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Only the Combined Treatment with Thyroxine and Triiodothyronine Ensures Euthyroidism in All Tissues of the Thyroidectomized Rat*

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ABSTRACT

We have recently shown that it is not possible to restore euthyroidism completely in all tissues of thyroidectomized rats infused with T_4 alone. The present study was undertaken to determine whether this is achieved when T_3 is added to the continuous sc infusion of T_4.

Thyroidectomized rats were infused with placebo or T_4 (0.80 and 0.90 μg/100 g BW·day), alone or in combination with T_3 (0.10, 0.15, or 0.20 μg/100 g BW·day). Placebo-infused intact rats served as euthyroid controls. Plasma and 12 tissues were obtained after 12 days of infusion. Plasma TSH and plasma and tissue T_4 and T_3 were determined by RIA. Iodothyronine deiodinase activities were assayed using cerebral cortex, pituitary, brown adipose tissue, liver, and lung.

Circulating and tissue T_4 levels were normal in all the groups infused with thyroid hormones. On the contrary, T_3 in plasma and most tissues and plasma TSH only reached normal levels when T_3 was added to the T_4 infusion. The combination of 0.9 μg T_4 and 0.15 μg T_3/100 g BW·day resulted in normal T_4 and T_3 concentrations in plasma and all tissues as well as normal circulating TSH and normal or near-normal 5′-deiodinase activities.

Combined replacement therapy with T_4 and T_3 (in proportions similar to those secreted by the normal rat thyroid) completely restored euthyroidism in thyroidectomized rats at much lower doses of T_4 than those needed to normalize T_3 in most tissues when T_4 alone was used. If pertinent to man, these results might well justify a change in the current therapy for hypothyroidism. (*Endocrinology* **137:** 2490–2502, 1996)

T4 IS WIDELY used for the replacement therapy of hypothyroidism in humans. Although thyroidal T_3 secretion is deficient in these patients, the current hypothesis is that peripheral conversion of the orally administered T_4 to T_3 in tissues is able to provide normal circulating and tissue concentrations of T_3 and would thus be able to ensure euthyroidism. The latter presumably would require normalization of both T_4 and T_3 (or at least T_3) in tissues (1). Attainment of euthyroidism is usually assessed on the basis of normalization of circulating TSH (2), which is actually only measuring the thyroid hormone status of the hypothalamus and thyrotrophs. It is implicitly admitted that to attain euthyroidism in hypothyroid patients with T_4 alone, it might be necessary to maintain circulating T_4 concentrations in the upper limits of the normal range (3). Indeed, circulating T_4 concentrations are higher in hypothyroid patients on T_4 than in normal individuals with similar concentrations of plasma T_3 and TSH (4). On the contrary, plasma T_3 concentrations in patients receiving T_4 treatment with normal plasma T_4 and TSH concentrations are only 80% of those in normal individuals (3).

We recently confirmed (1) in thyroidectomized rats receiving replacement therapy with T_4 alone that the dose of T_4 needed to normalize circulating T_3 and TSH levels results in supraphysiological concentrations of plasma T_4. Moreover, the resulting concentrations of T_4 in the 10 tissues studied is clearly higher than the normal range. Despite this, T_3 concentrations did not reach normal values in some tissues. Finally, the dose of T_4 needed to ensure a normal T_3 concentration (and, presumably, euthyroidism) is not the same for all tissues. It was evident that even if the undesirable effects of excessive T_4 concentrations were disregarded, peripheral conversion of T_4 to T_3 did not fully compensate for the absence of thyroidal secretion of T_3. Using thyroidectomized rats infused only with T_3 in doses ranging from 0.25–2.0 μg/100 g BW·day, it was not possible to normalize T_3 concentrations simultaneously in plasma and all tissues (5).

In the present study we compared the effects of infusion of T_4 alone with the effects of infusion of several combinations of T_4 plus T_3. As will be seen, T_3 must be added to T_4 to completely restore normal tissue concentrations of both T_4 and T_3 in thyroidectomized rats. The combination of 0.90 μg T_4 and 0.15 μg T_3/100 g BW·day was that which resulted in normal thyroid hormone concentrations in plasma and the 12 tissues studied as well as normal plasma TSH levels and normal or near-normal activities of 5′-iodothyronine deiodinase (5′D) in pituitary, liver, lung, cerebral cortex, and brown adipose tissue (BAT). This corresponds to a molar

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ratio of T_4 to T_3 similar to that reported by others (6–10) for the daily thyroidal production of T_4 and T_3 in normal adult rats. The addition of these small amounts of T_3 to the infusion allowed a significant reduction in the dose of T_4 needed to normalize T_3 in the majority of tissues with respect to the dose needed when T_4 alone is infused and avoided supraphysiological T_4 concentrations.

Materials and Methods

Experimental design

Young female Wistar rats, 120–150 g BW, were surgically thyroidectomized and received 100 μCi ^{131}I, ip, 1 week later. After 28 days, rats with complete body weight stasis were divided into groups of six rats each, and osmotic minipumps (model 2ML2, Alzet Corp., Palo Alto, CA) were implanted under the dorsal skin of the animals. Rats were infused with either placebo solution or T_4 at doses of 0.80 and 0.90 μg/100 g BW·day, alone or in combination with T_3 at doses of 0.10, 0.15 and 0.20 μg/100 g BW·day. The doses of T_4 were selected on the basis of results obtained in a previous study by our group (1), in which doses ranging from 0.6–1.0 μg/100 g BW·day tended to normalize plasma and tissue T_4 concentrations, whereas higher doses resulted in elevated T_4 concentrations in plasma and most tissues. The doses of T_3 that were added to the T_4 infused into the rats were selected as to cover a wide range of T_4 to T_3 molar ratios, from 3.6:1 to 7.6:1. One group of seven nonthyroidectomized rats, matched for sex and age and infused with placebo, served as the control euthyroid group.

After 12 days of infusion, the rats were slightly anesthetized with ether, bled extensively from the abdominal aorta after injection of a small amount (30–50 μl) of heparin (0.17% in 0.9% NaCl), and perfused with 50 ml PBS (0.05 M phosphate buffer containing 0.9% NaCl, pH 7.4). Samples of plasma (Pl), cerebral cortex (Cx), cerebellum (Cb), pituitary (P), BAT, heart (H), liver (L), lung (Lu), spleen (S), kidney (K), ovary (O), adrenal (A), and skeletal muscle (musculus quadriceps femoris; M), were obtained. Samples were immediately frozen on dry ice and stored at −20 C until analyzed, with the exception of aliquots of Cx, BAT, L, and Lu, which were stored at −80 C for measurement of iodothyronine deiodinase activity.

Determinations

T_4 and T_3 were measured in whole plasma by specific and highly sensitive RIAs, as previously described (11), and in tissues after extraction and purification of the iodothyronines, as detailed previously (12, 13). In brief, methanol is added to the still frozen tissue sample and homogenized. This avoids postmortem generation of T_3 from T_4 and degradation of T_4 or T_3 (13). Tracer amounts of [^{131}I]T_4 and [^{125}I]T_3 are added to each homogenate. This is followed by extraction of more than 90% of endogenous and added iodothyronines using chloroform-methanol (2:1). The iodothyronines are then back-extracted into an aqueous phase and purified by passing this aqueous phase through Bio-Rad AG 1 × 2 resin columns. After a pH gradient, the iodothyronines are eluted with 70% acetic acid, which is then evaporated to dryness. RIA buffer is added, and each extract is extensively counted to determine the recovery of the [^{131}I]T_4 and [^{125}I]T_3 added to each sample during the initial homogenization process; recovery usually varies between 50–60% for [^{131}I]T_4 and between 60–70% for [^{125}I]T_3. The samples are submitted to highly sensitive RIAs for the determination of T_4 and T_3; the limits of sensitivity are 2.5 pg T_4 and 1.5 pg T_3/tube. Cross-reactivities of different iodothyronines and metabolites in the T_4 and T_3 RIAs were recently reported (13). Each sample is processed in duplicate or triplicate at two or more dilutions. Concentrations are then calculated using the amounts of T_4 and T_3 found in the respective RIAs, the individual recovery of the [^{131}I]T_4 and [^{125}I]T_3 added to each sample during the initial homogenization process, and the weight of the tissue sample submitted to extraction. The amounts of tracers added are such that the radioactivities carried over into the RIA tubes are too low to interfere with the determinations, representing less than 2.5% of the radioactivity added as labeled antigen. Both T_4 and T_3 concentrations of a given tissue were determined in the same extraction run and a single RIA for each hormone.

TSH was measured in plasma using immunoreactants kindly provided by the Rat Pituitary Agency of the NIDDK, NIH (Bethesda, MD), as described previously (14). Results are expressed in weight equivalents of the NIDDK rat TSH RP-3 preparation.

