EXPERIMENTAL STUDY

Differences between the effects of thyroxine and tetraiodothyroacetic acid on TSH suppression and cardiac hypertrophy

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Abstract

Objective: We earlier reported marked qualitative differences between the effect of 3,5,3'-triiodothyroacetic acid (Triac) and tri-iodothyronine (T₃) on cardiac hypertrophy at equivalent thyroid-stimulating hormone (TSH)-suppressive doses. We have now extended these studies to specific cardiac parameters. Due to its rapid metabolic clearance rate, Triac is not suitable for TSH suppression and therefore the slowly metabolized 3,5,3',5'-tetraiodothyroacetic acid (Tetrac), the precursor of Triac, was studied.

Methods: Hypothyroid rats were infused over 13 days with 1.5–40.5 nmol Tetrac/day per 100 g body weight (BW) or with 0.5–13.5 nmol thyroxine (T₄)/day per 100 g BW.

Results: The responses of serum TSH and of hepatic monodeiodinase type 1 were parallel for both hormones, their potency ratios could therefore be compared. Tetrac was revealed as being only half as active on hepatic monodeiodinase type 1 despite a similar serum TSH levels. Tetrac can therefore be considered to have a preferential action on serum TSH suppression. The cardiac effects on Ca²⁺-ATPase (SERCA 2a) and monodeiodinase type 1 activity were qualitatively different and therefore one cannot give an overall quantitative estimate of these differences. The results showed clearly, however, that Tetrac is less efficient for all parameters studied, namely induction of cardiac hypertrophy, α -myosin heavy chain mRNA, monodeiodinase type 1 activity and mRNA levels of the sarcoplasmic SERCA 2a.

Conclusion: We postulate therefore that, in the rat and possibly in man, Tetrac could represent a favorable alternative for suppression of serum TSH levels.

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Introduction

In the mid 1950s 3,5,3',5'-tetraiodothyroacetic acid (Tetrac) was studied as a potential substitute for thyroxine (T₄) for treating myxedema and for its effects on peripheral lipid metabolism in humans (1–3). Except for the higher doses of Tetrac required, no clear differences in the clinical or lipid outcomes were found between Tetrac and T₄. More recent human studies of the ability of 3,5,3'-tri-iodothyroacetic acid (Triac), the active metabolite of Tetrac, to suppress thyroid-stimulating hormone (TSH) uncovered higher thyromimetic effects of Triac relative to T₄ on liver metabolism (4–7).

In the rat, studies with Triac revealed differences with tri-iodothyronine (T₃) for cardiac hypertrophy and cardiac monodeiodinase type 1 activity (8, 9). These differential peripheral sensitivities and actions of Triac

might be used for clinical benefit. The problem of its short half-life can be overcome by administering Tetrac, its slowly metabolized precursor, and Tetrac might even have an additional advantage over Triac and T₄ since it might allow excellent inhibition of serum TSH without inducing subclinical hyperthyroidism. This is because the intrapituitary conversion of T₄ to T₃ that is required for TSH suppression is reduced by the simultaneous inhibition of the monodeiodinase type 2 as physiologic levels of T₄ are achieved, decreasing the intrapituitary production of T₃. Therefore, larger than metabolically required replacement doses of T₄ are required to suppress TSH below normal levels. Such states of subclinical hyperthyroidism are the therapeutic goal for many patients with thyroid malignancies. Tetrac, on the other hand, because it does not inhibit, *in vitro*, monodeiodinase type 2, could possibly suppress TSH at less than its equivalent T₄ peripheral thyromimetic dose

by allowing the continued production of Triac with its similar potency to T_3 for suppressing TSH (10). The present studies assess the effects of Tetrac and T_4 on heart and pituitary at equivalent TSH-suppressing concentrations on gene regulation and enzyme activities *in vivo* in rats.

