A Study of Extrathyroidal Conversion of Thyroxine (T_4) to 3,3′,5-Triiodothyronine (T_3) *in Vitro**

INDER J. CHOPRA

Department of Medicine, UCLA Center for the Health Sciences, Los Angeles, California 90024

ABSTRACT. An *in vitro* model has been employed to study the nature of the process of peripheral monodeiodination of thyroxine to triiodothyronine (T_3) and the factors capable of modulating it. Thyroxine (T_4, 5μg/ml, 6×10^{-6}M) was incubated in 0.15M phosphate buffer (pH 7.35) with various rat tissue homogenates (approximately 0.13 g-equivalent) for 2 h at 37 C, and the T_3 generated during incubation was measured by a specific immunoassay of an ethanol extract of the homogenate. The liver and kidney homogenates produced more T_3 than various other tissues; the kidney was more active than the liver. The T_4 to T_3 converting activity in the liver homogenates was influenced by the concentration of the homogenate, duration of incubation, substrate (T_4) concentration, pH of incubation and temperature of incubation. It was unaffected by large concentrations of several agents including methimazole, hydrocortisone, sodium iodide, mono- or diiodotyrosine, 3,5-diiodothyronine, thyronine and methylated and halogenated analogues of 3,3′,5′-triidothyronine (reverse T_3, rT_3). However, various other agents including several thyroid analogues and propylthiouracil (PTU) inhibited T_4 to T_3 conversion in a dose-dependent manner. Inhibitory thyroid analogues, in order of their potency, were rT_3, 3′,5′-diiodothyronine, tetraiodothyroacetic acid, 3,3′-diiodothyronine and 3-monoiodothyronine; on a molar basis, the relative potency of these agents was approximately 100:100:5:1:1. PTU was about 3% as potent as rT_3 on a weight basis and only 1% as potent as rT_3 on a molar basis. Analyses of the data by Lineweaver-Burk plot suggested that rT_3 is a competitive inhibitor and PTU, an uncompetitive inhibitor of conversion of T_4 to T_3. The various data suggest that: a) monodeiodination of T_4 to T_3 is enzymic in nature, and b) rT_3 is a very potent inhibitor of conversion of T_4 to T_3. (*Endocrinology* **101**: 453, 1977)

SEVERAL recent studies have demonstrated monodeiodination of thyroxine (T_4) to 3,3′,5-triiodothyronine (T_3) in extrathyroidal tissues (1–6). It appears that this route of metabolism of T_4 may be the major source of T_3 in some animal species (6) as well as in man (4,7,8). However, there is at present little information regarding the nature of the process(es) responsible for peripheral monodeiodination of T_4 to T_3 or the factors capable of modulating it. The *in vitro* study described herein was undertaken to gather some insight into these matters. Furthermore, since recent studies have indicated that serum concentration and/or the daily production rate of another metabolite of T_4, *i.e.*, 3,3′,5′-triiodothyronine (reverse T_3, rT_3), may be increased in several situations where serum T_3 and/or daily production of T_3 are decreased (9–12), it appeared important to examine the effect of rT_3 on the conversion of T_4 to T_3. The studies have demonstrated that rT_3 is a potent inhibitor of peripheral conversion of T_4 to T_3.

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Materials and Methods

Animal tissues

Male Sprague-Dawley rats weighing 100 to 250 g were maintained on Purina rat chow and tap water *ad libitum*. Groups of 2 to 10 rats were killed by decapitation and various organs were dissected. The organs were washed in cold (4 C) 0.15M phosphate buffer (pH 7.35), blotted, weighed and homogenized in 2 volumes of buffer using a Waring blender. The homogenates were filtered through 4 layers of gauze and were either used within the next hour or frozen in 5 to 10 ml aliquots at −10 C and stored until use. Once thawed, any homogenate remaining after use was discarded. When it was planned to use the homogenate the same day, the container was kept surrounded by crushed ice. The tissue concentration in these homogenates was approximately 0.33 g wet weight equivalent (g-eq) per ml.

Other reagents

DL-rT_3 was obtained through the courtesy of Dr. Robert I. Meltzer of Warner-Lambert Research Institute. 3′,5′-Diiodo-DL-thyronine (3′,5′-T_2), 3,3′-diiodo-L-thyronine (3,3′-T_2) and 3-monoiodo-L-thyronine (3-T_1) were kind gifts of Dr. Paul Block, Jr. of River Research, Toledo, Ohio. Other thyroid analogues and propylthiouracil (PTU), phenylmethylsulfonyl fluoride, α-methyl-p-tyrosine were obtained from Sigma Chemical Company, St. Louis, Mo; tert-butylhydroperoxide was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin; powdered diphenylhydantoin sodium (Dilantin®) was obtained from Parke-Davis Company. Several other drugs were purchased from local commercial sources. Various agents were dissolved in water, 0.1M NaOH, assay buffer or ethanol depending on their solubility; concentrations for use in experiments were, however, prepared in the assay buffer or water.

