High Affinity of Triiodothyronine (T_3) for Nonphenolic Ring Deiodinase and High Affinity of Tetraiodothyroacetic Acid (TETRAC) for Phenolic Ring Deiodinase in Cultured Monkey Hepatocarcinoma Cells and in Rat Liver Homogenates

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Abstract

The metabolism of 3, 5-[3'- 125 I]triiodothyronine (T_3) and 3-[3', 5'- 125 I]triiodothyronine (rT_3) was studied in cultured monkey hepatocarcinoma cells (NCLP-6E), and the deiodinations of these iodothyronines were also investigated in cultured cell homogenates and in rat liver homogenates. The metabolites were analyzed by ion-exchange column chromatography.

For nonphenolic ring deiodination of 3, 5-[3'- 125 I]triiodothyronine, the order of the inhibitory effect of excess unlabeled iodothyronine or its analog was as follows: 3,3',5-triiodothyronine > triiodothyroacetic acid > tetraiodothyroacetic acid > thyroxine. This order did not differ between in the intact cells (NCLP-6E) and their homogenates.

The order of effectiveness of the excess unlabeled compounds on phenolic ring deiodination of 3-[3', 5'- 125 I]triiodothyronine in the intact cells was as follows: tetraiodothyroacetic acid > triiodothyroacetic acid, 3, 3', 5-triiodothyronine > thyroxine. This order was the same among monkey hepatocarcinoma cell homogenates, rat hepatoma cell homogenates and rat liver homogenates, and triiodothyroacetic acid was obviously more effective than 3, 3', 5-triiodothyronine.

It was concluded that 3, 3', 5-triiodothyronine had the highest affinity for nonphenolic ring deiodinase among iodothyronines and their analogs used in the present study and that tetraiodothyroacetic acid had the highest affinity for phenolic ring deiodinase. It seems, therefore, that the metabolites derived from the thyroid hormones might contribute to deiodinations which involve activation and inactivation of the hormones.

It is a current theory that 3, 3', 5-triiodothyronine (T_3) and 3, 3', 5'-triiodothyronine (rT_3) are mainly formed by the extra-thyroidal deiodinations of thyroxine (T_4). In the metabolism of T_4 , when monodeiodinations occur in the phenolic and non-phenolic rings of thyroxine, T_3 and rT_3 , respectively, are formed. The formation of T_3 means the activation of T_4 , and that of rT_3 , the inactivation. *In vivo* experiments (Pittman and Barker, 1959a, 1959b; Benua *et al.*, 1959) showed that rT_3 behaved as an antagonist of the thyroid hormones.

Recently, Coiro *et al.* (1980) investigated the exact effect of rT_3 on the conversion of T_4 to T_3 *in vivo*, and the same subject was studied in cultured monkey hepatocarcinoma cells (Sorimachi, 1980). These results and other data obtained from the experiments utilizing cell homogenates (Sorimachi and Robbins, 1979; Sorimachi *et al.*, 1980a) and tissue homogenates (Chopra, 1977; Kaplan and Utiger, 1978; Visser *et al.*, 1979) revealed that rT_3 has particularly high affinity for phenolic ring deiodinase among the thyroid hormones and their analogs.

3, 3', 5, 5'-tetraiodothyroacetic acid (TETRAC) and 3, 3', 5-triiodothyroacetic acid (TRIAC) were identified among the metabolites of the thyroid hormones (Roche *et al.*, 1956; Flock *et al.*, 1957; Galton and Pitt-Riverse, 1959; Refetoff *et al.*, 1972). However, we have no information on the physiological role of these compounds. Our previous study on the inhibitory effect of excess unlabeled iodothyronines on the deiodination of labeled rT_3 showed that TRIAC had higher affinity for phenolic ring deiodinase than T_3 in rat hepatoma cell homogenates (Sorimachi *et al.*, 1980a). We have therefore investigated the effect of TETRAC and TRIAC on both phenolic and nonphenolic ring deiodinations of ^{125}I -labeled iodothyronines in cultured monkey hepatocarcinoma cells which metabolized the thyroid hormones (Sorimachi and Robbins, 1977) and in the homogenates by ion-exchange column chromatography (Sorimachi and Ui, 1974, 1975).