Type I 5′D activity (5′D-I) was assayed in L, P, and Lu homogenates as previously described (15), using 400 nM rT_3 and 2 mM dithiothreitol (DTT) for L, and 2 nM rT_3 and 20 mM DTT for P and Lu, in 100 mM potassium phosphate buffer (pH 7.0). The reaction time was 10 min for L, and 60 min for P and Lu. Virtually all activity in L, P, and Lu was propylthiouracil (PTU) sensitive. Type II 5′D activity was assayed in Cx, P, and BAT (16) using 2 nM T_4, 1 μM T_3, and 20 mM DTT in the presence of 1 mM PTU, and the reaction time was 60 min. Before each assay [^{125}I]rT_3 or [^{125}I]T_4 was purified by paper electrophoresis to separate the contaminating iodide. The ^{125}I$^-$ released was separated by ion exchange chromatography on Dowex 50W-X2 columns equilibrated in 10% acetic acid. The production of equal amounts of iodide and 3′,3-diiodothyronine was checked in some assays. The protein content was determined by the method of Lowry *et al.* (17), after precipitation of the homogenates with 10% trichloroacetic acid to avoid interferences from DTT in the colorimetric reaction.

Drugs and reagents

T_4, T_3, 3,5-diiodothyronine, PTU, and DTT were obtained from Sigma Chemical Co. (St. Louis, MO). rT_3 and 3′,3-diiodothyronine were obtained from Henning Berlin (Berlin, Germany).

High specific activity [^{131}I]T_4, [^{125}I]T_3, [^{125}I]T_4, and [^{125}I]rT_3 (3000 μCi/μg) were synthesized in our laboratory, as previously described (12), and used for highly sensitive T_4 and T_3 RIAs, as recovery tracers for plasma and tissues extractions, and as substrates for 5′D.

Statistical analysis

One-way ANOVA and protected least significant difference test for multiple comparisons were used after validation of the homogeneity of variances by the Bartlett-Box F test (18). Square root or logarithmic transformations usually ensured homogeneity of variances when this was not found with the raw data. Results are expressed as the mean ± SE. $P < 0.05$ was considered significant in all comparisons. Statistical analyses were performed with the SPSS Base System Software for the Macintosh version 4.0 (SPSS, Chicago, IL).

Results

The absolute values of plasma T_4, T_3, and TSH; tissue T_4 and T_3; and 5′D-I and 5′D-II are shown in Tables 1, 2A and 2B. The statistically significant differences with respect to normal intact control rats infused with placebo are schematically summarized in Tables 3 and 4. To facilitate the comparisons between different tissues, the results are represented in the figures as percentages of the mean value for the control group of intact rats.

Circulating and tissue T_4 and T_3 and plasma TSH concentrations

Plasma T_4 concentrations were within the normal range in all groups infused with T_4, either alone or in combination with T_3, and low in the thyroidectomized rats infused only with placebo (Table 3 and Fig. 1A). Plasma T_3 was very low in the group infused with placebo, moderately low in the groups infused with T_4 alone, and normal in the groups infused with combinations of T_4 and T_3 (Table 3 and Fig. 1A). Plasma TSH levels were very high in the thyroidectomized rats infused with placebo and remained elevated when T_4

TABLE 1. Plasma T_4, T_3, and TSH; tissue concentrations of T_4 and T_3; and 5′D activities in control and thyroidectomized rats infused with placebo

Group	Control intact rats		Thyroidectomized rats	
	T_4	T_3	T_4	T_3
Plasma	32 ± 2	0.96 ± 0.02	<5	0.16 ± 0.01
Cerebral cortex	2.44 ± 0.11	1.79 ± 0.04	0.34 ± 0.09	0.14 ± 0.02
Pituitary	60 ± 3	44 ± 4	29 ± 0	26 ± 6
Liver	35.22 ± 3.05	4.29 ± 0.10	0.31 ± 0.09	0.34 ± 0.02
Cerebellum	11.06 ± 0.90	1.70 ± 0.05	2.43 ± 0.28	0.30 ± 0.01
Heart	4.43 ± 0.18	1.59 ± 0.04	0.14 ± 0.05	0.04 ± 0.00
Lung	7.45 ± 0.52	1.69 ± 0.03	0.55 ± 0.05	0.13 ± 0.02
Kidney	19.99 ± 1.02	6.04 ± 0.22	0.65 ± 0.06	0.08 ± 0.01
Spleen	3.99 ± 0.32	1.17 ± 0.03	0.78 ± 0.17	0.11 ± 0.02
Muscle	2.31 ± 0.14	0.77 ± 0.03	0.20 ± 0.03	0.24 ± 0.14
Adrenal	6.91 ± 0.76	1.34 ± 0.05	3.58 ± 0.64	0.64 ± 0.02
Ovary	6.19 ± 0.29	0.77 ± 0.07	0.75 ± 0.11	0.21 ± 0.01
BAT	5.49 ± 0.47	2.37 ± 0.14	0.59 ± 0.10	0.30 ± 0.01
Plasma TSH	0.59 ± 0.07		22.29 ± 3.79	
Cortex 5′D-II	20 ± 2		196 ± 96	
Pituitary 5′D-I	3881 ± 198		848 ± 149	
Pituitary 5′D-II	587 ± 25		1496 ± 31	
Liver 5′D-I	51 ± 1		11 ± 0	
Lung 5′D-I	493 ± 54		131 ± 31	
BAT 5′D-II	137 ± 21		430 ± 33	

Values are expressed as the mean ± SE. The following units have been used: plasma T_4, T_3, and TSH, nanograms per ml; tissue T_4 and T_3, nanograms per g, with the exception of pituitary T_4 and T_3, which are expressed as picograms per gland. The activities of 5′D are expressed as femtomoles per h/mg protein, with the exception of liver type I 5′D, which is expressed as picomoles per min/mg protein. All differences between control and thyroidectomized rats infused with placebo were statistically significant.

was infused alone or in combination with the smaller doses of T_3. It reached normal levels only in the groups infused with the combinations of 0.8 μg T_4 plus 0.20 μg T_3/100 g BW·day, 0.9 μg T_4 plus 0.15 μg T_3/100 g BW·day, and 0.9 μg T_4 plus 0.20 μg T_3/100 g BW·day (Table 3 and Fig. 1B).

The changes in tissue T_4 and T_3 were similar to those described for the circulating concentrations of iodothyronines. As a rule, T_4 reached normal or near-normal concentrations in all tissues of the groups infused with T_4, whether alone or in combination with T_3 (Table 3 and Figs. 2 and 3). On the contrary, T_3 levels were low in most tissues when T_4 was infused alone, although they were significantly higher than those in placebo-infused thyroidectomized rats. The addition of T_3 in the doses used here was effective in normalizing tissue T_3 concentrations (Table 3 and Figs. 2 and 3). The combination of 0.9 μg T_4 and 0.15 μg T_3/100 g BW·day was especially effective, as it resulted in normal levels of plasma T_4, T_3, and TSH, and normal concentrations of T_4 and T_3 in all the tissues studied (Table 3).

Although T_3 concentrations only became normal in most tissues when the infusion of T_4 was combined with that of T_3, in the case of Cx, BAT, Cb, and A, normal T_3 levels were also reached with T_4 alone with the dose of 0.8 μg or 0.9 mg T_4/100 g BW·day, or both (Table 3 and Fig. 2). This observation confirms the previous results (1) that, in contrast with other tissues, normal concentrations of T_3 in Cx, Cb, and BAT are reached even with the relatively low T_4 doses used here regardless of whether T_3 is also infused.

The molar T_3 to T_4 ratios in plasma and tissues are summarized in Table 5A and 5B. The combinations leading to normalization of the T_3 to T_4 ratios in the majority of tissues were 0.8 μg T_4 and 0.15 μg T_3/100 g BW·day, and 0.9 μg T_4 and 0.15 μg T_3/100 g BW·day. The latter would appear to be the combination of choice, when normalization of circulating TSH and of T_4 and T_3 in plasma and all tissues are also taken into consideration (Table 3).

Visual inspection of Figs. 1–3 shows that the pattern of changes in the concentrations of T_4 in all of the tissues studied resembles that observed for circulating T_4. The pattern of changes observed for T_3 concentrations in many tissues resembles that observed for circulating T_3, with the exception of Cx, Cb, and BAT, for which the changes were more similar to those in plasma T_4.

Type I and II 5′D activities in several tissues

The 5′D activities of several tissues are summarized in Table 4 and Fig. 4. Cerebral cortex 5′D-II activity was elevated in the group of thyroidectomized rats infused with placebo and normal in all groups infused with T_4, either alone or in combination with T_3. The 5′D-II activity of BAT was elevated in the group of thyroidectomized rats infused with placebo, normal in the groups infused with T_4 alone, and variable in the groups infused with T_4 plus T_3. The elevation of BAT 5′D-II activity found in some of the groups infused with combinations of T_4 and T_3 grossly paralleled the evolution of BAT T_3 concentrations (Figs. 2 and 4).