In vitro *study of conversion of T_4 to T_3*

Aliquots of tissue homogenates were incubated with non-radioactive T_4 for a certain period of time and the amount of T_3 generated was measured by radioimmunoassay (RIA) in an ethanol extract of tissue. In a typical experiment the following reagents were added in 10 × 75 mm disposable glass culture tubes: 1) 0.15M phosphate buffer, pH 7.35, volume to adjust the final volume to 1 ml; 2) non-radioactive T_4: 100 μl of a solution containing 50 μg T_4/ml; 3) tissue homogenate, 400 μl representing approximately 0.13 g-eq of tissue. The final pH of the incubation mixture was 7.2 (7.15–7.25). Tubes were mixed by swirling and placed for 120 min in an incubator at 37 C. The reaction was stopped by extraction of the incubation mixtures with 2 volumes of 95% ethanol. Each assay included 2 tubes (zero incubation tubes) which were handled exactly as described except that the tissue homogenate was added at the end of incubation immediately prior to extraction with ethanol. T_3 content of these tubes was considered to represent T_3 contained in the tissue homogenate and in commercial T_4 and that apparent from the cross-reaction of T_4 in T_3 assay. This amount of T_3, which varied between 5.2–7.6 ng/tube when 5 μg T_4 was employed, was subtracted from T_3 measured in test samples to derive how much T_3 was produced due to incubation of the tissue homogenate with T_4. The various results were arbitrarily expressed as the mean amount of T_3 generated per μg T_4 per g-eq of tissue per hour. This was done by multiplying the amount of T_3 produced per tube by 0.769 [1/0.13 × 5 × 2 = 0.769), where 0.13 was the amount (g-eq) of tissue employed, 2 was the duration (h) of incubation and 5 was the weight (μg) of T_4 in the incubation vessel].

When the effects of temperature of incubation or of duration of incubation on *in vitro* production of T_3 were to be studied, the temperature of the tissue homogenate was adjusted to that during incubation before adding it to the incubation vessel.

To study the effect of various agents on the conversion of T_4 to T_3, 100 μl of buffer in the incubation mixture were replaced by an equal volume of a solution containing the desired amount of the test agent; a duplicate set of zero incubation tubes containing the test agent was also prepared in addition to zero incubation tubes, which were prepared regularly. Additionally, in the case of thyroid analogues which could be converted to substances which could cross-react in the T_3 RIA, the effect of incubation of tissue with the thyroid analogue alone (without T_4) was also studied. Any apparent T_3 detected in these tubes was subtracted from the amount of T_3 measured in the tubes containing T_4 and the thyroid analogue under study in order to derive its effect on T_3 production from T_4.

To study the effect of changes in pH of incubation on the conversion of T_4 to T_3, livers from 5 rats were pooled, cut into small pieces, and minced at 4 C. The minced tissue was divided into aliquots and homogenized in 2 volumes (wt/vol) of two different buffer systems, *i.e.*, Sorensen's phosphate buffer (0.168M monopotassium disodium phosphate) covering the pH ranges from 5.5 to 8.2, and barbital-sodium acetate buffer (0.21M sodium acetate and sodium barbital plus 0.15M HCl) covering the pH ranges from 4.3 to 8.5 (13). The buffer solution employed during incubation was also changed from the 0.15M phosphate (pH 7.35) used regularly to the one used for homogenization. The remainder of the protocol of the incubation mixture was identical to that described above.

T_3 radioimmunoassay

The amount of T_3 generated during incubation of T_4 with tissue homogenates was measured in

ethanol extracts of the incubation mixture using RIA methods described previously (14,15). One hundred to 300 μl of ethanol extract representing 33.3 to 100 μl of the incubation mixture and containing 63% ethanol was used directly in the RIA in which the final volume was 1.0 ml; a volume of 63% ethanol equal to those of the unknowns (100 to 300 μl) was added to tubes representing the standard curve of the RIA to minimize the effects of ethanol on measurements of T_3 by immunoassay. Since ethanol extracts of tissues contained little or no protein, as evidenced by absence of a precipitate during treatment of the extracts with 10% trichloroacetic acid (TCA), use of 63% ethanol alone in the standard curve tubes was considered sufficient to render the unknowns comparable to the standards. Any effect of non-T_3 substances extracted from the tissues on measurements of T_3 by RIA was taken into account by measuring T_3 in zero-incubation tubes.

The sensitivity of the T_3 RIA varied between 30 to 50 pg per assay tube. Even when the sensitivity was 50 pg/tube, RIA allowed detection of 1.5 ng of T_3 in 1 ml of incubation mixture using only 100 μl of ethanol extract (or 33.3 μl of incubation mixture); 0.5 ng of T_3 could be detected when 300 μl of ethanol extract (or 100 μl of incubation mixture) was tested. These amounts, 1.5 and 0.5 ng, of T_3 represent very small fractions ($\leq$0.03%) of the amount of T_4 (5 μg) added to the incubation mixture.