Materials and Methods

Chemicals

Unlabeled T_4 and T_3 were purchased from the Wako Pure Chemical Industries (Osaka, Japan) and Sigma Chemical Co. (St. Louis, USA), respectively. TETRAC and TRIAC were kindly supplied by Dr. Hans J. Cahnmann of the National Institutes of Health, USA. Labeled compounds, 3,5-[3'- ^{125}I] triiodothyronine (^{125}I - T_3 , 1.2 mCi/ μ g) and 3-[3', 5'- ^{125}I] triiodothyronine (^{125}I - rT_3 , 0.9 mCi/ μ g), were purchased from New England Nuclear. Iodide contained in these labeled materials was removed by means of a small ion-exchange column just before use (Sorimachi and Cahnmann, 1977).

Cells

The procedures for cell cultivation of monkey hepatocarcinoma cells (NCLP-6E) and Reuber rat hepatoma cells (R117-21B) are described in detail elsewhere (Sorimachi *et al.*, 1980a, b). The former cells had been originally cultured in modified Eagle's minimum essential medium containing 10% fetal bovine serum (Sorimachi and Robbins, 1977), but now the concentration of serum has been decreased to 0.5%. Usually, the cells were cultured in glass

culture flasks and for the subsequent experiments they were grown in 3.5-cm diameter plastic culture dishes or in multi-well plates (24 wells, each 1.5-cm in diameter, NUNC, Denmark). After the cells reached near confluence, ^{125}I - T_3 (16 pCi) or ^{125}I - rT_3 (24 pCi) was incubated with the cells in the presence or absence of excess unlabeled iodothyronine or its analog for the stated intervals.

Homogenates

The cultured cells (3×10^6 - 6×10^6 cells) and the rat liver were homogenized in a Teflon-glass Potter-Elvehjem type homogenizer in 0.2 ml of H_2O at $0^\circ C$ (Sorimachi *et al.*, 1980a), and the rat liver homogenate was centrifuged at $600 \times g$ for 10 min at $4^\circ C$ to remove the connective tissues and the cell debris.

Deiodinase assay

The assay of phenolic ring deiodinase in the homogenates was done according to the method of Sorimachi and Robbins (1979) with a slight modification (Sorimachi *et al.*, 1980a). The homogenates was incubated with ^{125}I - T_3 (20-30 pCi) at pH 6.5 and $37^\circ C$, and the reaction was stopped by the addition of 0.5-ml 10% trichloroacetic acid. After centrifugation of the reaction mixtures the ^{125}I liberated from ^{125}I - rT_3 was measured. For the assay of nonphenolic ring deiodinase, the cell homogenates were incubated with ^{125}I - T_3 (42 pCi) at pH 7.9 and $37^\circ C$ (Sorimachi and Robbins, 1979), and the reaction was stopped by the addition of 1 ml of ethanol. After these procedures, the total volume was 1.5 ml. Then the reaction mixtures were centrifuged at $800 \times g$ for 20 min, and more than 95% of the total radioactivity was recovered by this ethanol extraction. The protein concentration of the homogenates and the cultured cells were determined by the method of Lowry *et al.*, (1951) with bovine serum albumin as the standard.

Ion-exchange column chromatography

For the analysis of iodothyronines and their metabolites, ion-exchange column chromatography using AG50W-X4 (30-35 μ m, Bio Rad) was carried out (Sorimachi and Ui, 1974, 1975). The elution system was a pH gradient obtained with NH_3OH (0-0.7 M). A portion of the incubation medium containing the metabolites was acidified by adding a drop of 10% acetic acid before application to the column. The ethanol extracts from the reaction mixtures containing the cell homogenates were also acidified with a few drops of 10% acetic acid, and then a portion of them was applied to the column.