Materials and methods

Materials

Thyroid hormones (3,3'-di-iodothyronine (T_2), T_3 , T_4) and their analogs Diac, Triac and Tetrac were obtained from Henning Co. (Berlin, Germany). All the chemical reagents used were purchased from Fluka (Buchs, Switzerland), Sigma Chemical Co. (St Louis, MO, USA) or Merck (Darmstadt, Germany). Osmotic mini-pumps (model 2002) were purchased from Alzet (Palo Alto, CA, USA).

Carrier-free [125 I]Na was purchased from Amersham International plc (Amersham, Bucks, UK) and [131 I]Na from Nuklear Reaktor (Würenlingen, Switzerland). T_3 , T_4 , Triac and Tetrac were labeled with 125 I by the chloramine-T method using 3,5- T_2 , L- T_3 , Diac and Triac as substrate respectively (11). Specific activities thus achieved were higher than 1200 μ Ci/ μ g.

Experimental protocol

Seventy-nine male SIVZ rats were purchased from the breeder (Institut für Labortierkunde des Universität Zürich, Zürich, Switzerland). They were rendered hypothyroid by a single injection of [131 I]Na (100 μ Ci/rat) and before and throughout the experiment 0.02% 2-mercapto-1-methylimidazol (MMI) was added to drinking water. In order to have a euthyroid control group of similar weight and identical age, ten rats received only MMI in the drinking water and, 2 weeks before implanting the minipumps, MMI was withdrawn. Osmotic minipumps (secretion rate 0.25 μ l/h, duration 14 days) were implanted i.p. 2 months after the injection of [131 I]Na. The minipumps were filled with T_4 or Tetrac dissolved in 0.05 M NaOH, 2% bovine serum albumin and 100 000 c.p.m. [125 I] T_4 or [125 I]Tetrac as a control of their secretion rates. The infusion lasted 13 days. Doses of 0, 1.5, 4.5, 13.5 and 40.5 nmol Tetrac/day per 100 g body weight (BW) or 0.5, 1.5, 4.5 and 13.5 nmol T_4 /day per 100 g BW ($n = 4$ /dose) were infused. A euthyroid control group was included. The animals were weighed every 2 days. Rats were killed by decapitation and blood was collected and stored at -20°C until use. Tissues (liver, heart, brown adipose tissue (BAT), cerebral cortex and pituitary) were removed rapidly, frozen in liquid nitrogen and stored at -70°C until use.

Hormone assays

Total serum T_4 , T_3 , Tetrac, Triac and TSH levels (intra-assay coefficient of variation 7–10%, interassay coefficient of variation 12–15%) were measured using in-house methods. Serum TSH measurements were performed with reagents kindly provided by the National Pituitary Program, NIAMDD, NIH (Bethesda, MD, USA). The detection limits were 0.80 ng TSH/ml, 9.5 pmol T_4 /ml, 0.16 pmol T_3 /ml, 10 pmol Tetrac/ml and 0.52 pmol Triac/ml.

Monodeiodinase type 1 and 2 activities

Monodeiodinase type 1 and type 2 activities were determined in tissue homogenates by measuring the release of radioiodide from [125 I]reverse T_3 (r T_3) according to the method of Leonard & Rosenberg (12).

Monodeiodinase type 1 activity was measured in liver homogenates using 1 μ M r T_3 and 1 mM DTT with 10 min of incubation, whereas for heart tissue, 0.01 μ M r T_3 and 10 mM DTT with 1 h of incubation were used. Monodeiodinase type 2 activity was measured in BAT and cerebral cortex using 1 nM r T_3 , 10 mM DTT and 1 mM propylthiouracil with 1 h of incubation. Protein concentrations were measured by the method of Bradford (Bio-Rad Laboratories GmbH, Munich, Germany) with bovine gammaglobulin as the protein standard.