Results

The amounts of T_3 produced during incubation of fresh homogenates of various rat tissues with T_4 are shown in Table 1. It is clear that kidney and liver homogenates yielded much greater amounts of T_3 than other tissues.

In order to examine the influence of differences in extractability of T_3 from various tissues, the recovery of radioactivity after adding radioactive T_3 and that of T_3 after adding non-radioactive T_3 to various tissue homogenates was studied. The recovery of the radioactivity added to 4 liver homogenates was (mean ± SE) 88.6 ± 0.60% and that of non-radioactive T_3 (50,100 and 200 ng/tube) incubated with 4 liver homogenates was 89.0 ± 3.0% (range in 12 experiments: 79–98). Similarly, the recovery of the radioactive and that of non-radioactive T_3 added to homogenates of kidney, muscle, heart, spleen, lung, brain and intestines varied between 86–96% in two experiments. While these data suggested that more T_3 may be formed than is actually detected, it is clear that the observed differences in the yield of T_3 from various tissues are attributable more to the differences in their relative capacity for conversion of T_4 to T_3 than to the differences in extractability of T_3 from various tissues. The coefficient of variation of T_3 production from T_4 was 4.0% for liver homogenates and 2.4% for kidney homogenates. Even though kidney homogenates appeared to be more potent than liver homogenates in conversion of T_4 to T_3 (Table 1), liver homogenates were employed for most of the subsequent experiments. This was done because of the availability of larger amounts of available liver tissue than of kidney tissue.

TABLE 1. Comparison of the amounts of T_3 generated during incubation of fresh homogenates of various rat tissues with T_4 at 37 C.

Tissue	T_3 generated during incubation (ng/μg T_4/h/g-eq tissue)
Liver	27 ± 0.6*
Kidney	49 ± 0.6†
Muscle	1.4 ± 0.5†
Heart	1.1 ± 0.1†
Spleen	1.3 ± 0.2†
Lung	0.0 ± 0.0†
Brain	1.7 ± 0.4†
Intestines	0.7 ± 0.1†

* Mean ± SE of quadruplicates.
† Compared to liver, $P < 0.001$.

Characterization of T_4 to T_3 converting activity

Since it is inconvenient to have to prepare the tissue before each experiment, the feasibility of using stored homogenates, frozen at −10 C, was studied. These studies revealed that while some loss of T_4 monodeiodinating activity may occur during such storage of homogenates, most of the activity is retained for up to 40 days (Table 2).

TABLE 2. Effect of freezing on *in vitro* conversion of T_4 to T_3 by rat tissue homogenates

Experiment	Tissue	Duration of storage at −10 C (days)	T_3 generated (ng/μg T_4/h/g-eq tissue)
I.	Liver	0	28.4 ± 0.92*
		2	24.1 ± 0.35
II.	Liver	2	33.1†
		40	25.3
III.	Liver	0	27.1 ± 0.54
		9	20.4 ± 1.14
IV.	Kidney	0	47.1
		30	34.1
V.	Kidney	0	48.6 ± 0.58
		9	25.1 ± 1.4

* Mean ± SE of quadruplicates.
† Mean of duplicate determinations.

Figure 1 presents the data on the production of T_3 during incubation of fresh rat liver homogenate with T_4 for varying lengths of time. T_3 production increased rapidly in the first 30 min and slowly thereafter.

In order to determine for how long the T_4 monodeiodinating activity remains viable *in vitro*, the effect of preincubation of fresh liver tissue for 1 to 8 h at 37 C was studied (Table 3). A preincubation period of 1 h

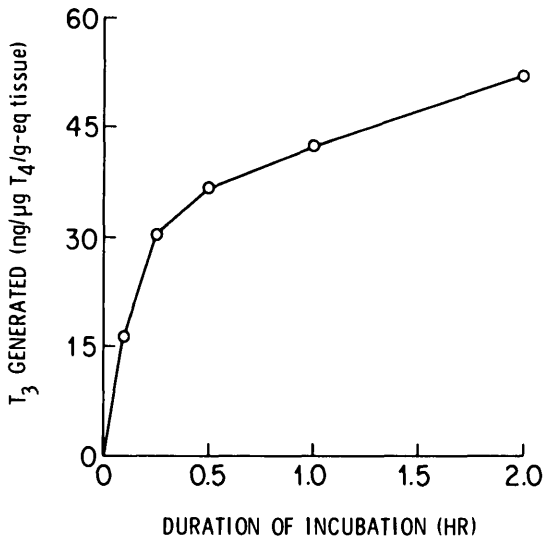

FIG. 1. Effect of duration of incubation at 37 C on the conversion of T_4 to T_3 by fresh rat liver homogenate. Data are averages of duplicate determinations.

TABLE 3. Effect of preincubation at 37 C on T_4 to T_3 converting activity of rat liver homogenate

Duration of preincubation (h)	T_3 generated during incubation (ng/μg T_4/h/g-eq tissue)
0	25.8*
1.0	22.4
2.0	9.7
4.0	3.0
8.0	0.7

* Mean of duplicate determinations.

influenced the T_4 to T_3 converting activity only minimally. However, more prolonged periods of pre-incubation caused an increasing reduction in this activity. These data suggested that T_4 to T_3 converting activity may remain stable for only about 3 h at 37 C.

