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ONE ENZYME FOR THE 5′-DEIODINATION OF 3,3′,5′-TRIIODOTHYRONINE AND 3′,5′-DIIODOTHYRONINE IN RAT LIVER

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Abstract—Many studies suggest that one enzyme is involved in the phenolic ring deiodination of iodothyronines in rat liver and kidney and another one in the tyrosyl ring deiodination. This study describes some characteristics of the phenolic ring (5′-) deiodination of rT_3 and 3′,5′-T_2 by rat liver microsomes. At pH 7.2 the K_m values of the 5′-deiodination of rT_3 and 3′,5′-T_2 were 0.103 and 0.77 μM, respectively. 3′,5′-T_2 and rT_3 inhibited the respective 5′-deiodination reactions competitively, the K_i values being 1.05 and 0.134 μM, respectively. Several radiographic contrast agents markedly inhibit the 5′-monodeiodination of rT_3 and 3′,5′-T_2, the type of inhibition being competitive. Of these compounds iopanoic acid, ipodic acid and iophenoxic acid are the most potent inhibitors with K_i values of approximately 2 μM for both reactions. The non-iodine containing compound 8-anilino-1-naphthalene sulphonic acid (ANS) appeared to be a very strong competitive inhibitor of both 5′-deiodinations (K_i 4.3–4.7 μM), whereas salicylic acid, which as ANS inhibits the binding of iodothyronines to T_4-binding globulin, inhibited these reactions to a much lesser extent (K_i 300–500 μM). On the other hand, diiodosalicylic acid was a very strong inhibitor. The β-adrenergic blocker D,L-propranolol was a weak noncompetitive inhibitor of both 5′-deiodinations (K_i 0.4–0.7 mM). These reactions were also inhibited by various 2,6-diiodophenol derivatives, triiodophenol being the strongest and diiodotyrosine the weakest inhibitor tested. Comparing the K_i values of various inhibitors for the 5′-deiodination of rT_3 and 3′,5′-T_2, a positive correlation between these values was found ($r = 0.97$). It was concluded that rT_3 (to 3,3′-T_2) and 3′,5′-T_2 (to 3′-T_1) monodeiodinating activities are very similar to each other and that there may just be one monodeiodinase catalyzing the 5′-deiodination of iodothyronines in rat liver.

Deiodination of T_4† is an important pathway for the production of T_3, rT_3 and lower substituted iodothyronines [1–3]. The deiodination reactions in rat liver are catalyzed by iodothyronine deiodinase activity, which is associated with the endoplasmic reticulum [4–6]. Since the deiodination is a reductive process and is enhanced by thiol compounds, reduced glutathione is thought to be the endogenous cofactor for this reaction [7–9].

Two types of deiodination reactions may be distinguished, viz. deiodination of the phenolic ring (5′- or 3′-deiodination) and deiodination of the tyrosyl ring (5- or 3-deiodination). Many investigators suggest that 5- and 5′-deiodinations in rat liver and kidney are mediated by separate enzymes, viz. an iodothyronine 5- and 5′- deiodinase, respectively [8, 10, 11]. However, subcellular fractionation [4], as well as solubilization and partial purification [12]‡ did not result in separation of the 5- and 5′-deiodinating activities. In this study, the 5-deiodination of rT_3 and 3′,5′-T_2 by rat liver microsomes has been studied to establish whether the effect of various inhibitors is similar by comparing the inhibitor constants on these reactions. The results in the present paper suggest that in rat liver one iodothyronine deiodinase catalyzes the 5′-monodeiodination of rT_3 and 3′,5′-T_2.

MATERIALS AND METHODS

Materials. All iodothyronines and thyronine except for 3-T_1 were purchased from Henning GmbH (Berlin, F.R.G.). 3-T_1 was obtained by courtesy of Dr. P. Block, Jr. (University of Toledo, OH, U.S.A.). Iopanoic acid (Telepaque) and sodium tyropanoate (Bilopaque) were kindly provided by Sterling Winthrop Laboratories (New York, NY). Sodium ipodate (Oragrafin), amidotrizoic acid (Urografin), iotroxic acid (Biliscopin), ioglycamic acid (Bilivistan) and iodipamide (Biligrafin) were a gift from Schering (Berlin, F.R.G.). Diatrizoic acid (Angiografin), iocetamic acid (Cholebrin), iodamide (Urombrin), metrizoic acid (Isopaque), acetrizoic acid (Plexombrine) and iophenoxic acid (Trilombrine) were kindly supplied by the Research Laboratories of Dagra (Diemen, Holland). Salicylic acid was purchased from B.D.H. (Poole, U.K.), sulfosalicylic acid from Pierce Chemical Company (Rockford, IL) and D,L-propranolol from I.C.I. (Macclesfield, U.K.). The following substances were obtained from Sigma (St. Louis, MO): 3,5-diiodotyrosine, 3,5-diiodosalicylic acid, DTT and ANS. 2,4,6-Triiodophenol, 2,6-diisopropylphenol and 3,5-diiodo-4-hydroxybenzoic acid were purchased from I.C.N. Pharmaceuticals (Plainview, NY) and 2,6-diiohydroquinone and 2,6-diiodo-4-nitrophenol were from Pfaltz & Bauer (Stamford, CT).