In order to study the effect of circulating Tetrac on the measurement of enzyme activities, two rats were injected i.p. with 10 μ Ci [125 I]Tetrac and killed 6 h later. The tissue homogenates were extracted with 3 vol ethanol- NH_3 (97:3). After 10 min centrifugation at 15 000 *g*, the supernatant was vacuum dried and applied on thin-layer chromatograms (Merck 254; toluol, acetic acid, water: 2:2:1). In order to calculate the tissue concentrations, the highest serum Tetrac levels were used (406 pmol/ml). The calculated tissue concentrations were: liver 1.9, heart 2.29 and brain cortex 0.47 fmol Tetrac/ μ g protein.

Total RNA extraction and Northern blot hybridization

Total RNA was extracted by the method of Chomczynski & Sacchi (13). The probe for monodeiodinase type 1 was kindly provided by Dr P R Larsen (Harvard Medical School, Boston, MA, USA), for monodeiodinase type 2 by Dr D L St Germain (Lebanon, NH, USA), the rat growth hormone (rGH) probe by Dr F DeNoto (University of California, San Francisco, CA, USA) and the probe for rat cardiac reticulum sarcoplasmic Ca^{2+} -ATPase (SERCA 2a) by Dr W H Dillmann and Dr R Sayen (Medical Center of University of California, San Diego, CA, USA). The probes for α - and β -myosin heavy chain (α -MHC and β -MHC) were synthesized by Microsynth GmbH (Balgach, Switzerland). Probes were

labeled by γ [^{32}P]dCTP using a random oligonucleotide priming kit (^{32}P QuickPrime™ Kit; Pharmacia Biotech, Uppsala, Sweden) to a specific activity of 1×10^9 c.p.m./ μg DNA except for α - and β -MHC using polynucleotide kinase T4 with γ [^{32}P]dATP. Hybridization was carried out at 42 °C.

The amount of labeled mRNA was quantified by scanning laser densitometry (Phosphorimager; Molecular Dynamics, Sunnyvale, CA, USA). Variations in RNA loading and transfer were corrected by densitometric quantification of a negative photograph of 28S and 18S ribosomal RNA bands from ethidium bromide-stained RNA gels.

The final values were obtained by using the groups treated with 0.5 and 1.5 nmol T_4 /day per 100 g BW (depending on the tissue) as control and expressed in arbitrary units. Each membrane contained a 0.5 and/or a 1.5 nmol T_4 /day per 100 g BW sample. For the RNA levels of pituitary monodeiodinase type 2 the densitometric value of the highest dose of T_4 was used as internal standard.

Statistical methods

A two-way analysis of variance (ANOVA) was used to estimate differences among the two treatment groups, over the increasing doses of the hormones (four levels), and the interaction between treatment and dose levels (SAS General Linear Models program) (14). The dose levels at a 1:3 molar ratio were considered for 1, 3, 9 and 27 times the lowest dose. A one-way analysis of covariance, with $\ln$ (TSH) as the covariate, was performed using BMDP software (IV), which also includes a test for parallel regression slopes (15). When the two treatment groups shared a common slope, adjusted mean values for each treatment group were calculated at the overall mean $\ln$ (TSH) concentration. Most variables were log transformed to improve linearity for these analyses. Significance was considered at $P < 0.05$.

Results

As may be seen in Table 1, animals treated with Tetrac and T_4 gained weight during the 13 days of treatment. With the highest doses of Tetrac and T_4 the final weight gain was inferior to the other groups. This can be explained by their hyperthyroid status induced by both hormones.

For both treatments, the increase in liver weight was parallel to the increase in BW (data not shown). However, the heart weight increased faster than BW with increasing infusion rates of T_4 . In contrast to T_4 , Tetrac induced a much milder cardiac hypertrophy ($P < 0.01$).

Due to the strong binding to prealbumin, serum Tetrac levels were high but serum Triac levels remain low. The ratio of serum Triac to Tetrac was lower than the T_3 to T_4 ratio (Table 2). Despite these high serum Tetrac levels, the contamination of the homogenates with Tetrac was not sufficient to affect the enzyme assay. In brain cortex homogenates, the concentration was less than 1 fmol/ μg protein. The addition of Tetrac to a monodeiodinase type 2 assay using microsomal protein from brain cortex showed, with 1 fmol Tetrac/ μg protein, 90.5% of the full activity. Similar results were obtained by the addition of T_4 or rT_3 .