The effect of tissue concentration on the production of T_3 from T_4 is shown in Fig. 2. It is apparent that T_3 production increases progressively with increasing concentration of liver homogenate up to 0.20 g-eq wet weight per ml.

Figure 3 describes the effect of pH of incubation on the production of T_3 using two different buffer systems. It is clear that T_3 production is influenced considerably by the pH of the buffer. Additionally, the nature of the ions in the buffer and the molarity of the buffer appear important in the generation of T_3 from T_4. Thus, although the two experiments in Fig. 3 employed the same pooled liver tissue and were run in parallel, the production of T_3 from T_4 was consistently greater in Sorensen's phosphate buffer than in barbital-sodium acetate buffer at all pH values studied. However, the production of T_3 was maximal or near maximal around neutral pH in both buffer systems.

The effect of substrate (T_4) concentration on the rate of production of T_3 was studied using fresh rat liver homogenate (Table 4). It was found that the fractional rate of conversion of T_4 to T_3 decreases as the concentration of T_4 is increased in the incubation mixture and that the actual amount of T_3 generated increases toward a maximum as

the T_4 concentration is increased to 5 μg/ml. Similar data were obtained in another 3 experiments in which frozen rat liver homogenates were used.

The effect of temperature during incubation on the production of T_3 is shown in Table 5. It is clear that the maximum yield of T_3 occurs at 37 C. T_3 production decreases markedly when the temperature of incubation is decreased to 4 C or increased to 56 C.

Effect of various thyroid analogues

The effect of varying doses of several thyroid analogues on the conversion of T_4 to T_3 is shown in Fig. 4. Reverse T_3, tetraiodothyroacetic acid (TETRAC), 3,3'-T_2, and 3-T_1 caused significant, dose-dependent inhibition of T_3 production with rT_3 being the most potent inhibitor; using the doses that caused 50% inhibition of T_3 production, the relative inhibitory potency of these various agents, on a molar basis, appeared to be approximately 100:5:1:1. In another experiment, the effects of 3',5'-DL-T_2 and rT_3 on production of T_3 from T_4 were compared; molar concentrations of these agents that caused 50% inhibition of T_3 production were similar. In contrast with these agents, several other thyroid analogues including monoiodotyrosine (MIT, up to 3×10^{-6}M), diiodotyrosine (DIT, up to 3×10^{-6}M) 3,5-diiodothyronine (3,5-T_2, up to 10^{-5}M), thyronine (T_0, up to 10^{-5}M) and sodium iodide (NaI, up to 6.7×10^{-3}M) (Fig. 4) and chlorinated, brominated or methylated analogues of rT_3 and methylated T_4 (up to 5×10^{-6}M) had no discernible effect on the

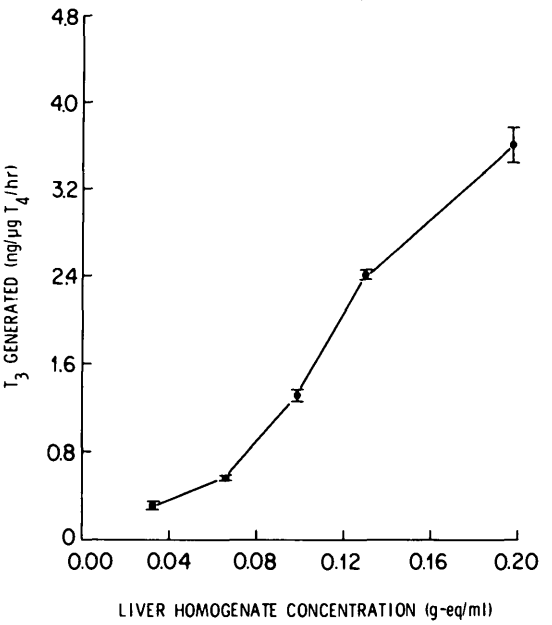

FIG. 2. Effect of the concentration of rat liver homogenate on the conversion of T_4 to T_3. Liver homogenate stored frozen at −10 C for 12 days was employed. Data are presented as mean ± SEM of quadruplicate determinations.