Results

Figure 1 shows the elution profile for iodide, monoiodotyrosine (MIT), diiodotyrosine (DIT), T_3 , T_4 , TETRAC and TRIAC on ion-exchange column chromatography. TRIAC was eluted between MIT and DIT, and TETRAC was at the same position as DIT in the present system. This result indicates that ion-exchange column chromatography using AG50W-X4 should be useful in the analysis of TETRAC and TRIAC which are metabolites of the thyroid hormones.

Figure 1. Elution profile of I^- , MIT, DIT, T_3 , T_4 , TRIAC and TETRAC on ion-exchange column chromatography (AG50W-X4). The amounts of samples applied to the column were: MIT, DIT, T_3 , 5 nmol; T_4 , 2 nmol; TRIAC, TETRAC, 3 mol. The elution system was a pH gradient obtained with increasing NH_4OH (0–0.7 M) (Sorimachi and Ui, 1975). The detection of iodocompounds was due to the sericarcenite reaction. The last compound (T_3) was eluted within 2.5 h by means of this column chromatography.

There were differences in radioactivity in the medium and the cells whether excess unlabeled iodothyronine or its analog was present in the medium or not (Fig. 2, left panel). In the 3, 5-[3'- ^{125}I] T_3 incubation with the cells, the radioactivity in the

medium was the highest at 8 h of incubation in the absence of the unlabeled compound, and the lowest radioactivity was recorded in the presence of excess unlabeled T_3 at 8 h and 24 h of incubation. The cellular radioactivity differed much among the samples after 24 h of incubation, and the highest value was observed in the presence of unlabeled T_3 and the lowest was recorded in the absence of unlabeled iodothyronine or its analog.

When 3-[3', 5'- ^{125}I] T_3 was incubated with monkey hepatocarcinoma cells, the radioactivity changes in the medium were not so clear at 8 h of incubation (Fig. 2, right panel). However, minor differences in radioactivity in the medium appeared at 24 h of incubation, and the differences were quite obvious in relation to the cellular uptakes among which the lowest radioactivity was found in the absence of the unlabeled hormone or its analog.

A portion of the incubation medium containing T_3 and its metabolites was applied on ion-exchange column chromatography (Fig. 3), and it was shown that nonphenolic ring deiodination and sulfation took place in the cultured cells. About 80% of the total radioactivity in the medium accounted for the iodothyronine sulfates at 8 h of incubation in the absence of the unlabeled compound. This means that nearly 80% of the T_3 was converted to 3, 3'- T_2 by nonphenolic ring deiodination. About 10% of the sulfates had accounted for 3'- T_1 sulfate (Sorimachi and Robbins, 1978b). Table I shows the percent distribution of the radioactivity due to the metabolites in the medium on the elution profiles obtained with ion-exchange column chromatography. The effectiveness of excess unlabeled iodothyronine and its analog on the nonphenolic ring deiodination of 3, 5-[3'- ^{125}I] T_3 was as follows: $T_3 > TRIAC > TETRAC > T_4$. A minute amount of iodothyronine glucuronides was detected on the elution profiles.

Figure 2. Radioactivity in the medium or in the cells after incubation of $^{125}\text{I}-\text{T}_3$ (left panel) or $^{125}\text{I}-\text{rT}_3$ (right panel) with monkey hepatocarcinoma cells. The initial concentrations of labeled T_3 and rT_3 were $5.8 \times 10^{-11}\text{M}$ and $1.6 \times 10^{-10}\text{M}$, respectively. A portion of the medium (0.5 ml), whose initial volume was 1 ml, was removed at 8 h of incubation, and the remaining medium was separated from the cells at 24 h. The cells were solubilized by 1% SDS solution for radioactivity counting. Each value is the mean of two culture wells. The amount of cellular proteins in the well was 0.33 mg.