Based on preliminary studies, which indicated a relative TSH-suppressive potency of 1–3, infusions at a molar T_4 to Tetrac ratio of 1:3 were chosen. For this reason, in the ANOVA calculations, the molar ratios were used. In Fig. 1 the effects of the two hormones on the two cardiac parameters, monodeiodinase type 1 activity and SERCA 2a mRNA levels, are shown. Inspection of Fig. 1 demonstrates the weak response to increasing doses of Tetrac compared with T_4 ($P < 0.001$). In the ANOVA, a significant interaction between the two hormone treatments ($P = 0.003$ for heart monodeiodinase type 1, $P = < 0.001$ for SERCA 2a) verifies this difference in response. This represents one of the two major findings of this study. For α - and β -MHC there was, however, no interaction with dose and treatment (Fig. 2).

Table 1 Whole body and heart weights. Values are means $\pm$ S.E.M.

	Infusion (nmol/100 g BW per day)	Molar ratio	Body weight (g)		Heart (g)	Heart/BW (%)
			At day 0	Increase after 13 days		
Tetrac infusion	1.5	1	229.9 $\pm$ 6.3	14.0 $\pm$ 1.8	0.76 $\pm$ 0.02	0.31 $\pm$ 0.01
	4.5	3	228.2 $\pm$ 7.0	27.1 $\pm$ 1.3	0.83 $\pm$ 0.04	0.33 $\pm$ 0.02
	13.5	9	230.0 $\pm$ 4.3	33.9 $\pm$ 2.2	0.94 $\pm$ 0.03	0.36 $\pm$ 0.01
	40.5	27	235.0 $\pm$ 14.9	15.5 $\pm$ 4.8	0.98 $\pm$ 0.06	0.39 $\pm$ 0.02 ^a
T_4 infusion	0.5	1	230.0 $\pm$ 14.3	18.5 $\pm$ 2.1	0.86 $\pm$ 0.06	0.35 $\pm$ 0.02
	1.5	3	231.2 $\pm$ 5.3	31.1 $\pm$ 3.0	0.90 $\pm$ 0.04	0.34 $\pm$ 0.01
	4.5	9	230.8 $\pm$ 8.9	32.1 $\pm$ 2.3	1.19 $\pm$ 0.03	0.45 $\pm$ 0.02
	13.5	27	230.0 $\pm$ 10.1	14.8 $\pm$ 6.4	1.32 $\pm$ 0.11	0.54 $\pm$ 0.02
Euthyroid			233.4 $\pm$ 9.4	60.8 $\pm$ 5.4	1.04 $\pm$ 0.05	0.38 $\pm$ 0.01
Hypothyroid			226.4 $\pm$ 8.9	1.3 $\pm$ 1.9	0.61 $\pm$ 0.03	0.27 $\pm$ 0.01

Table 2 Serum values of TSH, T₄, T₃, Triac and Tetrac. Values are means $\pm$ S.E.M.

	Molar ratio	Infusion (nmol/100 g BW per day)	Serum values				
			TSH (ng/ml)	T ₃ (pmol/ml)	T ₄ (pmol/ml)	Triac (pmol/ml)	Tetrac (pmol/ml)
Tetrac infusion	1	1.5	90.3 $\pm$ 8.6.			1.1 $\pm$ 0.3	168 $\pm$ 16
	3	4.5	4.6 $\pm$ 0.5			1.1 $\pm$ 0.2	332 $\pm$ 19
	9	13.5	1.2 $\pm$ 0.3			2.4 $\pm$ 0.5	458 $\pm$ 27
	27	40.5	<0.8			6.3 $\pm$ 1.7	406 $\pm$ 57
T ₄ infusion	1	0.5	110.2 $\pm$ 10.8	0.7 $\pm$ 0.0	43.1 $\pm$ 2.8		
	3	1.5	11.8 $\pm$ 3.0	1.2 $\pm$ 0.1	77.1 $\pm$ 9.4		
	9	4.5	1.0 $\pm$ 0.2	3.4 $\pm$ 0.2	129.7 $\pm$ 9.5		
	27	13.5	<0.8	24.0 $\pm$ 0.7	353.4 $\pm$ 31.7		
Euthyroid			7.9 $\pm$ 1.1	2.1 $\pm$ 0.2	81.9 $\pm$ 6.1		
Hypothyroid			76.7 $\pm$ 6.4	0.2 $\pm$ 0.0			