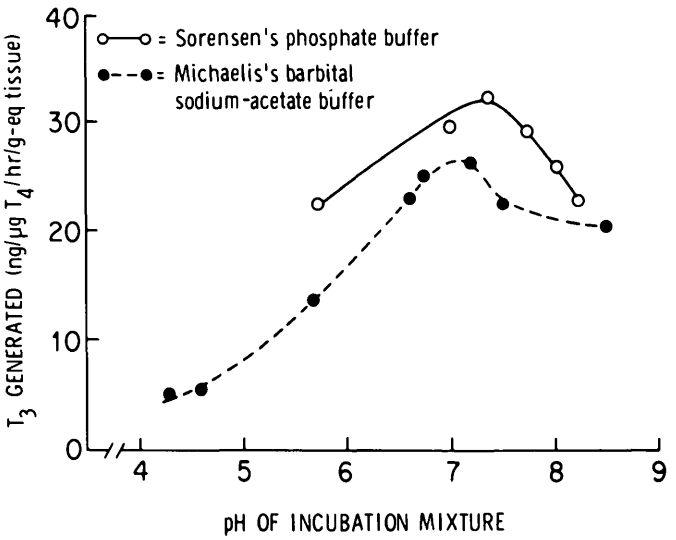

FIG. 3. Effect of pH on conversion of T_4 to T_3 by fresh rat liver homogenate. Individual points are averages of duplicate determinations.

TABLE 4. Effect of the substrate (T_4) concentration on the conversion of T_4 to T_3 by liver homogenates*

Substrate (T_4) concentration in incubation mixture† (μg/ml)	T_3 generated during incubation (ng/ml)	Rate of production of T_3 (ng/μg T_4/h/g-eq tissue)
0.05	1.36	105
0.10	2.40	92.3
0.25	5.90	90.7
0.50	9.90	76.1
1.00	10.8	41.5
2.00	18.1	34.6
5.00	31.8	24.6
10.0	27.0	10.4
50.0	28.0	1.92

* Data are averages of duplicate determinations.

† Liver homogenate concentration was 0.13 g-eq tissue per ml. The final volume of incubation mixture was 1.0 ml, and the final pH was 7.15, temperature 37 C and duration of incubation, 2 h.

TABLE 5. Effect of temperature of incubation on the conversion of T_4 to T_3 by rat liver homogenate* *in vitro*

Temperature C	T_3 generated (ng/μg T_4/h/g-eq tissue)
4.0	1.15 ± 0.34†
23.4	6.23 ± 0.18
37.0	15.7 ± 0.44
56.0	2.31 ± 0.13

* Homogenates stored frozen at −10 C for 12 days were employed in this experiment.

† Mean ± SE of quadruplicate determinations.

conversion of T_4 to T_3; 3,3',5-triiodothyroacetic acid (TRIAC) and triiodothyropropionic acid (TRIPROP) could not be studied adequately because these substances cross-reacted excessively in the T_3-RIA (14).

Marked inhibition of the conversion of T_4 to T_3 by rT_3 was intriguing. To examine whether this effect of rT_3 is unique to liver or whether other tissues can also be affected, the influence of rT_3 on the monodeiodination of T_4 to T_3 was studied using kidney homogenate (Fig. 5). It was found that just as in case of liver homogenate, rT_3 can also inhibit the production of T_3 by the kidney; under identical conditions, much larger doses of MIT do not have any appreciable effect on conversion of T_4 to T_3.

Studies were next conducted to evaluate the nature of the inhibition of conversion of T_4 to T_3 by rT_3. Liver homogenates were incubated with varying amounts (1 to 10 μg) of T_4 with and without two concentrations (10 and 40 ng/ml) of rT_3, and the amount of T_3 generated during a 2 h incubation at 37 C was quantitated. Although initial

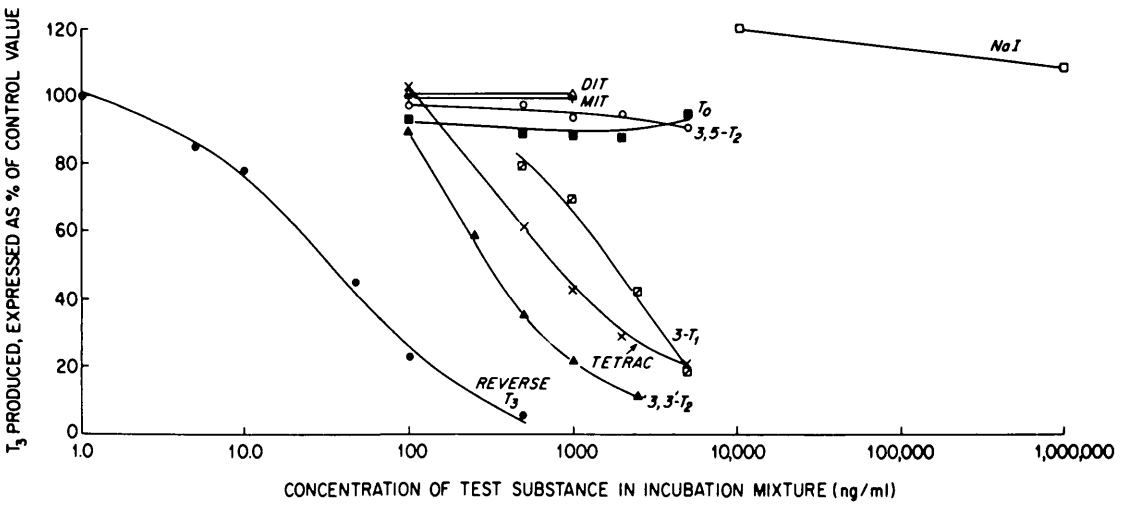

FIG. 4. Effect of some thyroid analogues on *in vitro* production of T_3 from T_4 by rat liver homogenate. T_3 produced from T_4 in the absence of test agents (control value) has been expressed as 100%. T_3 produced in the presence of the test agents is expressed as per cent of control value and is plotted against the logarithm of the concentration of the test agent in incubation mixture. Individual points represent the average of duplicate determinations.

