3,5,3'-triiodothyronine (T₃) and 3,5,3',5'-tetraiodothyronine (thyroxine, T₄) were purchased from the Henning Co. (Berlin, Germany). According to the manufacturer, TRIAC contained no T₃ and less than 0.05% 3,5-Diac; T₃ contained less than 0.01% T₄. For TRIAC this was confirmed by ourselves by HPLC. 2-Mercapto-l-methyl-imidazole (MMI) was purchased from Sigma Inc., Buchs, Switzerland. Carrier-free [125I]NaI, [125I]T₃ (3000 mCi/mg) and [125I]T₄ (3000 mCi/mg) and α-[32P]-dCTP (3000 Ci/mmol) were purchased from Amersham International (Bucks, UK). Reverse T₃ (rT₃) and TRIAC were labeled with 125I by chloramine-T method (12). Mini-osmotic pumps (Alzet 2002, Alza Co., Palo Alto, CA, USA, delivering for 14 days) were used for T₄, T₃ and TRIAC infusions. TRIAC, T₃ and T₄ were dissolved in 0.05 mol/l NaOH containing 0.9% NaCl and 2% BSA with 50 000 c.p.m. of either [125I]TRIAC, [125I]T₃ or [125I]T₄ in each minipump. For each animal the exact amounts of hormone were added so that the infusion rate would correspond to the expected one using the body weight (BW) 1 day before the implantation. The added 125I counts allowed verification of the infusion rate.

### Experimental procedures

Fifty-six rats weighing 120–150 g each were rendered hypothyroid by injection of 0.5 mCi [131I]NaI/100 g BW. One week later, and throughout the whole experiment, MMI (200 mg/l) was added to the drinking water. Before implanting the minipumps, serum T₄ levels were determined by obtaining tail blood. In all rats except the euthyroid animals, the minipumps were implanted at the time of implantation for TRIAC and T₄ were 2, 4 and 8 nmol/100 g BW per day, and for T₃ 1, 2 and 4 nmol/100 g BW per day. Serum metabolic changes in BW at the end of experiment.

Resting metabolic rates (RMR)

RMR was only measured with the highest dose of either hormone by an open-circuit calorimeter equipped with a sensitive mass flowmeter before implantation and 6 and 13 days later (8). RMR in the euthyroid litter mates were measured when they weighed the same as the hypothyroid rats at the moment of implantation (weight-matched). The measurement was repeated at the end of the experiment (age-matched).

### Radioimmunoassays

Serum T₄, T₃ and TRIAC levels were measured with published in-house RIAs, their intra- and inter-assay coefficients of variation ranging from 6 to 10%. The TRIAC radioimmunoassay was done with TRIAC standards. [125I]TRIAC and the T₃ antiserum. Serum TSH measurements were performed with reagents kindly provided by the National Institutes of Health (NIH, Bethesda, MD, USA). The detection limits were 0.1 pmol T₄/ml, 0.3 pmol TRIAC/ml, 3 pmol T₃/ml and 0.8 ng TSH/ml.

### Deiodination assays and protein measurements

Monodeiodinase type I (5'D1) and type II (5'D2) assays were performed according to the method of Leonard & Rosenberg (9) and were based on the release of radioiodide from [125I]T₃ (10). The assay for monodeiodinase type III (5'D3) activity of brain cortex was performed with the same homogenates as for 5'D2 using inner ring labeled [125I]T₃ as substrate (11).

### Total RNA extraction and Northern blot hybridization

The total RNAs from liver, kidney and pituitary were extracted using the guanidinium thiocyanate method (12). Aliquots of total RNA (20 μg) were denatured and subjected to electrophoresis through 1% agarose. The RNA was transferred to nylon membrane (Nytran-N, Rockford, Illinois, San Francisco, CA, USA, respectively) were purchased in-house RIAs, their intra- and inter-assay coefficients of variation ranging from 6 to 10%. The detection limits were 0.1 pmol T₄/ml, 0.3 pmol TRIAC/ml, 3 pmol T₃/ml and 0.8 ng TSH/ml.
the amount of each mRNA was quantitated by scanning laser densitometry (Molecular Dynamics, Sunnyvale, CA, USA). Each membrane contained RNA samples from 2 nmol T4 per day/100 g BW treated rats as controls.