Table I. Relative distribution of radioactivity due to ^{125}I -labeled compounds in the medium after incubation of $3,5-[3'-^{125}\text{I}]\text{T}_3$ with monkey hepatocarcinoma cells.

	Metabolites		T_3
	Sulfates	Glucuronides	
Control	77.5	1.6	20.9 ^a
T_4	51.6	1.6	46.6 ^b
TETRAC	44.9	1.4	53.7 ^b
T_3	19.8	1.4	78.8 ^b
TRIAC	40.3	1.5	58.9 ^b

The incubation period was 8 h at 37°C , and the initial concentration of labeled T_3 was $5.8 \times 10^{-11}\text{M}$. The amount of cellular protein was 0.33 mg/well. A portion of the mixture of the medium obtained from two culture wells was applied to the column. The concentration of the inhibitors was 10^{-7}M .

(a) This value is due to the mixture of $3'-\text{T}_1$, 3 , $3'-\text{T}_2$ and T_3 (Fig. 3).

(b) The production rate of 3 , $3'-\text{T}_2$ depends on the concentration of the excess unlabeled inhibitors, whereas the consumption rate of 3 , $3'-\text{T}_2$ (the sulfation process) is nearly independent of the concentration (Sorimachi, 1980) since the latter reaction is much faster than the former in the presence of excess unlabeled iodothyronine or its analog. Thus, the peaks due to 3 , $3'-\text{T}_2$ and $3'-\text{T}_1$ are undetectable.

When $3,5-[3'-^{125}\text{I}]\text{T}_3$ was incubated with monkey hepatocarcinoma cell homogenates, nonphenolic ring diiodination occurred, resulting in 3 , $3'-\text{T}_2$ formation (Fig. 4). However, no clear liberation of $^{125}\text{I}^-$ was observed, since T_3 was not a good substrate of phenolic ring diiodinase (Sorimachi and Robbins, 1979; Sorimachi, 1980). The sulfation which required high energy did not take place in the homogenates used in the present system, and a similar result had been reported before (Sorimachi and Rob-

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Figure 3. Elution profile of the metabolites of $^{125}\text{I}-\text{T}_3$ incubated with monkey hepatocarcinoma cells for 8 h. The initial concentration of labeled T_3 was $5.8 \times 10^{-11}\text{M}$. The sample applied on the column was the mixture of the medium obtained from two culture wells. "S" and "G" represent iodothyronine sulfates and glucuronides, respectively. Each fraction contained 50 drops of the eluents.

bins, 1979). Table II shows the effect of excess unlabeled iodothyronine and its analog on the nonphenolic ring deiodination of 3, 5-[3'-¹²⁵I]T₃ in the cell homogenates. The effectiveness was as follows: T₃ > TRIAC > TETRAC > T₄. A small difference in Table II would be a reliable indicator, because the accuracy and reproducibility of this column method was very good (Sorimachi and Ui, 1974).

Table. II. Relative distribution of radioactivity due to ¹²⁵I-labeled compounds after incubation of 3, 5-[3'-¹²⁵I]T₃ with monkey hepatocarcinoma cell homogenates on column chromatography.

	Metabolites		T ₃
	3'-T ₁	3, 3'-T ₂	
Control	2.4	57.8	39.8
T ₄		24.5	75.5
TETRAC		19.2	80.8
T ₃		12.0	88.0
TRIAC	0.6	16.1	83.3

The homogenate (protein amount: 0.82 mg) was incubated with labeled T₃ (3.2×10^{-10} M) for 30 min at 37°C. A portion of the mixture of two samples was applied to the column. The concentration of unlabeled compounds was 10^{-7} M.

Figure 4. Elution profile of the metabolites of ¹²⁵I-T₃ incubated with monkey hepatocarcinoma cell homogenates for 30 min. The initial concentration of labeled T₃ was 3.2×10^{-10} M. The protein amount of homogenates used for deiodination was 0.82 mg.