Pituitary 5′D-II activity was elevated in thyroidectomized rats infused with placebo and T_4 alone, and only decreased to normal in the groups infused with 0.8 μg T_4 plus 0.20 μg T_3/100 g BW·day, 0.9 μg T_4 plus 0.15 μg T_3/100 g BW·day, and 0.9 μg T_4 plus 0.20 μg T_3/100 g BW·day (Table 4 and Fig. 4). The changes in pituitary 5′D-II activity resembled those in circulating TSH in the same groups, but no concordance was found with the changes in plasma T_4 and T_3 (Table 3 and Fig. 1). In contrast, pituitary 5′D-I activity was low in thyroidectomized rats infused with placebo and T_4 alone, and only became normal in the groups infused with 0.8 μg T_4 plus

TABLE 2A. Plasma T_4, T_3, and TSH; tissue concentrations of T_4 and T_3; and 5′D activities in thyroidectomized rats infused with 0.8 μg T_4/100 g · day, alone or in combination with T_3

Dose of T_4: / Dose of T_3:	0.80 μg/100 g · day / 0.00 μg/100 g · day		0.80 μg/100 g · day / 0.10 μg/100 g · day		0.80 μg/100 g · day / 0.15 μg/100 g · day		0.80 μg/100 g · day / 0.20 μg/100 g · day	
	T_4	T_3	T_4	T_3	T_4	T_3	T_4	T_3
Plasma	36 ± 2	0.59 ± 0.04	35 ± 1	0.83 ± 0.04	36 ± 5	0.90 ± 0.06	32 ± 2	1.10 ± 0.03
Cerebral cortex	2.46 ± 0.13	1.68 ± 0.09	2.28 ± 0.13	2.05 ± 0.07	2.04 ± 0.07	2.06 ± 0.05	2.32 ± 0.20	2.47 ± 0.17
Pituitary	54 ± 3	34 ± 1	61 ± 1	64 ± 2	60 ± 2	51 ± 3	62 ± 2	47 ± 4
Liver	28.20 ± 1.09	2.62 ± 0.13	29.99 ± 1.06	4.20 ± 0.13	28.12 ± 4.06	4.12 ± 0.22	27.92 ± 1.97	3.72 ± 0.43
Cerebellum	11.15 ± 0.36	1.51 ± 0.02	10.48 ± 0.36	1.59 ± 0.03	10.25 ± 0.68	1.69 ± 0.07	10.11 ± 0.62	1.79 ± 0.07
Heart	4.46 ± 0.10	0.69 ± 0.08	4.97 ± 0.24	1.17 ± 0.11	4.19 ± 0.44	1.31 ± 0.02	3.46 ± 0.23	1.51 ± 0.14
Lung	8.33 ± 0.23	0.94 ± 0.06	6.60 ± 0.12	1.42 ± 0.10	6.92 ± 0.83	1.53 ± 0.07	5.73 ± 0.28	1.61 ± 0.13
Kidney	18.54 ± 0.50	3.48 ± 0.17	20.61 ± 0.55	6.91 ± 0.90	19.19 ± 1.06	5.62 ± 0.19	20.37 ± 0.96	5.63 ± 0.57
Spleen	4.04 ± 0.16	0.77 ± 0.03	4.25 ± 0.27	1.17 ± 0.06	4.35 ± 0.28	1.00 ± 0.02	4.07 ± 0.20	1.15 ± 0.08
Muscle	1.95 ± 0.15	0.38 ± 0.02	2.60 ± 0.14	0.75 ± 0.03	2.30 ± 0.17	0.79 ± 0.13	1.85 ± 0.06	1.65 ± 0.40
Adrenal	8.58 ± 0.88	0.87 ± 0.05	4.43 ± 0.65	1.58 ± 0.43	4.64 ± 0.14	1.55 ± 0.08	3.91 ± 0.40	1.42 ± 0.26
Ovary	6.27 ± 0.33	0.40 ± 0.03	5.61 ± 0.26	0.57 ± 0.03	6.04 ± 0.39	0.64 ± 0.11	5.40 ± 0.35	0.67 ± 0.09
BAT	6.89 ± 0.43	1.92 ± 0.12	7.42 ± 1.20	2.42 ± 0.05	5.65 ± 0.42	2.16 ± 0.11	5.21 ± 0.75	2.03 ± 0.21
Plasma TSH	9.09 ± 1.79		2.74 ± 0.23		3.21 ± 1.02		1.24 ± 0.26	
Cortex 5′D-II	17 ± 1		24 ± 3		34 ± 4		27 ± 1	
Pituitary 5′D-I	1724 ± 21		2772 ± 244		3163 ± 307		2891 ± 270	
Pituitary 5′D-II	826 ± 31		864 ± 54		1059 ± 27		727 ± 78	
Liver 5′D-I	30 ± 2		37 ± 2		45 ± 2		63 ± 3	
Lung 5′D-I	303 ± 18		311 ± 59		418 ± 38		482 ± 16	
BAT 5′D-II	179 ± 38		251 ± 28		152 ± 14		128 ± 16	

Values are expressed as the mean ± SE. The following units have been used: plasma T_4, T_3, and TSH, nanograms per ml; tissue T_4 and T_3 nanograms per g, with the exception of pituitary T_4 and T_3, which are expressed as picograms per gland. The activities of 5′D are expressed as femtomoles per h/mg protein, with the exception of liver type I 5′D, which is expressed as picomoles per min/mg protein. The statistical differences between control and thyroid hormone-infused rats are presented in Tables 3 and 4.

TABLE 2B. Plasma T_4, T_3, and TSH: tissue concentrations of T_4 and T_3; and 5′D activities in thyroidectomized rats infused with 0.90 μg T_4/100 g · day, alone or in combination with T_3

Dose of T_4: / Dose of T_3:	0.90 μg/100 g · day / 0.00 μg/100 g · day		0.90 μg/100 g · day / 0.10 μg/100 g · day		0.90 μg/100 g · day / 0.15 μg/100 g · day		0.90 μg/100 g · day / 0.20 μg/100 g · day	
	T_4	T_3	T_4	T_3	T_4	T_3	T_4	T_3
Plasma	36 ± 2	0.60 ± 0.03	32 ± 3	0.87 ± 0.06	38 ± 2	0.97 ± 0.05	29 ± 1	1.11 ± 0.02
Cerebral cortex	2.49 ± 0.04	1.85 ± 0.02	2.53 ± 0.18	2.11 ± 0.15	2.36 ± 0.14	1.90 ± 0.07	2.21 ± 0.06	2.084 ± 0.10
Pituitary	61 ± 5	29 ± 2	68 ± 5	39 ± 2	52 ± 7	43 ± 2	54 ± 3	52 ± 3
Liver	29.03 ± 2.62	2.60 ± 0.08	25.07 ± 2.44	3.58 ± 0.30	28.59 ± 1.94	3.85 ± 0.18	30.37 ± 1.41	4.02 ± 0.11
Cerebellum	9.93 ± 0.30	1.34 ± 0.06	10.93 ± 0.30	1.66 ± 0.09	11.96 ± 0.83	1.80 ± 0.11	10.86 ± 0.30	1.79 ± 0.04
Heart	4.67 ± 0.40	0.77 ± 0.05	3.98 ± 0.23	1.29 ± 0.13	4.71 ± 0.12	1.53 ± 0.11	4.57 ± 0.27	1.77 ± 0.09
Lung	8.34 ± 0.33	0.85 ± 0.08	7.98 ± 0.14	1.32 ± 0.06	6.98 ± 0.26	1.66 ± 0.03	7.97 ± 0.24	1.77 ± 0.09
Kidney	19.74 ± 0.72	2.43 ± 0.11	19.56 ± 0.43	4.14 ± 0.08	18.27 ± 0.53	5.41 ± 0.38	19.19 ± 1.22	5.27 ± 0.52
Spleen	4.18 ± 0.04	0.62 ± 0.04	3.81 ± 0.15	1.06 ± 0.08	4.12 ± 0.27	1.11 ± 0.04	3.48 ± 0.10	1.14 ± 0.05
Muscle	2.55 ± 0.22	0.37 ± 0.03	2.20 ± 0.11	1.22 ± 0.42	2.39 ± 0.15	1.28 ± 0.30	2.01 ± 0.27	1.11 ± 0.11
Adrenal	6.31 ± 0.20	0.90 ± 0.08	8.88 ± 0.42	1.89 ± 0.34	7.19 ± 0.56	1.64 ± 0.13	6.79 ± 0.53	2.43 ± 0.48
Ovary	6.81 ± 0.30	0.33 ± 0.03	7.02 ± 0.36	0.71 ± 0.15	6.02 ± 0.23	0.97 ± 0.07	5.10 ± 0.22	0.60 ± 0.05
BAT	6.96 ± 0.26	1.61 ± 0.26	6.10 ± 0.43	3.35 ± 0.32	6.97 ± 0.57	1.89 ± 0.53	5.50 ± 0.40	2.79 ± 0.18
Plasma TSH	9.25 ± 1.68		2.65 ± 0.83		0.61 ± 0.11		0.79 ± 0.21	
Cortex 5′D-II	27 ± 4		29 ± 1		17 ± 2		28 ± 4	
Pituitary 5′D-I	2469 ± 344		2813 ± 340		2807 ± 137		3461 ± 170	
Pituitary 5′D-II	1317 ± 106		850 ± 48		530 ± 45		565 ± 62	
Liver 5′D-I	30 ± 1		53 ± 3		66 ± 4		50 ± 2	
Lung 5′D-I	373 ± 44		509 ± 24		443 ± 49		407 ± 48	
BAT 5′D-II	221 ± 35		363 ± 22		148 ± 48		318 ± 15	

Values are expressed as the mean ± SE. The following units have been used: plasma T_4, T_3, and TSH: nanograms per ml; tissue T_4 and T_3 nanograms per g, with the exception of pituitary T_4 and T_3, which are expressed as picograms per gland. The activities of 5′D are expressed as femtomoles per h/mg protein, with the exception of liver type I 5′D, which is expressed as picomoles per min/mg protein. The statistical differences between control and thyroid hormone-infused rats are presented in Tables 3 and 4.