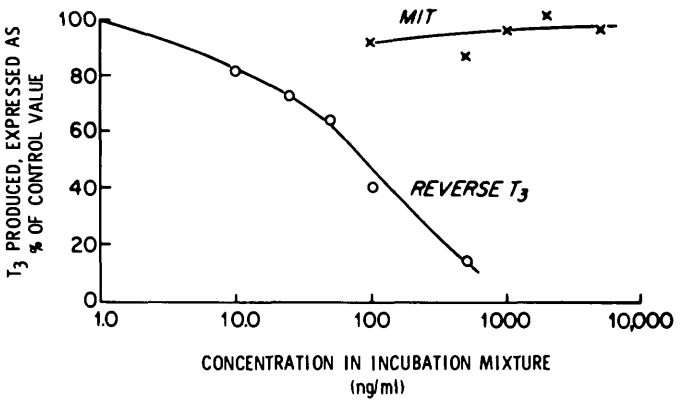

FIG. 5. Effect of monoidotyrosine (MIT) and reverse T_3 (rT_3) on the production of T_3 during incubation of T_4 with rat kidney homogenate. Data are presented as described in Fig. 4.

velocity conditions were not used, the data, when examined by Lineweaver-Burk plot (16), suggested that rT_3 is a competitive inhibitor of conversion of T_4 to T_3 (Fig. 6).

Effect of agents other than thyroid analogues

Conversion of T_4 and T_3 by rat liver homogenates was unaffected by several nonthyroidal agents tested including ethylene diamine tetraacetic acid (EDTA, up to 2.7×10^{-2}M), ascorbic acid (up to 5.7×10^{-3}M), methylene blue (up to 2×10^{-2}M), NAD (10^{-6}M), NADH (10^{-6}M) as well as several drugs, *e.g.*, methimazole (up to 4.4×10^{-2}M), hydrocortisone (2.3×10^{-5}M), dexamethasone (2.5×10^{-4}M), DL-α-methyl-*p*-tyrosine (up to 5×10^{-4}M), diphenylhydantoin (up to 3.6×10^{-4}M), thyrotropin (Thytropar®, up to 1 U/ml), insulin (up to 50 U/ml) and glucagon (up to 2.8×10^{-4}M). Inhibition of T_3 production was, however, observed with large doses of propylthiouracil (PTU), sodium azide, calcium chloride, mercury, tert-butylhydroperoxide and phenylmethyl sulfonyl fluoride (Table 6); PTU was the most potent inhibitor among these agents. However, when the T_4 to T_3 conversion inhibiting potency of PTU was compared with that of rT_3 using the same liver homogenate, PTU was much less potent than rT_3. Fifty per cent inhibition of conversion of T_4 to T_3 was caused by about 900 ng/ml (5.3×10^{-6}M) of PTU and by only 33 ng/ml (5.0×10^{-8}M) of rT_3.

To evaluate the nature of the inhibition of conversion of T_4 to T_3 by PTU, studies similar to those described above for rT_3 were conducted. Thus, liver homogenates were

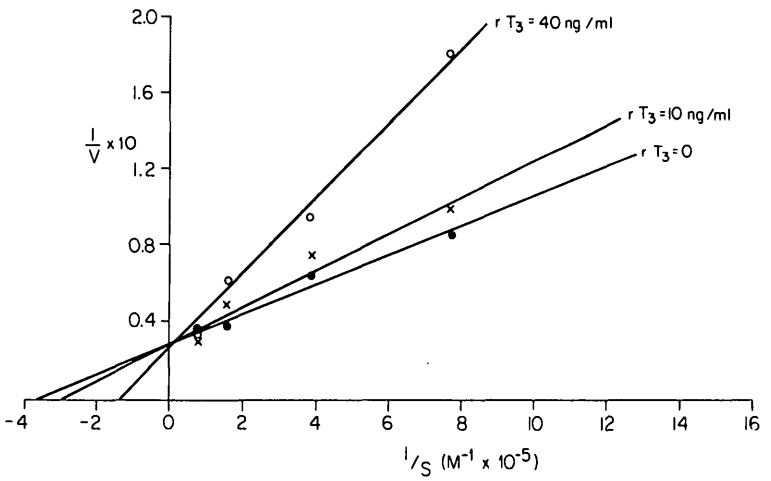

FIG. 6. Lineweaver-Burk plot of the data on the production of T_3 from T_4 by rat liver homogenate in the presence and absence of two concentrations (10 and 40 ng/ml) of reverse T_3 (rT_3). The liver homogenate used in this experiment had been stored frozen at −10 C for 9 days before use. V = velocity of reaction *i.e.*, T_3 (ng) generated during 2 h incubation of T_4 with 0.13 g-eq liver at 37 C; S = molar concentration of T_4 in the incubation mixture.