Monkey hepatocarcinoma cells metabolized rT₃ by phenolic ring deiodination, and formed 3, 3'-T₂. The production rate of 3, 3'-T₂ is much lower than the consumption rate of this compound by sulfation. Thus, only a minor peak of 3, 3'-T₂ was detected after the peak of T₃ on the elution profiles of the rT₃ metabolites, although the existence of 3, 3'-T₂ is not clear from Fig. 5. The phenolic ring deiodination of 3-[3', 5'-¹²⁵I]T₃ forms 3-[3'-¹²⁵I]T₂ and ¹²⁵I⁻. Therefore, the amount of ¹²⁵I⁻ and ¹²⁵I-labeled sulfates represents the activity of phenolic ring deiodinase in the present system using the intact cells. The phenolic ring deiodination of 3-[3', 5'-¹²⁵I]T₃ was most strongly inhibited by TETRAC among T₃, T₄, TETRAC and TRIAC (Table III), and their effectiveness on the deiodination was as follows: TETRAC > TRIAC, T₃ > T₄. A small amount of iodothyronine glucuronides was detected.

Figure 5. Elution profile of the metabolites of ¹²⁵I-rT₃ incubated with monkey hepatocarcinoma cells for 24 h. The initial concentration of labeled rT₃ was 1.6×10^{-10} M. The cellular protein concentration was 0.33 mg/well. "S" and "G" represent iodothyronine sulfates and glucuronides, respectively.

Table. III. Relative distribution of radioactivity due to ^{125}I -labeled compounds after incubation of 3-[3', 5'- ^{125}I] T_3 with monkey hepatocarcinoma cell on column chromatography.

	Metabolites			rT_3
	I- + Sulfates	Glucuronides	3, 3'- T_2	
Control	70.6	4.4	0.4	24.6
T_4	50.6	3.6	3.4	42.4
TETRAC	30.5	6.6	4.9	58.0
T_3	39.3	3.3	5.0	52.4
TRIAC	39.5	5.7	1.9	52.9

The initial concentrations of labeled rT_3 and unlabeled inhibitors were 1.6×10^{-10} M and 10^{-6} M, respectively, and the incubation period was 24 h at 37°C . The amount of the cellular proteins was 0.33 mg/well. A portion of the mixture of the medium obtained from two culture wells was applied to the column.

Table. IV. Inhibitory effect of excess unlabeled iodothyronine or its analog on phenolic ring deiodination of 3-[3', 5'- ^{125}I] T_3 with various homogenates.

	Inhibitors (M)							
	T_4		TETRAC		T_3		TRIAC	
	10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-7}
Rat liver	54.7	87.5	22.6	53.6	43.4	85.2	31.9	67.2
R117-21B	69.5	101	22.1	51.2	43.9	88.8	25.8	68.3
NCLP-6E	69.8	93.1	22.0	67.1	52.2	86.4	38.5	65.8

The concentration of labeled rT_3 was 2.2×10^{-10} M. The amount of proteins in the homogenates used for the deiodination was: rat liver homogenate, 0.06 mg; rat hepatoma cell (R117-21B) homogenate, 0.40 mg; monkey hepatocarcinoma cell (NCLP-6E) homogenate, 0.60 mg. The values are percentages of the control in which the unlabeled compounds were not added to the reaction system, and the mean of duplicate experiments.

The inhibitory effect of excess unlabeled iodothyronine and its analog on the phenolic ring deiodination of 3-[3', 5'- ^{125}I] T_3 was investigated with a few kinds of homogenates: monkey hepatocarcinoma cell homogenate, rat hepatoma cell homogenate, and rat liver homogenate (Table IV). For the assay of phenolic ring deiodinase in the homogenates, a simple method using a small column (Sorimachi and Robbins, 1979; Sorimachi *et al.*, 1980a) was used. The effectiveness of excess unlabeled iodothyronine and its analog was as follows: $\text{TETRAC} > \text{TRIAC} > \text{T}_3 > \text{T}_4$. This order was the same among the three kinds of homogenates and at the two different concentrations of these inhibitors (10^{-6} M and 10^{-7} M). In the experiments using the homogenates, TRIAC was obviously more effective than T_3 in the phenolic ring deiodination of labeled rT_3 .