0.15 μg T_3/100 g BW·day, 0.8 μg T_4 plus 0.20 μg T_3/100 g BW·day, and 0.9 μg T_4 plus 0.20 μg T_3/100 g BW·day (Table 4 and Fig. 4), showing no clear concordance with either plasma TSH, T_4, and T_3 or pituitary T_4 and T_3 contents.

Liver and lung 5′D-I activities were low in thyroidectomized rats infused with placebo and T_4 alone (Tables 3 and 4 and Figs. 1 and 3). Although liver 5′D-I activity changed irregularly depending on the combination of T_4 and T_3 infused, showing elevated activities in the groups infused with 0.8 μg T_4 plus 0.20 μg T_3/100 g BW·day, and 0.9 μg T_4 plus 0.15 μg T_3/100 g BW·day, lung 5′D-I activity paralleled the changes in plasma T_3 with the exception of low activity in the

TABLE 3. Schematic representation of the changes with respect to intact control rats in the plasma concentrations of T_4, T_3, and TSH, and tissue levels of T_4 and T_3 in thyroidectomized rats infused with different doses of T_4, alone or in combination with different doses of T_3 (micrograms per 100 g BW/day)

Dose of T_4:	0.80			0.80			0.80			0.80			0.90			0.90			0.90			0.90		
Dose of T_3:				0.10			0.15			0.20						0.10			0.15			0.20		
Determination:	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH
Plasma	=	▼	▲	=	=	▲	=	=	▲	=	=	=	=	▼	▲	=	=	▲	=	=	=[a]	=	=	=
Cerebral cortex	=	=[a]	=	=	=	=		=	▲		=	=[a]		=	▽		=	=[a]		=	=			
Pituitary	=	▽		=	▽		=	=[a]		=	=[a]		=	▼		=	=[a]		=	=		=	=	
Liver	=	▼		=	=[a]		▼	=		=	=[a]		=	▼		▽	▽		=	=[a]		=	=[a]	
Cerebellum	=	=[a]		=	=[a]		=	=[a]		=	=[a]		=	▽		=	=[a]		=	=[a]		=	=[a]	
Heart	=	▼		=	▽		=	=[a]		▽	=		=	▼		=	▽		–	=[a]		=	=[a]	
Lung	=	▼		=	▽		=	=[a]		▽	=		=	▼		=	▽		=	=[a]		=	=[a]	
Kidney	=	▼		=	=[a]		=	=[a]		=	=[a]		=	▼		=	▼		=	=[a]		=	=[a]	
Spleen	=	▼		=	=[a]		=	▽		=	=[a]		=	▼		=	=[a]		=	=		=	=[a]	
Muscle	=	▼		=	=[a]		=	=[a]		=	▲		=	▼		=	=[a]		=	=[a]		=	=[a]	
Adrenal	▽	▼		▼	=		▼	=		▼	=		=	=[a]		▽	=		=	=[a]		=	▲	
Ovary	=	▼		=	=[a]		=	=[a]		=	=[a]		=	▼		=	=[a]		=	=		▽	=	
BAT	=	=[a]		▲	=		=	=[a]		=	=[a]		=	▼		=	▲		=	=		=	=	

The symbols represent the comparison of the mean values of the groups infused with thyroid hormones with the mean values of the control group: =, no statistically significant difference; ▼, a decrease; ▲, an increase (compared to controls $P < 0.05$). Open *arrows* are used when the change with respect to controls is relatively small (within ±30% of the mean of the control group).

[a] In addition to normal T_4 and T_3 concentrations, the molar T_3/T_4 ratio was not different from that in the control group.

TABLE 4. Schematic representation of 5′D activity in thyroidectomized rats infused with different doses of T_4, alone or in combination with different doses of T_3 (micrograms per 100 g/day), with respect to age- and sex-matched controls

Dose of T_4:	0.80		0.80		0.80		0.80		0.90		0.90		0.90		0.90	
Dose of T_3:			0.10		0.15		0.20				0.10		0.15		0.20	
	5′D-I	5′D-II	5′D-I	5′D-II	5′D-I	5′D-II	5′D-I	5′D-II	5′D-I	5′D-II	5′D-I	5′D-II	5′D-I	5′D-II	5′D-I	5′D-II
Cerebral cortex		=		=		=		=		=		=		=		=
BAT		=		▲		=		=		=		▲		=		▲
Pituitary	▼	▲	▽	▲	=	▲	=	=	▼	▲	▽	▲	▽	=	=	=
Liver	▼		▽		=		▽		▼		=		▽		=	
Lung	▼		▼		=		=		▽		=		=		=	

The symbols represent the comparison of the mean values of the groups infused with thyroid hormones with the mean values of the control group; their meaning is explained in Table 3.

group infused with 0.8 μg T_4 plus 0.10 μg T_3/100 g BW·day (Tables 3 and 4 and Figs. 1 and 3).

Discussion

The experimental design

The aim of a hormonal replacement therapy is to ensure an adequate supply of the missing hormone in a manner that mimics the normal supply as closely as possible and results in normal biological effects, both qualitatively and quantitatively. When the thyroid gland is absent or not capable of synthesizing or secreting T_4 and T_3 in humans, treatment with T_4 alone is preferred over that with T_3 alone because the former is the main secretory product of the gland and generates T_3 in many tissues, some of which are a source of systemic T_3 distributed throughout the body by the bloodstream. However, as thyroidal secretion of T_3 is still missing when this therapeutic approach is used, combined T_4 and T_3 treatment seems to be a more physiological approach.

The present experimental design was based on results previously obtained in thyroidectomized rats on replacement therapy with T_4 or T_3 alone (1, 5). In the rat it is difficult to quantify doses given orally, either in food or drinking water, and we have, therefore, used continuous sc infusion of the iodothyronines as the route of administration of choice. Compared to intermittent ip or iv injections, continuous sc infusion avoids the wide daily fluctuations in the plasma and tissue concentrations of both T_4 and T_3 (19), as well as fluctuations in a biological end point of action, such as circulating TSH (20), which have been described with intermittent injections of iodothyronines. Moreover, the use of osmotic minipumps does not require restraint of the animals, thus allowing free access to food and water. The period of infusion in our study was 12 days, which is 17 times the mean residence time of T_4 in adult rats (17.4 h) (21). Previous studies by others (22, 23) have shown that adult rats receiving a constant infusion of radiolabeled T_4 are in equilibrium by 6 (euthyroid rats) or 8 (hypothyroid rats) days of infusion, when the amounts of labeled metabolites excreted daily into the feces and urine become constant and their sum is equivalent to the amount of radioactivity infused daily. The corresponding periods are 3 and 8 days, respectively, when labeled T_3 is infused. In the present study infusion into hypothyroid animals of T_4, with or without T_3, was, therefore, extended beyond the period when restoration to euthyroidism is accompanied by changes in thyroid hormone metabolism. Obviously, with this mode of administration diurnal rhythms of circulating T_4 and T_3, which might be dependent on the circadian variations in TSH (24), would be abolished. Those rhythms that are caused by intra-

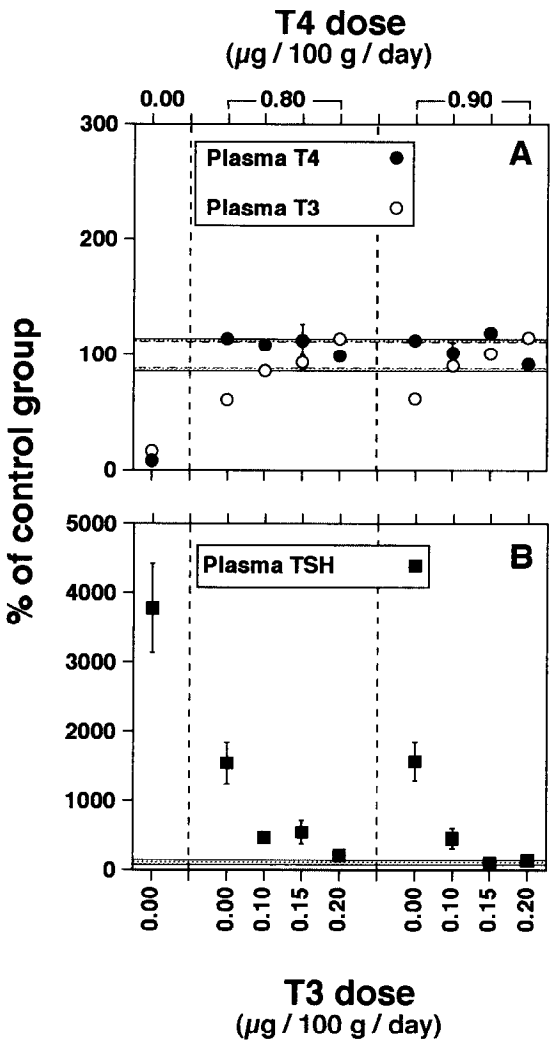

FIG. 1. A, Changes in plasma T_4 and T_3 concentration (*full and empty circles*, respectively) in thyroidectomized rats infused with placebo, T_4 alone, or T_4 in combination with T_3 as a function of the doses of T_4 and T_3. Values shown are means (±SE) and are expressed as percentages of the mean value found for control intact animals. The *areas enclosed by horizontal lines* represent the 95% confidence intervals for plasma T_4 (*full lines, dotted area*) or T_3 (*dotted lines, white area*) in intact control rats. B, Corresponding changes in circulating TSH, superimposed on levels in normal controls (*horizontal dotted area*).

cellular events, such as those described for deiodinase activities in several areas of the rat brain (25) or the pineal gland (26), might, however, still be operative.