Serum TSH values were significantly lower in the Tetrac-treated rats when compared with the T₄-treated rats with the same molar ratio infusion rate ($P < 0.05$).

Figure 1 Effect of Tetrac and T₄ treatments on the (a) cardiac monodeiodinase type 1 activity and (b) mRNA levels of SERCA 2a. For both parameters, the incremental increase in response to Tetrac is much weaker than for T₄ ($P < 0.001$), and response differences between the treated groups are not consistent over the increasing dose levels (interaction between dose and treatment, $P < 0.005$ for both parameters). For the definition of the densitometric quantification, see Materials and methods. EU (WM), euthyroid; Hypo, hypothyroid. Values are means $\pm$ S.E.M.

Figure 2 (a) α -MHC mRNA values were not significantly different between the two treatment groups. (b) β -MHC values were significantly different between the treatment groups ($P < 0.001$) and this difference was consistent over the three dose levels considered. In (a) the lowest dose and in (b) the highest dose were not included in the statistical calculations. EU (WM), euthyroid; Hypo, hypothyroid; N.D., not determined. Values are means $\pm$ S.E.M.

In contrast to the cardiac effects, the hepatic response (monodeiodinase type 1 activity and mRNA levels) to increasing doses of the two hormones was parallel and in addition, T_4 was more potent at all dose levels ($P = 0.001$ and for liver mRNA $P = 0.053$) (Fig. 3).

There was no treatment difference for the response of rGH mRNA (Fig. 4a). In Figs 4b and 5 the response of monodeiodinase type 2 is illustrated. The inhibition of the pituitary mRNA levels of monodeiodinase type 2 was less efficient with Tetrac than with T_4 ($P = 0.001$) (Fig. 4b). The monodeiodinase type 2 activity of brain cortex was also less affected by Tetrac than by T_4 ($P = 0.001$) (Fig. 5a) and in BAT the difference was present ($P < 0.001$) but less striking (Fig. 5b).

Since serum TSH concentrations were found to be higher in the T_4 -treated rats compared with the Tetrac-treated rats (mean of 31 vs 24 ng/ml, $P = < 0.04$), the data were reanalyzed using an analysis of covariance with the natural log of TSH ($\ln$ (TSH)) as the covariate. For those variables where the two treatment groups had a similar response slope compared with their $\ln$ (TSH) concentrations, adjusted group means could be calculated at the overall group $\ln$ (TSH) concentration. Fig. 6 demonstrates the plot of $\ln$ (TSH) against $\ln$ (hepatic monodeiodinase type 1 activity). It was therefore possible to predict, at a mean serum TSH concentration, the mean effect of T_4 or Tetrac on the parameters studied as an indication of the relative

Figure 3 Effect of Tetrac and T₄ treatments on (a) the hepatic monodeiodinase type 1 (D1) activity and (b) its mRNA levels. In both cases, the increase in response is consistent across all dose levels. EU (WM), euthyroid; Hypo, hypothyroid; N.D., not determined. Values are means $\pm$ S.E.M.

activities of these two hormones on these parameters. The results are summarized in Table 3. They indicate that at an equal serum TSH concentration, hepatic monodeiodinase type 1 activity and mRNA levels were less stimulated in Tetrac-treated rats (59 and 49% of the value in T₄-treated rats). This means that for this parameter we had overestimated the potency of Tetrac by using a 1:3 molar infusion ratio for comparison. Tetrac was also less active than T₄ in reducing the excessive monodeiodinase type 2 activity characteristic for hypothyroid cortex. In Table 3 the results are expressed in terms of remaining activity and, therefore, the activity of the enzyme was higher with the poorer inhibitor.