Discussion

The application of the radioimmunoassay method to the analysis of the thyroid hormones and their metabolites has extensively contributed to the investigation of the metabolism of the hormones not only in *in vivo* but also in *in vitro* experiments. Generally speaking, this method can distinguish the structural difference among T_4 , T_3 and rT_3 . However, the antibody of T_3 showed a large close reaction with TRIAC (Chopra, 1972; Visser *et al.*, 1975). These results suggest that the radioimmunoassay method could not be applicable for the analysis of mixtures of T_4 , T_3 , TETRAC and TRIAC. On the other hand, for the analysis of their mixtures ion-exchange column chromatography must be much better, since these compounds are completely separated from

each other (Fig. 1)

We have already reported that, in the metabolism of 3,5-[3'-¹²⁵I]T₃, the radioactivity changes in the medium incubated with monkey hepatocarcinoma cells reflected the disappearance of T₃ (Sorimachi and Robbins, 1977), and that this phenomenon was due to the different degrees of cellular uptake of iodothyronines and their analogs (Sorimachi and Robbins, 1978a). In the latter experiments, the cellular uptake of T₃ was the highest among the iodotyrosines, iodothyronines and iodothyronine sulfates, and that of iodothyronine sulfates was extremely low or absent. Table I shows that a large portion of 3,5-[3'-¹²⁵I]T₃ was metabolized by nonphenolic ring deiodination followed by sulfation at 8 h of incubation with the intact cells in the absence of excess unlabeled iodothyronine and its analog. Thus, the radioactivity in the medium was the highest in the control (Fig. 2). However, since T₃ has a high affinity for nonphenolic ring deiodinase, the addition of excess unlabeled T₃ strongly inhibited the conversion of 3,5-[3'-¹²⁵I]T₃ to 3-[3'-¹²⁵I]T₂. Therefore, the cellular accumulation of T₃ increased in the presence of excess unlabeled T₃ and the radioactivity in the medium decreased, as shown in Fig. 2. On the other hand, the cellular uptake of rT₃ is not so high compared with T₃ (Sorimachi and Robbins, 1978a) that the radioactivity changes in the medium were not large.

When intact cells were used for the experiments in which the inhibitory effect of various compounds on the deiodinations of iodothyronines, the different degrees of the cellular uptake of the inhibitors should be considered to indicate their effectiveness. However, the experiments using the intact cells would be more physiological than that utilizing the homogenates. The comparison of the data obtained from the intact cells and from the homogenates should give a better understanding of *in vivo* phenomena.

The order of effectiveness of excess unlabeled iodothyronines and their analogs on the deiodinations was the same as between the intact cells and the homogenates (Table V), although differences in the inhibitory effect were small among T₃, TRIAC, T₄ and TETRAC, as shown in Tables I, II, III and IV. Unlabeled T₃ was most effective on nonphenolic ring deiodination not only in the intact cultured cells but also in the cell homogenates, while the effect of T₄ was the smallest among inhibitors. The present data are consistent with our previous data which showed that T₃ was more effective on nonphenolic ring deiodination than T₄ in monkey hepatocarcinoma cells (Sorimachi, 1980) and in their homogenates (Sorimachi and Robbins, 1979).

Phenolic ring deiodination was most strongly affected by TETRAC among the four compounds examined in the present study, in the intact cells and in the homo-

Table V. Order of effectiveness of excess unlabeled iodothyronine or its analog on phenolic and nonphenolic ring deiodinations.