The two doses of T_4 used for the present study were selected from the observation (1) that when T_4 alone is infused, doses ranging from 0.6–1.0 μg/100 g BW·day tended to normalize plasma and tissue T_4 concentrations, whereas higher doses resulted in supraphysiological T_4 concentrations in plasma and most tissues. The doses of T_3 that were added to the T_4 infusion were selected so as to cover a wide range of T_4 to T_3 molar ratios, from 3.6:1 up to 7.6:1. This ought to include the value of 5.7 ± 0.5:1 assessed in five different groups of normal adult male rats (6–9), and the single value of 6.5:1 reported for normal adult female rats (10). Also, all doses of T_3 tested here were lower than that needed to normalize T_3 in plasma and most tissues when T_3 alone was infused (5).

Maintenance of euthyroidism in tissues

Several criteria might be considered for defining euthyroidism, or normal thyroidal status, of a tissue. One criterion might be that the biological effects of thyroid hormones are qualitatively and quantitatively the same as those in tissues from normal animals; another could be that normal concentrations of thyroid hormones are provided to the tissue. The latter criterion assumes that normalization of the iodothyronine content of the tissue would be followed by normalization of the biological effects. It is at present very difficult to apply the first of these two criteria, because of the paucity of biological end point that we can attribute to direct local effects of the thyroid hormones in different tissues. At present, we have to rely on the second criteria for euthyroidism, which encloses two possibilities, namely 1) that both T_4 and T_3 have to be normal; or 2) that it is enough to ensure normal T_3 concentrations to elicit qualitatively and quantitatively normal biological end points. We have shown that using T_4 or T_3 alone it is not possible to meet either one of these two possibilities simultaneously for plasma and tissues (1, 5) despite the wide range of doses used for the studies (from 0.2–8.0 μg T_4/100 g BW·day, and 0.25–2.0 μg T_3/100 g BW·day). Moreover, when infusing T_4 alone, supraphysiological T_4 concentrations have to be reached in most tissues to normalize their T_3 concentrations, and this occurs at different T_4 doses for different tissues (1). When using T_3 alone, T_4 concentrations in plasma and tissues are always very low, and supraphysiological T_3 concentrations have to be reached in the circulation to normalize T_3 levels in many tissues (5).

The present data, on the contrary, show that the combined infusion of appropriate amounts of T_4 plus T_3 is able to completely restore euthyroidism simultaneously in all tissues of thyroidectomized rats. This demonstration is not only based on the restoration of both T_4 and T_3 concentrations in plasma and tissues, but also on the normalization of some of their biological effects, as assessed by plasma TSH levels, and 5′D activities in some tissues.

As assessed from plasma TSH, 1.6 μg T_4/100 g BW·day are needed to decrease to normal the elevated TSH levels in thyroidectomized rats (1). The T_4 doses used here (0.8 or 0.9 μg/100 g BW·day are inadequate despite the fact that all of the animals had normal plasma T_4 levels. In these conditions, the normalization of circulating TSH was clearly related to the dose of T_3 infused together with T_4 and the resulting circulating T_3 levels, in agreement with a previous study by Emerson *et al.* (27).

The addition of the small doses of T_3 used here decreases the amount of T_4 (0.8–0.9 μg/100 g BW·day) needed to normalize T_3 in the majority of tissues by about 50% compared to the amount (1.6–2.0 μg/100 g BW·day) necessary when T_4 alone is used (1). A possible explanation for this finding is that 5′D-I activity, which is low in hypothyroid animals, is regulated by T_3 (28). As a consequence, when hypothyroid animals are infused with T_4 alone, plasma T_3 is low, and doses of 1.6–2 μg T_4/100 g BW·day are needed to normalize it, as most of systemic T_3 is contributed by tissues with 5′D-I activity. When T_3 is infused in the doses reported

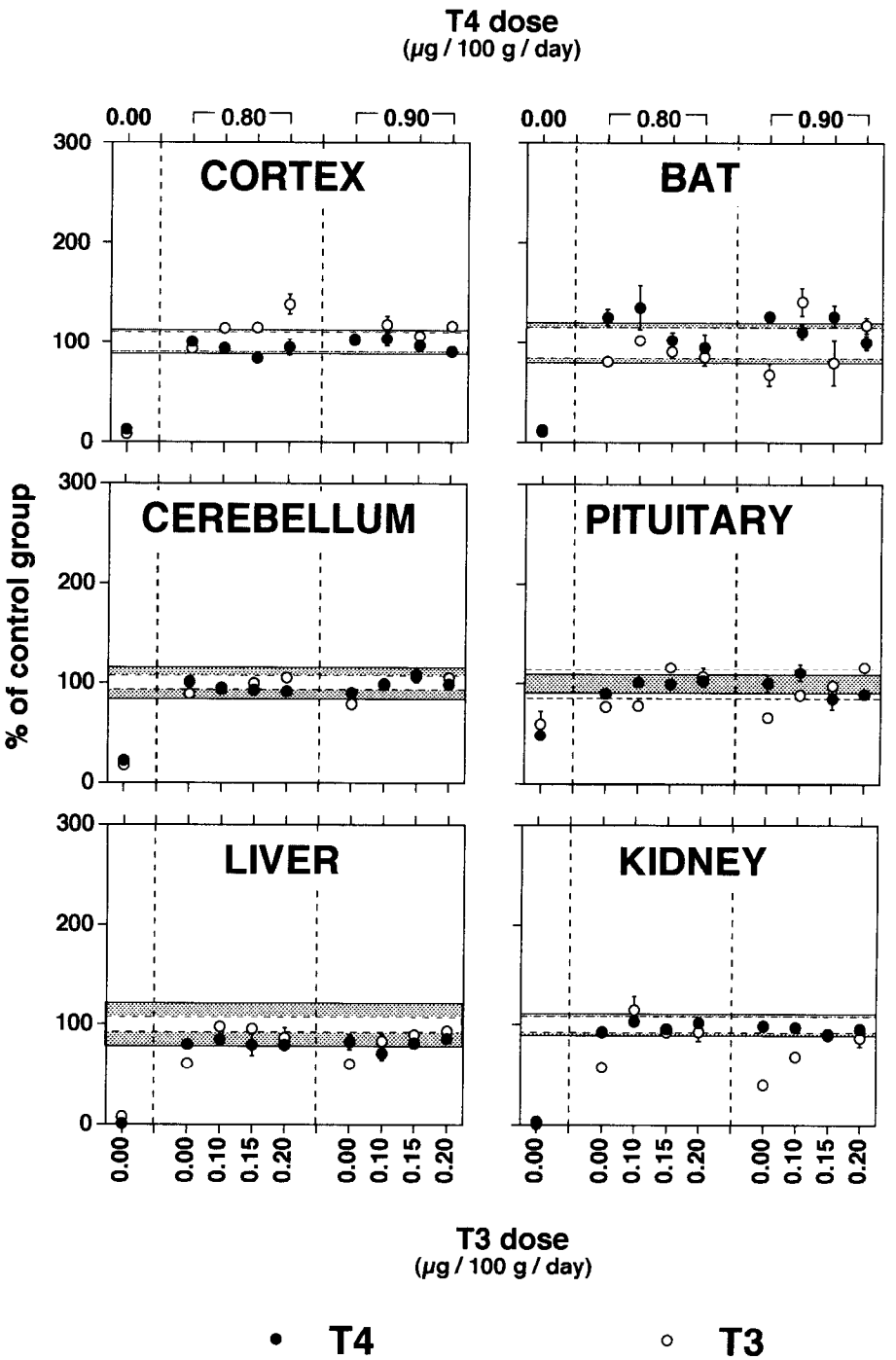

FIG. 2. The changes in concentrations of T_4 (*full circles*) and T_3 (*empty circles*) in different tissues of thyroidectomized rats infused with placebo, T_4 alone, or T_4 in combination with T_3 are shown as function of the doses of T_4 and T_3. The expression of data and the meaning of *dotted and white areas* are explained in Fig. 1A.

here, 5'D-I activity increases rapidly (5). Addition of T_3 to a dose of T_4 thus enhances the formation of T_3 generated from T_4 in 5'D-I-containing tissues and, as a consequence, contributes to systemic T_3 to a greater degree than does the same dose of T_4 alone. The T_3 generated from T_4 would also contribute to a further increase in 5'D-I activity, so that more T_3 would be generated from the same amount of T_4. Because of these mutually potentiating effects, much less T_4 would be necessary for normal T_3 levels to be reached in most tissues.

Moreover, with the present approach, T_4 concentrations are not increased above normal values in any of the tissues studied. This appears desirable, as we do not know whether long term adverse effects might result from chronically elevated intracellular T_4 concentrations or from permanent stimulation or suppression of iodothyronine deiodinases. With the combination of 0.9 μg T_4 and 0.15 μg T_3/100 g BW·day, overstimulation of TSH secretion and of the compensatory mechanisms needed to convert T_4 into T_3 is no longer necessary to ensure euthyroidism in all tissues.