TABLE 6. Inhibitory effect of some non-thyroidal agents on conversion of T_4 to T_3 by rat liver homogenate *in vitro*

Agent	Molar concentration of the agent tested	T_3 generated: Control	T_3 generated: in presence of test agent
		(ng/μg T_4/h/ g-eq tissue)	
Propyl-thiouracil	—	23.8*	
	5.9×10^{-5}		3.50
	1.2×10^{-5}		8.50
	2.4×10^{-6}		16.4
Sodium azide	—	20.7	
	1.5×10^{-2}		4.2
Calcium chloride	—	21.2	
	0.1		1.15
	10^{-2}		20.3
	10^{-3}		22.1
Mercuhydrin®	—	19.8	
	500.0†		3.00
	50.0†		9.69
Tert-butylhydroperoxide	—	16.5	
	1.1×10^{-3}		1.95
	2.8×10^{-4}		8.31
	1.1×10^{-4}		13.2
	1.1×10^{-5}		16.5
Phenylmethyl sulfonyl fluoride‡	—	17.6	
	1.1×10^{-3}		11.4
	5.7×10^{-4}		17.6

* Each value is the average of duplicate determinations.

† μg/ml as mercury.

‡ Dissolved in ethanol; equivalent amount of ethanol was also added to control sample.

incubated with varying doses of T_4 in the presence and absence of two concentrations (400 ng/ml and 2.0 μg/ml) of PTU, and the data on T_3 produced during incubation were examined by Lineweaver-Burk plot (Fig. 7). The relationship was very different from that in the presence of rT_3 (Fig. 6). Data in Fig. 7 suggest that, in contrast with rT_3, PTU is an uncompetitive inhibitor of conversion of T_4 to T_3.

Discussion

The conversion of T_4 to T_3 *in vitro* has been demonstrated in several studies (17–26). Some of these studies have provided information which is helpful in analysis of the data of the present study. Since TRIAC cross-reacted significantly (40%) with the T_3-antibody (14), its presence in incubation medium could influence the results of the present study; TRIAC could be formed from metabolism (oxidative deamination and decarboxylation) of T_3 formed from T_4 or from 5′-monodeiodination of TETRAC that may be formed from metabolism of T_4 added to the medium. In the absence of chromatographic identification of T_4 metabolites, it is difficult to assess fully the contribution of TRIAC to T_3 values measured in this study. However, in previous studies, (22,23), in which radioactive T_4 was incubated with liver or kidney tissue, only minimal amounts (less than 0.3%) of radioactivity were found by chromatography to be in the form of TRIAC; this proportion of TRIAC could cause a maximum overestimation of the T_3 measured in this study of about 0.12% of T_4. Since the fractional rate of production of apparent T_3 decreases with increase in substrate (T_4) concentration (Table 4, 27), and since the amount of T_4 employed in this study was much greater than in previous studies employing radioactive T_4 (22,23), it would seem that the contribution of TRIAC to T_3 measurements in this study would actually be much less than the 0.12% referred to above. There is no evidence that the fractional rate of production of TRIAC (or pyruvic acid or propionic acid derivatives of T_3) increases during incubation of T_4 with tissues capable of conversion of T_4 to T_3 (22,23,27).

Although, the conversion of T_4 to T_3 has been demonstrated in many tissues (Table 1; 17–26), the nature of this phenomenon has not been clearly elucidated. In the present study, it was observed that factors modulating the conversion of T_4 to T_3 include the temperature and the pH of incubation,

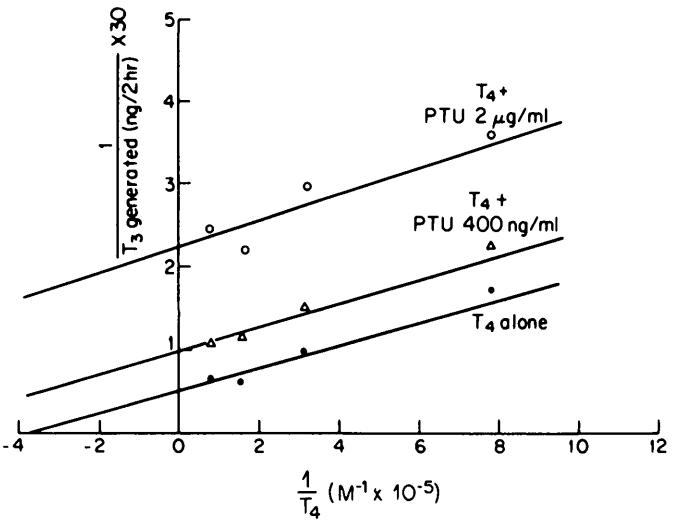

FIG. 7. Lineweaver-Burk plot of the data on the production of T_3 from T_4 rat liver homogenate in the presence and absence of two concentrations (400 ng/ml and 2.0 μg/ml) of propylthiouracil (PTU). The liver homogenate used in this experiment had been stored frozen at −10 C for 5 days before use. T_4 = molar concentration of T_4 in the incubation mixture. V = velocity of reaction, *i.e.*, T_3 (ng) generated during 2 h incubation of T_4 with 0.13 g-eq liver at 37 C. Individual points represent the average of duplicate determinations.

and the concentration of the substrate (T_4). These characteristics suggest that T_4 5′-monodeiodinating activity is enzymic in nature. A similar conclusion was drawn from a previous study employing kidney slices (27).