		Inhibitors			
		T ₃	TRIAC	T ₄	TETRAC
Nonphenolic ring deiodination	Intact cells	1	2	4	3
	Homogenates	1	2	4	3
Phenolic ring deiodination	Intact cells	(3 or 2)	(2 or 3)	4	1
	Homogenates ^a	3	2	4	1

When a difference in the inhibitory effectiveness of the unlabeled compounds on the deiodination was very small and it was difficult to determine the order because the differences are outside the limits of measurement by the ordinary technique used in the present study, the numbers were parenthesized.

(a) Monkey hepatocarcinoma cell homogenate, rat hepatoma cell homogenate and rat liver homogenate were used.

genates, while the effect of T_4 was the smallest (Table V). Furthermore, the present investigation revealed that T_3 was more effective on phenolic ring deiodination than T_4 . The same result was obtained in the previous experiments using the intact cells (Sorimachi, 1980), but the opposite result was reported with the cell homogenates (Sorimachi and Robbins, 1979). In the present study, the order of the inhibitory effect on the deiodinations was the same in the intact cells and in the homogenates. This suggests that the affinity of iodothyronines and their analogs for deiodinases would be more important than their cellular uptake. The difference in the order of the inhibitory effects between phenolic and nonphenolic ring deiodinations further supports that the degree of the inhibitory effect would be due to affinity of the inhibitors for deiodinases.

Nonphenolic ring deiodinase contributes to the inactivation of T_4 ; this enzyme converts T_4 to rT_3 and T_3 to 3,3'- T_2 . The previous (Sorimachi and Robbins, 1979; Sorimachi, 1980) and present data show that T_3 has the highest affinity for nonphenolic ring deiodinase among various iodothyronines and their analogs including TETRAC and TRIAC. If we consider that T_3 has a much higher hormonal activity than T_4 , nonphenolic ring deiodinase would play an important role in regulating the T_3 degradation. Since TETRAC and TRIAC have a higher affinity than T_4 for nonphenolic ring deiodinase, these deaminated compounds could partially contribute to the regulation of nonphenolic ring deiodination of the thyroid hormones.

In our previous study (Sorimachi *et al.*, 1980a), TRIAC had a higher affinity for phenolic ring deiodinase than T_3 , although the affinity of rT_3 for phenolic ring deiodinase was higher than that of TRIAC. The present investigation also confirmed that TRIAC has a higher affinity for phenolic ring deiodinase than T_3 , and newly

revealed that the affinity of TETRAC for this enzyme was higher than that of TRIAC. The existence of the inhibitory effect of TETRAC on the conversion of T_4 to T_3 was also shown by Chopra (1977). Thus, judging from the fact that TETRAC and TRIAC derived from the metabolism of the thyroid hormones have a higher affinity for phenolic ring deiodinase which converts T_4 to T_3 than their original compounds, these deaminated derivatives might be of considerable physiological importance.

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References

- Benua, R. S., S. Kumaoka, R. D. Leeper and R. W. Rawson (1959). The effect of dl-3,3',5'-triiodothyronine in Grave's disease. *J. Clin. Endocrinol. Metab.* 19, 1344-1346.
- Chopra, I. J. (1977). A study of extrathyroidal conversion of thyroxine (T_4) to 3, 3', 5-triiodothyronine (T_3) *in vitro*. *Endocrinology* 101, 453-463.
- Chopra, I. J., R. S. Ho and R. Lam (1972). An improved radioimmunoassay of triiodothyronine in serum: Its application to clinical and physiological studies. *J. Lab. Clin. Med.* 80, 729-739.
- Coiro, V., A. Harris, H. M. Goodman, V. Vagenakis and L. Braverman (1980). Effect of pharmacological quantities of infused 3,3',5'-triiodothyronine on throxine monodeiodination to 3,5,3'-triiodothyronine. *Endocrinology* 106, 68-75.
- Flock, E. V., J. L. Bollman, J. H. Grindlay and B. F. McKenzie (1957). Metabolites of radioactive L-thyroxine and L-triiodothyronine. *Endocrinology* 61, 461-473.
- Galton, V. A. and R. Pitt-Riverse (1959). The identification of the acetic acid analogues of thyroxine and tri-iodothyronine in mammalian tissues.