Of the different combinations tested in the present study, the most effective in restoring euthyroidism in thyroidectomized rats has been 0.9 μg T_4 plus 0.15 μg T_3/100 g BW·day,

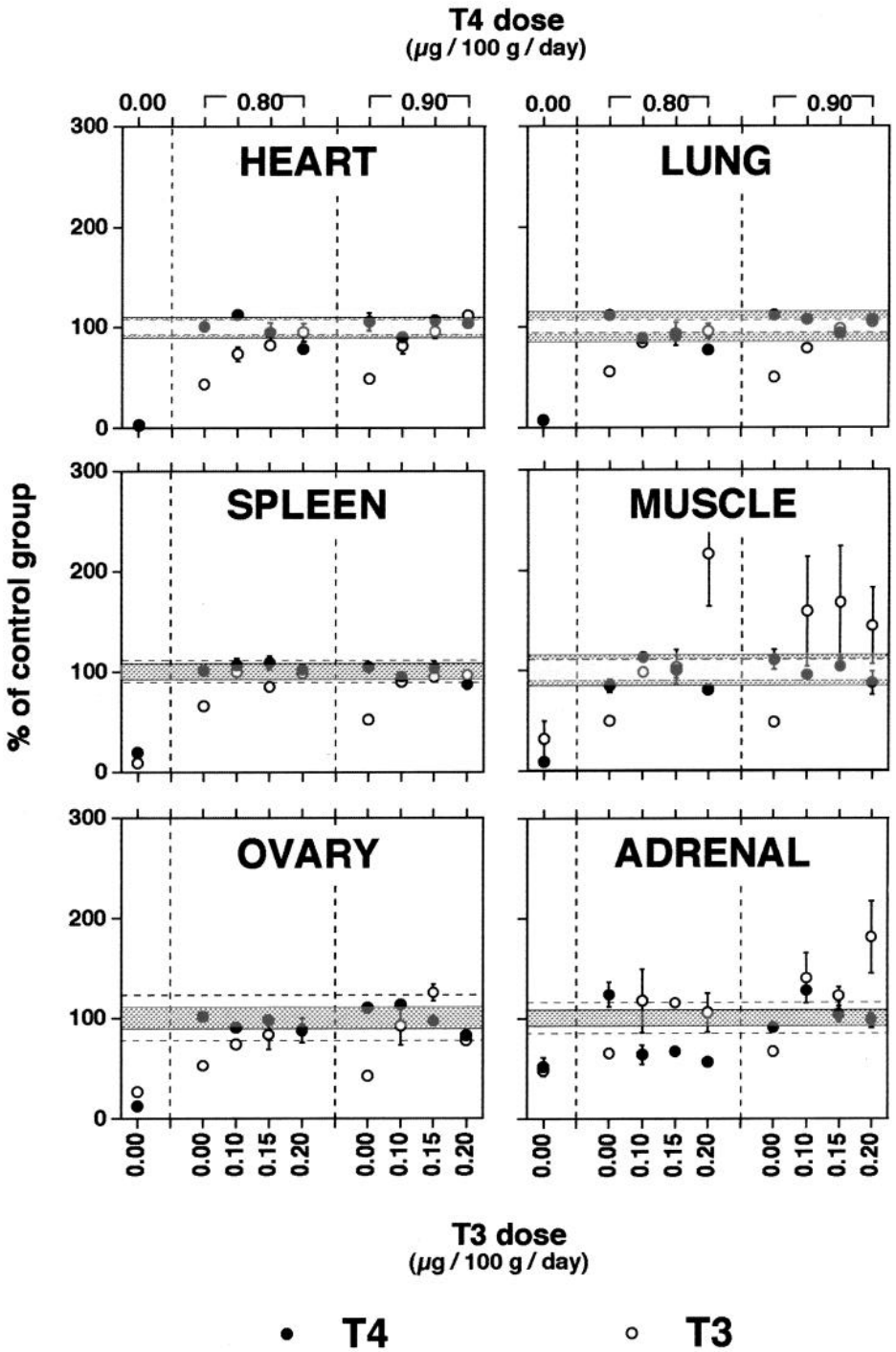

FIG. 3. The changes in concentrations of T_4 (*full circles*) and T_3 (*empty circles*) in different tissues of thyroidectomized rats infused with placebo, T_4 alone, or T_4 in combination with T_3 are shown as function of the doses of T_4 and T_3. The expression of data and the meaning of *dotted and white areas* are explained in Fig. 1A.

in which T_4 and T_3 are in a 5.0:1 molar ratio, similar to that present in the normal thyroidal secretion of the rat, which is approximately 5.7 ± 0.5:1, as cited above (6–9).

The amounts of both T_4 and T_3 infused daily with the above combination into the thyroidectomized rats of the present study are somewhat higher than values reported for the daily thyroidal production rates in adult rats. The daily T_4 production rate, as assessed by isotopic equilibrium with labeled T_4 infused iv, has been reported to be 0.68 ± 0.23 μg T_4/100 g BW (range, 0.63–0.76) for male rats (6–9) and 0.73 ± 0.05 μg T_4/100 g BW (range, 0.73–0.87) for females (10, 29, 30). The average value derived by pulse kinetics after iv injection of a single tracer dose of labeled T_4 is quite similar (0.75 μg T_4/100 g BW) (21). There are fewer studies reporting the thyroidal secretion rate of T_3, as most studies did not determine the T_3 secreted by the gland but, rather, the total T_3 production rate, which also includes T_3 generated from T_4 in peripheral tissues. The values reported to date from one laboratory for the daily thyroidal secretion rate are 0.10 ± 0.09 μg T_3/100 g BW (range, 0.08–0.14) for males (6–9) and 0.09 μg T_3/100 g BW for females (10), which represent about 37% of the total T_3 production (0.28 ± 0.01 μg T_3/100 g BW).

TABLE 5A. Molar T_3/T_4 ratios in thyroidectomized rats infused with 0.80 μg T_4/100 g · day, alone or in combination with T_3

	Control intact rats	Thyroidectomized rats			
Dose of T_4:	Placebo	0.80 μg/100 g · day	0.80 μg/100 g · day	0.80 μg/100 g · day	0.80 μg/100 g · day
Dose of T_3:	Placebo	0.00 μg/100 g · day	0.10 μg/100 g · day	0.15 μg/100 g · day	0.20 μg/100 g · day
Plasma	0.035 ± 0.001	0.019 ± 0.000 ▼	0.027 ± 0.001 =	0.032 ± 0.004 =	0.042 ± 0.003 ▲
Cerebral cortex	0.855 ± 0.050	0.824 ± 0.061 =	1.084 ± 0.071 ▲	1.232 ± 0.093 ▲	1.337 ± 0.062 ▲
Pituitary	0.830 ± 0.094	0.710 ± 0.055 =	0.734 ± 0.050 =	1.036 ± 0.041 =	0.911 ± 0.098 =
Liver	0.157 ± 0.012	0.112 ± 0.006 =	0.176 ± 0.012 =	0.195 ± 0.029 =	0.160 ± 0.002 =
Cerebellum	0.187 ± 0.011	0.163 ± 0.009 =	0.173 ± 0.002 =	0.203 ± 0.024 =	0.179 ± 0.022 =
Heart	0.444 ± 0.020	0.175 ± 0.025 ▼	0.306 ± 0.028 ▼	0.362 ± 0.030 =	0.482 ± 0.022 =
Lung	0.283 ± 0.024	0.135 ± 0.005 ▼	0.252 ± 0.021 =	0.296 ± 0.020 =	0.334 ± 0.015 ◇
Kidney	0.366 ± 0.026	0.209 ± 0.003 ▼	0.453 ± 0.086 =	0.343 ± 0.017 =	0.348 ± 0.021 =
Spleen	0.384 ± 0.029	0.213 ± 0.005 ▼	0.347 ± 0.017 =	0.290 ± 0.022 ◇	0.379 ± 0.010 =
Muscle	0.445 ± 0.033	0.216 ± 0.023 =	0.327 ± 0.017 =	0.462 ± 0.056 =	1.251 ± 0.310 ▲
Adrenal	0.286 ± 0.053	0.140 ± 0.014 ▼	0.400 ± 0.057 =	0.389 ± 0.053 =	0.471 ± 0.071 ▲
Ovary	0.137 ± 0.009	0.082 ± 0.009 =	0.122 ± 0.011 =	0.168 ± 0.019 =	0.152 ± 0.023 =
BAT	0.436 ± 0.030	0.340 ± 0.033 =	0.469 ± 0.030 =	0.510 ± 0.045 =	0.444 ± 0.057 =

Molar ratios were calculated using a mol wt of 652 g for T_3 and 777 g for T_4. The symbols represent the comparison of the mean values of the groups infused with thyroid hormones with the mean values of the control group; they are explained in Table 3. The T_3: T_4 ratio for the placebo-infused thyroidectomized was not calculated, as the concentrations of both iodothyronines were very low often, near the detection limits, and small variations could lead to spurious differences.