Statistical methods
ANOVA with the Neuman–Keuls test was used when all 11 groups of rats were compared. A two-way ANOVA was used to study the effects of treatment and dose (ratio) and their interaction. The three treatments were the infusions of T4, T3 and TRIAC. The dose effect was analyzed at three levels of ratio to the lowest dose (i.e. 1, 2 and 4). In addition, data from rats treated with T3 or T4 were compared by analysis of covariance, using serum T3 values as the covariate. This analysis tests for the equality of the slopes of regression for the two treatments. The adjusted values were then used to compare the two treatment groups (T3 rats vs T4 rats). Since the T4 treatment appeared to be the major cause of strong interaction in the ANOVA, we reanalyzed the data with only the two treatments, T3 and TRIAC. Significance was considered as P < 0.05. Data are expressed as means ± S.E.M.

Results
Effect on the body weight gain, heart to BW ratio and RMR
BW was equal in all hypothyroid rats before treatment (208 ± 15 g). The increase in BW in thyroid hormone treated rats was 52 ± 4.5 g and was independent of the type of hormone or dosage. Euthyroid age-matched rats were significantly heavier than the hypothyroid and treated rats (346 ± 6.5 g). The ratio of heart to BW showed a dose-dependent increase. However, 8 nmol/100 g BW per day of TRIAC were needed to obtain a similar heart to BW ratio as in euthyroid rats while the same effect was obtained with 2 nmol T4 or 1 nmol T3/100 g BW per day. RMR was only measured with the highest dose. The RMR with 4 nmol/100 g BW per day of T3 was significantly higher than for 8 nmol/100 g BW per day of TRIAC, while there was no significant difference between TRIAC and T4 (Table 1).

Serum thyroid hormone and TSH levels
Serum T3 levels before implantation were less than 3 pmol/ml. Under the infusions of different concentrations of T3, T4 or TRIAC the respective serum concentrations increased. The increase was not linear, reflecting a dose-dependent increase of MCR. T3 levels under 2 nmol T3/100 g BW per day were slightly higher than in age-matched euthyroid rats (80.2 ± 7.3 vs 69.5 ± 1.4 pmol T3/ml; P < 0.05); but the serum T3 levels were still significantly lower than euthyroid controls, confirming the role of the intact thyroid in T3 secretion. With an infusion of 1 nmol T3/100 g BW per day, the T3 levels were slightly but insignificantly higher than in age-matched euthyroid rats (P < 0.05) (Table 1).

Serum TSH levels show important differences between the treated groups. Serum TSH levels with 2 nmol TRIAC/100 g BW per day were markedly increased (35.5 ± 5.7 ng/ml) while for 2 nmol/100 g BW per day T4 and 1 nmol T3, they were less than 0.8 ng/ml (Table 1, P < 0.001). There was however no significant difference between the medium dose of TRIAC (1.4 ± 0.26 ng TSH/ml for 4 nmol/100 g per day) and the lowest dose of T4 or T3 mentioned above. Unmeasurable serum TSH levels were found in rats treated with 2 and 4 nmol/100 g BW per day T4, and 8 nmol/100 g BW per day T3, and 8 nmol of TRIAC/100 g BW per day. On a molar basis TRIAC was at least four times less active than T3 and two times less than T4.

Peripheral effects of thyroid hormones
In this case the effects of increasing doses were stimulatory and allowed a comparison of the dose–response curves. In order to make the comparison between the incremental effects of the hormones easier, the dose ratios were also compared. These were for all three hormones 1 : 2 : 4.

In order to study the T3- and T4-infused rats, analysis of covariance was performed, serum T3 being the covariate. The slopes for heart and hepatic 5'D1 activity and Me mRNA vs serum T3 were parallel for T4 and T3 treated rats (Figs 1, 2 and 3). For heart 5'D1 activity and Me mRNA the adjusted mean values of the activities were not significantly different, in other words, if serum T3 is taken as covariate, the effects of T4 and T3 are identical. For hepatic 5'D1 activity, the adjusted means in T4 treated rats were significantly greater then in T3 treated rats (P < 0.001). Hence, for the same serum T3 levels, T4 treatment was 1.4 times more active than T3 (Fig. 3).

The comparison of T3 and TRIAC effects on heart 5'D1 activity by two-way ANOVA showed a significant interaction between ratio level and treatment. The difference between the two treatments (P < 0.001) increased with higher doses and the incremental effect for increasing doses of TRIAC was much weaker than for T3 (Fig. 4). These results are in full agreement with the small effect of TRIAC on heart weight.