Discussion

Similar to T₄, Tetrac is a good substrate for monodeiodinase type 1 and is converted to Triac (16). According to the *in vitro* affinities to T₃ receptors it is also suggested that Tetrac acts as a prohormone and

that Triac is the active compound with a high affinity for T₃ receptors (17–19). It may be puzzling to find a smaller Triac to Tetrac serum ratio compared with the T₃ to T₄ ratio. We have published the metabolic clearance rate of Triac which, in the rat, does not differ from T₃ (28 ml/h per 100 g BW) (9). Using the present serum Triac levels, the above-mentioned MCR and the infusion rates of Tetrac, one can estimate the conversion rate. It is approximately 30% and therefore similar to the one of T₄ to T₃. We assume that the explanation for the difference in serum ratio is the consequence of a very high affinity of transthyretin for Tetrac (11).

Few direct effects of T₄ have been well documented, the marked inhibition of monodeiodinase type 2 activity being the best example (20, 21). The physiological relevance of this effect has been clearly established and probably plays a crucial role for an increased intrapituitary conversion of T₄ to T₃ in the hypothyroid state while normal serum T₄ concentrations

Figure 4 Effect of Tetrac and T_4 treatments on the expression of (a) rGH mRNA in the pituitary. There was no significant difference between treatments over the dose levels considered ($P = 0.14$). (b) Tetrac treatment was significantly weaker in suppressing pituitary monodeiodinase type 2 mRNA levels of the pituitary, $P = 0.001$. EU (WM), euthyroid; Hypo, hypothyroid; N.D., not determined. Values are means $\pm$ S.E.M.

inhibit the enzyme activity and, thus, the intrapituitary conversion of T_4 to T_3 is decreased (22, 23). Tetrac differs from T_4 since it has been shown that a large excess of Tetrac is not able to inhibit monodeiodinase type 2 activity in glial cell cultures. In our present experiments the *in vitro* results were not fully reproduced. In terms of activity, this is clearly seen by some inhibition of monodeiodinase type 2 activity of brain cortex with high Tetrac concentrations (Fig. 5a). There was insufficient pituitary material for measurements of enzyme activity and RNA extraction. It is, however, known that monodeiodinase type 2 activities of cerebral cortex and pituitary correlate well (24). In addition, the results of the Northern blot of pituitary

monodeiodinase type 2 mRNA show a difference between the effect of T_4 and Tetrac which suggests that a long-term non-nuclear effect such as the inhibition of monodeiodinase type 2 activity will affect mRNA expression.

We are therefore able to conclude that in hypothyroid and euthyroid conditions, Tetrac is clearly a weaker inhibitor of monodeiodinase type 2 activity than T_4 . If this difference could also be found in humans, it would be of major clinical importance since it follows that for a similar peripheral effect of T_4 and Tetrac one can expect a more pronounced inhibition of TSH secretion.

Concerning the nuclear effects and assuming that they are mediated by Triac, one might assume that

Figure 5 Effect of Tetrac and T₄ treatments on the monodeiodinase type 2 (D2) activity of (a) cerebral cortex and (b) BAT. In these tissues, T₄ treatment was significantly stronger than Tetrac in suppressing D2 activity ($P < 0.001$). This difference in response, however, was not consistent over the dose levels considered. (Interaction between dose and treatment < 0.001 for cortex, $P < 0.01$ for BAT.) EU (WM), euthyroid; Hypo, hypothyroid. Values are means $\pm$ S.E.M.

Table 3 The adjusted group means for each variable were estimated by analysis of covariance at the mean ln (TSH) concentration. For SERCA 2a and cardiac monodeiodinase type 1 the slopes between T₄ and Tetrac treatment were different and therefore the mean potency could not be estimated. The mRNA levels are expressed in arbitrary units (Phosphoimager).