The present study indicates that while T_4 5′-monodeiodinating activity of the liver is lost rapidly (within hours) at 37 C, it is quite stable for several days during storage at −10 C. A previous study has suggested absence of T_4 to T_3 converting activity in frozen rat livers (28). The reasons for this discrepancy are unclear but may pertain to the method of tissue freezing as well as to the differing conditions of incubation. Nejad *et al.* (28) had employed dry ice for freezing liver tissue; its extremely low temperature may have been detrimental to T_4 monodeiodinating activity.

The exact nature of 5′-monodeiodinating activity in the liver remains unclear. However, the finding that it is strongly inhibited by tert-butyl hydroxyperoxide (Table 6) raises the possibility that participation of thiol activated transhydrogenase may be involved (29). A previous study has suggested that tyrosine hydroxylase may take part in monodeiodination of T_4 to T_3 (30). However, even large doses (up to 5×10^{-4}M) of α-methyl-*p*-tyrosine failed to cause a noticeable decrease in conversion of T_4 to T_3 in liver homogenates. While these observations do not exclude the possibility that tyrosine hydroxylase may be involved in conversion of T_4 to T_3 in chromaffin tissue, they do suggest that enzymes other than tyrosine hydroxylase are probably involved in the case of the liver.

The present study includes an examination of the effect of several agents on the conversion of T_4 to T_3. Thus, while as described previously *in vivo* (31,32), PTU was found to be a potent inhibitor of T_4-5′-monodeiodinase activity, large concentrations of sodium iodide, methimazole, ascorbate and a variety of other agents had no significant effect; these results are similar to those observed in some other recent studies which employed liver slices (22) and kidney homogenates (23).

Perhaps the most interesting observation in this study was the finding of inhibition of conversion of T_4 to T_3 by rT_3. Reverse T_3 appeared to inhibit the conversion of T_4 to T_3 with a potency which was about 100 times more than PTU on a molar basis. Furthermore, T_4 5′-monodeiodination was inhibited competitively by rT_3 and uncompetitively by PTU (Fig. 6 and 7). Inhibition of the conversion of T_4 to T_3 by rT_3 has also been noted in a previous report

where conversion of radioactive T_4 to radioactive T_3 was studied (33). However, the doses of rT_3 tested in that study were much larger, about 100 times more than those clearly active in this study.

Reverse T_3 had little or no inhibitory effect when the T_4 to rT_3 concentration ratio (T_4/rT_3) in the incubation medium was 200 (and corresponding T_4/rT_3 molar ratio, 167) or more. As expected of a competitive inhibitor, it manifested an increasing inhibitory effect on the conversion of T_4 to T_3 as the T_4/rT_3 concentration ratio decreased; inhibition was clearly apparent when T_4/rT_3 concentration ratios were 100 or less. In this regard, it appears interesting to recall that the ratio of serum concentration of T_4 to rT_3 which normally approximates 215 (9) is low (about 90) in certain situations (*e.g.*, hepatic cirrhosis and the newborn) where serum T_3 is generally clearly subnormal (8,9,11,34). The data of the present study raise an interesting possibility for further study that high rT_3 may be involved, in part at least, in determining low serum T_3 in systemic illnesses and at the time of birth. Additionally, the present data offer an explanation for antithyroxine effects of rT_3 noted previously (35–38). Thus, Pittman and Barker (35) have noted that rT_3 antagonizes the metabolism-stimulating effect of T_4 administered to the thyroidectomized rat. Decrease in basal metabolic rate (BMR) has also been noted after administration of rT_3 to T_4 treated myxedematous patients (36,37). Similarly, Benua *et al.* (38) administered rT_3 to hyperthyroid patients and observed an improvement in clinical status and a fall in BMR. The present studies suggest that antithyroid effects of rT_3 in these various situations may have been due to its ability to inhibit conversion of T_4 to biologically more potent T_3.

Just like rT_3, 3′,5′-T_2 is also a potent inhibitor of conversion of T_4 to T_3. Recent studies suggest that this may be another iodothyronine present in human serum (39). However, since the information about its serum concentration relative to T_4 is not available, it is difficult even to speculate about its possible significance as an agent that may be involved in regulating conversion of T_4 to T_3 in health or disease. Future studies may shed some light on this matter.

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