- Biochem. J.* 72, 319-321.
- Kaplan, M. M. and R. D. Utiger (1978). Iodothyronine metabolism in rat liver homogenates. *J. Clin. Invest.* 64, 459-471.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
- Pittman, C. S. and S. B. Baker (1959a). Inhibition of thyroxine action by 3, 3', 5'-triiodothyronine. *Endocrinology* 64, 466-468.
- Pittman, C. S. and S. B. Baker (1959b). Antithyroxine effects of some thyroxine analogues. *Am. J. Physiol.* 197, 1271-1274.
- Refetoff, S., R. Matalon and M. Bigazzi (1972). Metabolism of L-thyroxine (T_4) and L-triiodothyronine (T_3) by human fibroblast in tissue culture: Evidence for cellular binding proteins and conversion of T_4 to T_3 . *Endocrinology* 91, 934-947.
- Roche, J., R. Michel, P. Jouan and W. Wolf (1956). The recovery of 3:5:3'-triiodothyroacetic acid and 3:3'-diiodothyronine from rat kidney after injection of 3:5:3'-triiodothyronine. *Endocrinology* 59, 425-435.
- Sorimachi, K. (1980). Inhibitory effect of unlabeled iodothyronine on the deiodination of labeled thyroid hormones by cultured hepatocarcinoma cells. *Endocrinol. Japon.* 27, 209-214.
- Sorimachi, K. and H. J. Cahmann (1977). A simple synthesis of [3, 5- ^{125}I] diiodo-L-thyronine and [3, 5- ^{125}I]-L-thyroxine of high specific activity. *Endocrinology* 101, 1276-1280.
- Sorimachi, K., A. Niwa and Y. Yasumura (1980a). Phenolic ring deiodination in cultured rat hepatoma cells, and subcellular localization of deiodinases in cultured rat hepatoma, monkey hepatocarcinoma cells and normal rat liver homogenates. *Biochim. Biophys. Acta* 630, 469-475.
- Sorimachi, K., A. Niwa and Y. Yasumura (1980b). Activation and inactivation of thyroxine by cultured rat hepatoma cells. *Biochim. Biophys. Acta.* 633, 134-143.
- Sorimachi, K. and J. Robbins (1977). Metabolism of thyroid hormones by cultured monkey hepatocarcinoma cells. Nonphenolic ring deiodination and sulfation. *J. Biol. Chem.* 252, 4458-4463.
- Sorimachi, K. and J. Robbins (1978a). Uptake and metabolism of thyroid hormones by cultured monkey hepatocarcinoma cells. Effect of KCN and dinitrophenol. *Biochim. Biophys. Acta* 542, 515-526.
- Sorimachi, K. and J. Robbins (1978b). Thyroxine inactivation by starvation in cultured hepatocarcinoma cells. Formation of reverse triiodothyronine. *Horm. Metab. Res.* 10, 528-531.
- Sorimachi, K. and J. Robbins (1979). Phenolic and nonphenolic ring deiodinations of iodothyronines in cultured monkey hepatocarcinoma cell homogenate. *Biochim. Biophys. Acta* 583, 443-453.
- Sorimachi, K. and N. Ui (1974). An improved chromatographic method for the analysis of iodoamino acids in thyroglobulin. *J. Biochem.* 76, 39-45.
- Sorimachi, K. and N. Ui (1975). Ion-exchange chromatographic analysis of iodothyronines. *Anal. Biochem.* 67, 157-165.
- Visser, T. J., D. Fekkes, R. Docter and G. Hennemann (1979). Kinetics of enzymic reductive deiodination of iodothyronines. Effect of pH. *Biochem. J.* 179, 489-495.
- Visser, T. J., I. van der Does-Tobé, R. Docter and G. Hennemann (1975). Conversion of thyroxine into tri-iodothyronine by rat liver homogenate. *Biochem. J.* 150, 489-493.