Measurement	Organ	n	Adjusted means*		T ₄ /Tetrac†	Tetrac/T ₄ ‡	P value
			Tetrac-infused rats	T ₄ -infused rats			
Monodeiodinase type 1 activity	Liver	62	106	180		59%	<0.001
Monodeiodinase type 1 mRNA	Liver	27	2.48	5.1		49%	<0.03
Monodeiodinase type 2 activity	Brain cortex	61	27.7	5.02	18%		<0.001
Monodeiodinase type 2 activity	BAT	61	28	11.8	42%		<0.001
β -MHC mRNA	Heart	20	5.3	1.13	21%		<0.001
α -MHC mRNA	Heart	24	328	495		66%	<0.19
GH mRNA	Pituitary	30	20 280	28 644		71%	<0.07
Monodeiodinase type 2 mRNA	Pituitary	20	2.00	1.25	60%		<0.001

* Each measurement in T₄- and Tetrac-treated rats.

† Activity of T₄ in % of Tetrac.

‡ Activity of Tetrac in % of T₄.

Figure 6 A reciprocal plot of the natural log of serum TSH vs the natural log of hepatic monodeiodinase type 1 (D1) activity. The slopes of the two curves are not significantly different allowing for the calculation of the adjusted group means of the hepatic monodeiodinase activity for T_4 -treated rats and Tetrac-treated rats at the overall mean $\ln$ (TSH) of 1.43 $\ln$ (TSH) of 1.43 ng/ml. See Table 3 for results.

some small differences between Triac and T_3 would be found since Triac has a higher affinity for $\beta 1$ and $\beta 2$ receptors than T_3 , yet only by a factor of approximately 2 (19, 25, 26). There has been a recent report indicating that, based on *in vitro* transfection studies, Triac could selectively stimulate T_3 receptor β (TR- β) and be a preferential treatment for patients with thyroid hormone resistance (18). Our *in vivo* experiments indicate that the slopes of response of hepatic monodeiodinase type 1 and of serum TSH are not different, suggesting a similar mechanism of action. However, at the level of relative potencies, the *in vivo* data do not agree with what could be expected from *in vitro* data, since rat liver has mainly TR-1 receptors, an ideal substrate for Triac. Yet, according to Table 3, the potency of Triac on hepatic monodeiodinase type 1 is only about 50% of its effect on serum TSH levels. We have therefore to assume that *in vivo* the action of the hormones is the result of many factors, such as their metabolism, cellular and nuclear uptake and export. The relative importance of these factors may vary greatly and may differ from tissue to tissue.

Finally, none of the above-mentioned variables could explain the remarkable differences between the two hormones on the three heart parameters, monodeiodinase type 1, β -MHC and, most importantly, SERCA 2a which confirm our studies with continuous Triac infusions. The fact that here the response to increasing doses of Tetrac is clearly less than for T_4 indicates a fundamental difference in the mode of cardiac action for these two hormones. In this context, it is most interesting to note that a recently synthesized T_3 analogue, GC-1, which has a high TR- β specificity, shows similar discordant effects (27–30).

Our results indicate two major findings for the action of Tetrac. On one hand, we have confirmed *in vivo* that Tetrac is a weak inhibitor of monodeiodinase type 2 activity and we show that for this reason, Tetrac is more potent than T_4 in inhibiting serum TSH concentrations. Secondly, we have demonstrated that Tetrac is qualitatively different and less potent than T_4 in stimulating cardiac hypertrophy and mRNA levels of SERCA 2a. The serum binding proteins of rats are different from humans; in the rat, transthyretin is the major binding protein which binds with high-affinity Tetrac and Triac but also T_4 and T_3 . One may therefore predict some differences between species. Nevertheless, it is possible that this substance may have the ideal profile for chronic treatment of patients with thyroid hormones, particularly if the treatment has, as an objective, complete serum TSH inhibition and its use should therefore be investigated in man.

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