The two hepatic parameters, 5'D1 mRNA levels and enzyme activity and Me mRNA levels responded similarly to T3 and TRIAC treatments since the incremental effects of increasing T3 or TRIAC infusions were parallel. For 5'D1 mRNA and Me mRNA levels, T3 was on a molar basis approximately twice as active as TRIAC (Figs 5 and 6).

5'D2 activity in brain cortex of hypothyroid rats was six times higher than in the age-matched euthyroid rats. With 2 nmol T4/100 g BW per day, the activity was significantly decreased and represented 50% of the one
in euthyroid animals. With 8 nmol T₄/100 g BW per day, 5D₂ activity was no longer measurable (Fig. 7). In T₃ treated animals, the 5D₂ activity was significantly lower than in hypothyroid rats and 3- to 4-fold higher than in age-matched euthyroid rats. There was no dose-dependency. Interestingly, TRIAC had no effect at all on 5D₂ activity.

Table 1 Steady state levels of thyroid hormones, TSH, resting metabolic rate and heart to body weight ratio (means ± S.E.M.).

<table>
<thead>
<tr>
<th></th>
<th>Euthyroid rats</th>
<th>Hypothyroid rats</th>
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<tbody>
<tr>
<td><strong>T₃</strong> (nmol/100 g BW per day)</td>
<td>1 2 4</td>
<td>2 4 8</td>
</tr>
<tr>
<td>Serum T₃ or TRIAC (pmol/ml)</td>
<td>1.34 ± 0.12ᵇ</td>
<td>2.39 ± 0.19ᵇ</td>
</tr>
<tr>
<td>Serum T₄ (pmol/ml)</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>MCR (ml/min per 100 g BW)</td>
<td>24.7 ± 2.3</td>
<td>27.7 ± 1.9</td>
</tr>
<tr>
<td>Serum TSH (ng/ml)</td>
<td>2.58 ± 0.9</td>
<td>ND</td>
</tr>
<tr>
<td>Heart to BW ratio</td>
<td>0.35 ± 0.01</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>RMR (ml O₂/min per 100 g BW)</td>
<td>—</td>
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**Euthyroid rats**

<table>
<thead>
<tr>
<th><strong>T₄</strong> (nmol/100 g BW per day)</th>
<th>2 4 8</th>
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<tbody>
<tr>
<td>Serum T₄ or TRIAC (pmol/ml)</td>
<td>3.82 ± 0.34</td>
</tr>
<tr>
<td>Serum T₃ (pmol/ml)</td>
<td>1.15 ± 0.03</td>
</tr>
<tr>
<td>MCR (ml/min per 100 g BW)</td>
<td>18.1 ± 1.4</td>
</tr>
<tr>
<td>Serum TSH (ng/ml)</td>
<td>35.5 ± 5.7</td>
</tr>
<tr>
<td>Heart to BW ratio</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>RMR (ml O₂/min per 100 g BW)</td>
<td>—</td>
</tr>
</tbody>
</table>

**Hypothyroid rats**

<table>
<thead>
<tr>
<th><strong>TRIAC</strong> (nmol/100 g BW per day)</th>
<th>2 4 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum T₃ or TRIAC (pmol/ml)</td>
<td>69.5 ± 1.4</td>
</tr>
<tr>
<td>Serum T₄ (pmol/ml)</td>
<td>—</td>
</tr>
<tr>
<td>MCR (ml/min per 100 g BW)</td>
<td>5.03 ± 0.93</td>
</tr>
<tr>
<td>Serum TSH (ng/ml)</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>Heart to BW ratio</td>
<td>—</td>
</tr>
<tr>
<td>RMR (ml O₂/min per 100 g BW)</td>
<td>5.39 ± 0.09ᶜ</td>
</tr>
</tbody>
</table>

ᵃP < 0.05 vs lowest dose;ᵇP < 0.05 vs middle dose;ᶜP < 0.05 andᵈP < 0.01 compared to TRIAC and T₄; ND, not detected.
In hypothyroid animals brain 5'D3 activity was markedly decreased compared with the age-matched euthyroid rats. There was a highly significant difference between the three treatments and a significant interaction between the treatment and doses, indicating that all three hormones have different effects. T4 was strongly stimulating 5'D3 activity (Fig. 8). With 2 nmol T4/100 g BW per day, the activity was already significantly higher than in the age-matched euthyroid rats (P < 0.05). The increase was dose-dependent.

Treatment with T3 had only a minor effect on the 5'D3 activity. TRIAC was more potent than T3 (P < 0.05) but less potent than T4 (P < 0.05).