TABLE 5B. Molar T_3/T_4 ratios in thyroidectomized rats infused with 0.90 μg T_4/100 g · day, alone or in combination with T_3

	Control intact rats	Thyroidectomized rats			
Dose of T_4:	Placebo	0.90 μg/100 g · day	0.90 μg/100 g · day	0.90 μg/100 g · day	0.90 μg/100 g · day
Dose of T_3:	Placebo	0.00 μg/100 g · day	0.10 μg/100 g · day	0.15 μg/100 g · day	0.20 μg/100 g · day
Plasma	0.035 ± 0.001	0.021 ± 0.001 ▼	0.030 ± 0.002 =	0.031 ± 0.002 =	0.044 ± 0.003 ◇
Cerebral cortex	0.855 ± 0.050	0.887 ± 0.007 =	0.905 ± 0.027 =	1.008 ± 0.028 =	1.194 ± 0.074 ▲
Pituitary	0.830 ± 0.094	0.551 ± 0.026 ▼	0.715 ± 0.084 =	1.097 ± 0.171 ▲	1.086 ± 0.047 ▲
Liver	0.157 ± 0.012	0.111 ± 0.010 =	0.156 ± 0.016 =	0.167 ± 0.011 =	0.159 ± 0.006 =
Cerebellum	0.187 ± 0.011	0.168 ± 0.014 =	0.193 ± 0.016 =	0.160 ± 0.015 =	0.197 ± 0.005 =
Heart	0.444 ± 0.020	0.208 ± 0.029 ▼	0.388 ± 0.053 =	0.400 ± 0.049 =	0.471 ± 0.025 =
Lung	0.283 ± 0.024	0.108 ± 0.009 ▼	0.192 ± 0.012 ▼	0.264 ± 0.016 =	0.271 ± 0.018 =
Kidney	0.366 ± 0.026	0.143 ± 0.008 ▼	0.253 ± 0.004 =	0.340 ± 0.007 =	0.367 ± 0.032 =
Spleen	0.384 ± 0.029	0.186 ± 0.008 ▼	0.332 ± 0.024 =	0.316 ± 0.020 ◇	0.396 ± 0.022 =
Muscle	0.445 ± 0.033	0.139 ± 0.020 –	0.661 ± 0.218 =	0.626 ± 0.128 =	0.650 ± 0.068 =
Adrenal	0.286 ± 0.053	0.170 ± 0.011 =	0.282 ± 0.054 =	0.322 ± 0.052 =	0.362 ± 0.030 =
Ovary	0.137 ± 0.009	0.055 ± 0.003 ▼	0.105 ± 0.011 =	0.193 ± 0.033 ▲	0.141 ± 0.015 =
BAT	0.436 ± 0.030	0.301 ± 0.054 =	0.696 ± 0.045 ▲	0.264 ± 0.034 ▼	0.608 ± 0.053 ▲

Molar ratios were calculated using a mol wt of 652 g for T_3 and 777 g for T_4. The symbols represent the comparison of the mean values of the groups infused with thyroid hormones with the mean values of the control group; they are explained in Table 3. The T_3/T_4 ratio for the placebo-infused thyroidectomized was not calculated, as the concentrations of both iodothyronines were very low often, near the detection limits, and small variations could lead to spurious differences.

These values have been obtained by the more direct steady state kinetic approach presently available, using the simultaneous infusion of T_4 and T_3 labeled with different isotopes. These results and our present ones contrast with a recent report (31) which concluded that the thyroid gland is the major source of circulating T_3 in the rat. The approach was less direct than the steady state kinetic method indicated above; conclusions were drawn from differences in circulating T_3 between selenium-supplemented and selenium-deficient rats. The latter have very low hepatic activity of the selenoprotein 5′D-I.

We cannot at present explain why the daily doses of both T_4 and T_3 that we must infuse to ensure euthyroidism in all tissues are higher than these calculated thyroidal production rates. There are many possible differences between laboratories, such as strain and age of the animals used, or food-related differences in fecal loss of the iodothyronines. Moreover, the route of infusion has been different (sc in our experiments *vs.* iv in others), and this might result in different degrees of absorption of the infused doses. It is also possible that the difference is related to the fact that our choice of the combination of 0.9 μg T_4 plus 0.15 μg T_3/100 g BW·day as adequate to compensate for the absence of thyroidal secretion is based on data obtained in plasma and 12 different tissues as well as on several biological effects, whereas the calculated production rates summarized above have been derived exclusively from plasma iodothyronine concentration data.

Compensatory mechanisms

The present combined T_4 plus T_3 replacement therapy with 0.9 μg of T_4 plus 0.15 μg T_3/100 g BW·day results in normalization or near-normalization of compensatory mechanisms, such as changes in circulating TSH and 5′D activities, operating in the placebo-infused thyroidectomized rats. The addition of T_3 to the T_4 dose should not prevent a normal response of these mechanisms when increased plasma and tissue T_3 concentrations are needed to meet higher demands. However, it might be argued that the addition of T_3 to the T_4

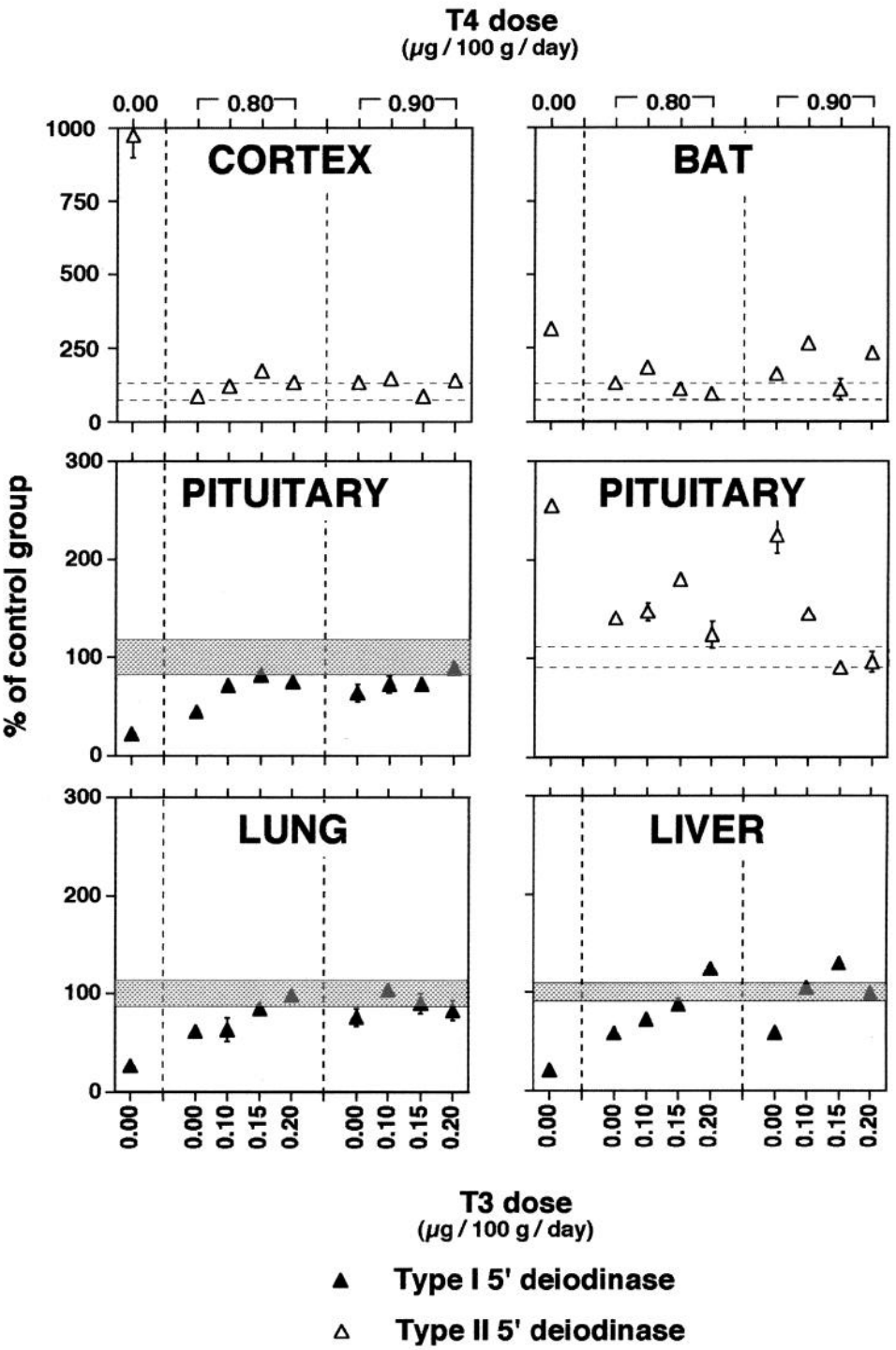

FIG. 4. The changes in the activities of 5′D-I (*full triangles*) and 5′D-II (*empty triangles*) in different tissues of thyroidectomized rats infused with placebo, T_4 alone, or T_4 in combination with T_3 are shown as function of the doses of T_4 and T_3. The *areas enclosed by horizontal lines* represent the 95% confidence intervals for type I 5′D (*full lines, dotted area*) or type II 5′D (*dotted lines, white area*) in intact control rats.

infusion dose would impair the adaptation to decreased demands for T_3. This might be the case for a hypothyroid animal receiving thyroid hormone replacement therapy faced with conditions, such as food restriction or nonthyroidal illnesses, in which a decreased concentration in tissues is considered a life-saving or protective response (32, 33). The decreased release of TRH from the hypothalamus followed by decreased plasma TSH and decreased sensitivity of thyrotrophs to TRH and, consequently, a decrease in the thyroidal secretion of T_4 and T_3 is as typical a response to severe nonthyroidal illness as is the decreased deiodination of T_4 to T_3 (32, 34). As evidenced here by the increase in plasma and tissue T_3 concentrations in the rats infused with T_4 alone compared to levels in rats receiving a placebo infusion, an important part of the T_3 found in rats receiving the combined infusion is derived from the conversion of T_4 to T_3, and this fraction should still be under metabolic control even if T_3 is added to the T_4 infusion. Moreover, in rat models of nonthyroidal illness, such as streptozotocin-induced diabetes and food restriction, the daily production of T_3 from T_4 is

indeed markedly reduced, but the major part of this decrease is related to an approximately 50% decrease in the thyroidal secretion of T_4, leading to a marked reduction of the T_4 pool required for generation of T_3 (8). Such results strongly suggest that the amount of T_4 would have to be markedly reduced to solve the problem posed by severe nonthyroidal illness in hypothyroid patients receiving replacement therapy, and that the mere withdrawal of T_3 from the replacement therapy would have a relatively minor effect. In any case, if replacement therapy has to be adjusted during the illness, the patient could be given T_4 alone.

Possible clinical implications

The aim of substitution therapy for hypothyroidism in humans is to replace the thyroidal secretion of thyroid hormones. The widely accepted approach to this treatment is the oral administration of T_4 alone (2), for reasons summarized in the introduction. This treatment might not be the most appropriate in view of our recent (1) and present results in thyroidectomized rats, which show that combined therapy with T_4 plus T_3 is a more physiological approach.

Secretion of T_3 by the normal human thyroid gland represents a smaller proportion of the total secretion of hormone than that in the rat; the reported T_4 to T_3 molar ratios are 14:1 for man (35) and 5.7:1 for the rat (6–9). This suggests that T_3 concentrations in human extrathyroidal tissues might be affected to a lesser extent than those in the rat by changes in the amount of T_3 secreted by the gland. Moreover, in patients, some residual functioning thyroid tissue may be present, and secretion of T_3 may be preferentially preserved over that of T_4 (36). However, any remnant thyroid function would be substantially reduced once TSH levels became normal with T_4 therapy. Despite these differences between the present experimental model and the situation encountered in clinical practice, there are similarities which suggest that patients might also benefit from an approach comparable to that used here. The scarce data available from a mixed population of hypothyroid patients receiving oral T_4 replacement therapy indicate that for similar concentrations of plasma T_3 and TSH, circulating levels of T_4 are elevated compared to those in matched controls (4). Our results in rats (1) suggest that the same might apply for their tissues. If so, the addition of small proportions of T_3 to the replacement therapy with T_4 might improve simultaneous attainment of euthyroidism in all tissues and avoid high T_4 concentrations and the chronic overstimulation of compensatory mechanisms.

For years, the suggested dose for replacement therapy in hypothyroid patients was 200–400 μg T_4, administered orally (37). At present, however, with the advent of highly sensitive plasma TSH assays and improved evaluation of the potency of T_4 preparations (38), the recommended dose has been reduced to 100–150 μg/day (2). Approximately 80% of the orally administered T_4 is absorbed (4); therefore, this replacement dose is not very different from the thyroidal T_4 secretion rate for a normal adult. Pilo *et al.* (35) assessed the mean daily thyroidal production rate of T_4 and T_3 by simultaneously injecting T_4 and T_3, labeled with different isotopes. To our knowledge, this is the only study in humans in which the thyroidal secretion of T_3 can be evaluated; in most other studies (21), only the total body production rate of T_3 can be calculated. The study was performed in a group of 14 normal adults from an area with a normal iodine intake, which included both men and women between 19–65 yr of age with a mean body wt and surface area of 70 kg and 1.79 m^2, respectively. The mean daily thyroidal production of T_4 is 56.2 $\mu g/m^2$, corresponding to 101 μg T_4, a value in agreement with previous assessments (21) of the T_4 production rate (96 μg/day) in man. The thyroidal production rate of T_3 is 3.3 $\mu g/m^2$, which corresponds to an average of 6 μg T_3. This amount of T_3 is approximately one fourth of the total T_3 production rate (thyroidal secretion plus extrathyroidal generation from T_4) of 26 μg T_3/day. According to these estimates, the total thyroidal production of thyroid hormones in man would be 101 μg T_4 and 6 μg T_3.

The relatively small difference between the replacement dose of T_4 usually administered and the thyroidal secretion rate appears to contrast with present results. The preferred doses of T_4 and T_3 infused sc into thyroidectomized rats are higher than the reported thyroidal secretion rates for normal animals, and the amount of T_4 infused to ensure normal T_3 levels for the majority of tissues is decreased almost 50% by concomitant addition of a small amount of T_3. In this respect, however, we should like to point out that the criteria for defining the adequate dose are also different. The T_4 dose is usually adjusted to the individual patient on the basis of achievement of normal circulating TSH and T_4 levels (36), because subtle changes in the feeling of well-being of the patient are more difficult to quantify. In the experimental studies, the preferred dose also takes into account the concentrations of T_4 and T_3 in tissues as well as some biological end points. If such results (1) are pertinent to man, restoration to normal of plasma T_3 and TSH levels, even in the presence of supraphysiological plasma T_4 concentrations, might not ensure normal T_3 concentrations in all tissues, and the doses of T_4 presently used might be inadequate to attain euthyroidism in some tissues.

It would appear that thyroid hormone substitution therapy in a hypothyroid patient ought to ensure that the amounts of T_4 and T_3 continuously absorbed into the bloodstream are at least equivalent to the corresponding thyroid secretion, namely approximately 100 μg T_4 and 6 μg T_3/day. The actual amounts that would have to be administered to achieve this are likely to be higher, depending on the route of administration and the degree of absorption of the iodothyronines. It should be possible to adjust treatment using simultaneous normalization of circulating T_4, TSH, and T_3 as a guideline.

Although at present, therapy with T_4 alone is preferred (2, 3, 36, 39), the daily oral administration of combinations of T_4 and T_3 has already been used for treatment of hypothyroid patients, but was largely abandoned, because it was associated with several problems related to 1) wide fluctuations in circulating T_3 concentrations and, possibly, 2) the addition of an excessive amount of T_3 to the daily T_4 dose. Such problems have been avoided in the present experiment by the use of continuous delivery and combinations of T_4 and T_3 in relative proportions resembling those normally secreted by the gland.

1) The mean residence time of T_4 in man is 310 h (13 days) (21). Treatment with a daily dose of T_4 means that 13 doses are given during this period, and fluctuations in circulating

T_4 are likely to be buffered. Delivery of T_4 into the bloodstream would be relatively comparable to the delivery of T_4 by the continuous sc infusion used by us. On the contrary, the mean residence time of T_3 in man has been calculated to be 59.3 h (2.5 days) (21). If the T_3 supplement were given once daily, only 2.5 doses would be administered during the mean residence period. This is quite different from the continuous delivery by constant infusion used for the present study and is likely to lead to the much wider and more frequent fluctuations in circulating T_3 concentrations compared to those of T_4 administered once daily, which have been described in hypothyroid patients receiving oral T_4 plus T_3 combination replacement therapy (34). The route of administration may also play a role, as the intestinal absorption of T_3 is faster than that of T_4, further contributing to the appearance of peaks of elevated plasma T_3 (40). The amount of T_3 reaching tissues might well be excessive during part of the interval between the daily doses and lead to undesirable thyroid hormone effects, especially in those tissues deriving most of their T_3 supply directly from plasma, such as the heart (23).

2) The preparations used for combined therapy with T_4 plus T_3, such as Liotrix (in the U.S.), Diotroxin (Glaxo Laboratories, Madrid, Spain), and Novothyral (Merck Laboratories, Darmstadt, Germany), probably contained an excess of T_3 compared to T_4; the molar ratios of T_4 to T_3 were 3.4:1 (4:1 by wt), 7.6:1 (9:1 by wt), and 4.2:1 (5:1 by wt), respectively, whereas the molar ratio for secretion by the human thyroid is 14:1 (35). Liotrix [Euthyroid, Parke-Davis Laboratories (Detroit, MI) and Thyrolar, Armour Laboratories (Kankakee, IL)] was available in several formulations with a wide range of doses, which contained from 12.5 μg T_4 plus 3.1 μg T_3 (Thyrolar-1/4) to 180 μg T_4 plus 45 μg T_3 (Euthyroid-3) (41). Diotroxin contained 90 μg T_4 plus 10 μg T_3 (42), and Novothyral contained 100 μg T_4 plus 20 μg T_3. The higher intestinal absorption rate of T_3 (~90%) (38) compared to that of T_4 (~80%) (4) would further contribute to the relative excess of T_3. Thus, the human daily thyroidal production rate of 101 μg T_4 and 6 μg T_3 cited above (35) would not be mimicked with any of these combinations.

If more extensive studies in hypothyroid patients support our tentative conclusion that therapy with a combination of T_4 and T_3 might be better than substitution with T_4 alone, the problems previously encountered should be solved. First, assuming similar absorption rates of T_4 and T_3, the preparation should contain T_4 and T_3 in a molar proportion of approximately 14:1 and deliver into the bloodstream 101 μg T_4 and 6 μg T_3/day, thus mimicking human thyroid secretion (35). Second, the route of administration should warrant a constant steady supply of both iodothyronines. This might be achieved by combining the oral administration of T_4 with that of sustained enteric release forms of T_3, also given orally. Other possible approaches might involve implantation of im preparations with sustained release of T_4 and T_3 or the transdermal delivery of both iodothyronines.

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