**GH and β-TSH mRNA levels**

The effect of three hormones on growth hormone mRNA levels are shown in Fig. 9. GH mRNA was unmeasurable in hypothyroid animals and markedly stimulated by all three hormones. There was no clear dose-dependency.

β-TSH mRNA levels were detectable under 2 nmol TRIAC/100 g BW per day. They were not measurable with higher TRIAC doses and in all groups treated with either T4 or T3 (data not shown).
Discussion

Despite circumstantial evidence for some qualitative differences between T$_3$ and TRIAC (14–16), a good *in vivo* comparison of the effects of these similar thyroid hormones has been hampered by their different half-lives. To overcome this limitation we decided to infuse these hormones over a very long period of time insuring a kinetic equilibrium and a constant effect. In addition, T$_4$ was also studied since it is thought to play a special role in inhibiting 5'-D2 activity and might be crucial for the inhibition of serum TSH. Also, it is postulated that for certain organs such as heart and liver the intracellularly produced T$_3$ is in free exchange with serum T$_3$. For these organs, the serum T$_3$ concentrations under T$_4$ or T$_3$ treatment should correlate with the thyroid hormone effects.

This was in fact so since analysis of covariance clearly showed that there was no difference in the T$_3$ or T$_4$ effect on cardiac and hepatic 5'-D1 activity as well as on Me mRNA levels. There was however a slightly higher activity of T$_4$ on hepatic 5'-D1 activity. This cannot be easily explained but might indicate a small preferential intrahepatic conversion of T$_4$ to T$_3$ which would not be sufficient to affect the mRNA levels.

![Figure 7](image1.jpg)

**Figure 7** Effect of treatment on brain cortex 5'-D2 activity (mean±S.E.M.), **P<0.01 compared with the hypothyroid value.**

![Figure 8](image2.jpg)

**Figure 8** Effect of treatment on brain cortex 5' D3 activity. With ANOVA, there was a significant interaction between treatment and doses, indicating that the three hormones had different effects.
More importantly, the results for the dose ratios of T₃ and TRIAC on Me mRNA levels and hepatic 5₀D₁ activity strongly suggest that for the two liver parameters the two hormones act identically even though in molar terms TRIAC had at least half the activity of T₃ and was as equally potent as T₄. In an earlier study, TRIAC was found to be less active than T₄ and T₃ despite similar or better affinities for the T₃ receptors (17). Such differences were explained by the more rapid MCR of TRIAC so that after single injections of TRIAC the occupancy of the nuclear receptors was only short lived. With continuous infusions, the MCR can be calculated more accurately and unexpectedly no difference between the MCR of T₃ and TRIAC could be found. Therefore, the MCR cannot explain the molar ratio and we suggest differences in intracellular metabolism and/or differences in cellular and nuclear uptake of the two hormones. In this respect, recent work indicates that TRIAC is a superior substrate for sulfation and consequent deiodination than T₃, while for cellular or nuclear uptake, contradictory results have been reported (18–22).

TSH secretion is assumed to be controlled by at least two thyroid hormone related mechanisms: the role of the local conversion of T₄ to T₃ and the abundance of β₁, β₂ and possibly α₁ T₃ receptors. TRIAC has a higher affinity for these receptors than T₃ yet the role of local conversion is probably more important and may partly explain the relative lower potency of TRIAC for TSH inhibition than T₄ (15). It cannot explain, however, the difference with T₃. These results are also in opposition to human studies where TRIAC seems to be slightly better than T₄ for inhibiting serum TSH (4). It is also interesting to note that T₄ was not a preferential inhibitor of TSH compared with T₃ which has been postulated on the basis of its specific intrapituitary conversion to T₃.

The most interesting findings are the difference between TRIAC and T₃ on heart to BW ratio and deiodinase. We hypothesize that the small increase of heart weight with increasing doses of TRIAC attest for a generalized difference of action between the two hormones, the 5₀D₁ activity being only one example. Even for T₄ for which there was no major difference in molar ratio with TRIAC, heart weight increased more than with TRIAC. It is well known that hyperthyroidism can induce cardiac hypertrophy and the RMR is an expression of the hyperthyroid state of the animals. Nevertheless, one does not find a difference between RMR of 8 nmol per day of T₄ or TRIAC, the only other parameter responding less well to TRIAC than to T₄ or T₃ is serum TSH. These findings suppose a specific difference of action for TRIAC on heart and in view of the clinical importance of cardiac effects of these hormones during TSH suppressive therapy further work on this topic will be necessary.

**Acknowledgements**

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