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† Abbreviations: T_4, thyroxine; T_3, 3,5,3′-triiodothyronine; rT_3 (reverse T_3), 3,3′,5′triiodothyronine; 3′,5′-T_2, 3′,5′-diiodothyronine; 3,3′-T_2, 3,3′-diiodothyronine; 3,5-T_2, 3,5-diiodothyronine; 3′-T_1, 3′-monoiodothyronine; 3-T_1, 3-monoiodothyronine; DTT, dithiothreitol; ANS, 8-anilino-1-naphthalene sulphonic acid.

‡ Fekkes, Hennemann and Visser, in preparation.

Preparation of rat liver microsomes. Male Wistar rats weighing approx. 200 g were used. Preparation of rat liver microsomal fraction in 25 mM Tris/HCl–3 mM EDTA–3 mM DTT (pH 7.4) was done essentially as described before [7]. The protein content of this fraction was measured after solubilization in 0.5 M NaOH, by using the method of Spector [13] with bovine serum albumin as a standard.

Deiodination studies. Conversion of rT_3 and T_3 into 3,3′-T_2 and of 3′,5′-T_2 into 3′-T_1 by rat liver microsomal fraction were studied essentially as by Visser *et al.* [11]. In short, the iodothyronine was reacted with microsomes (5–15 μg protein/ml) and various substances to be tested for 10–20 min at 37° in 0.1 M sodium phosphate–3 mM EDTA–3 mM DTT (pH 6.5 or 7.2), unless stated otherwise. The reaction was stopped by the addition of 4 vol. 0.06 M barbitone buffer (pH 8.6 at 20°) containing 0.1% (w/v) sodium dodecyl sulphate (SDS) and 0.1% (w/v) bovine serum albumin. The amounts of 3,3′-T_2 and 3′-T_1 produced were measured in duplicate with a specific radioimmunoassay in 50 μl of the extract [14, 15]. The amounts of 3,3′-T_2 and 3′-T_1 measured varied between 2 and 200 pg/tube (detection limit for both iodothyronines is 1 pg/tube). Cross-reactivities of the precursors and of the various agents tested with the products in the radioimmunoassay were in most instances negligible [14, 15]. Only in the case of the effect of rT_3 on the 5′-deiodination of 3′,5′-T_2, the amount of 3,3′-T_2 produced from rT_3 cross-reacted considerably in the radioimmunoassay of 3′-T_1. In this special case, different amounts of 3,3′-T_2 were included in the 3′-T_1 standards (this resulted in a modified 3′-T_1 standard curve), both the amounts of 3,3′-T_2 generated from rT_3 and of 3′-T_1 from 3′,5′-T_2 were measured and the actual amount of 3′-T_1 formed was gathered from the modified 3′-T_1 standard curve. In control experiments substrate was added only after the addition of the barbitone–SDS buffer. The amounts of 3,3′-T_2 and 3′-T_1 measured in the control experiments were less than 5 and 20%, respectively of those produced in the complete reaction mixture and were subtracted from these latter values. Under all conditions tested, both 3,3′-T_2 and 3′-T_1 were degraded for less than 5% during the incubation period.

Treatment of data. For the determination of K_m and V_{max} values the straight lines of double reciprocal plots were drawn by the method of least squares applied to unweighted means. In case of competitive inhibition K_i values were estimated by using the equation: apparent $K_m = K_m (1 + [I]/K_i)$, where the apparent K_m is -1/intercept on the abscissa in the Lineweaver–Burk plot and $[I]$ the concentration of the inhibitor. In case of noncompetitive inhibition K_i values were determined by using the equation: 1/apparent $V_{max} = 1/V_{max}\ (1 + [I]/K_i)$, where the apparent V_{max} is 1/intercept on the ordinate in the Lineweaver–Burk plot.

RESULTS

Deiodination studies of rT_3 and 3′,5′-T_2. Table 1 shows the K_m, V_{max} and K_i values of the 5′-deiodination of rT_3 and 3′,5′-T_2 at pH 6.5 and 7.2. The results demonstrate that at both pH values 3′,5′-T_2 inhibits the 5′-deiodination of rT_3 competitively with a K_i value being close to its K_m value in the 5′-deiodination to 3′-T_1. rT_3 also inhibits the 5′-deiodination of 3′,5′-T_2 competitively with a K_i value similar to the K_m value of the rT_3 5′-deiodination. The data shown in Table 1 were obtained in the presence of 3 mM DTT. Repetition of these experiments with varying concentrations of DTT yielded 2–3-fold higher K_m and V_{max} values after extrapolating the data to infinite DTT concentrations. The K_m values of DTT varied between 2 and 4 mM for both 5′-deiodinations (results not shown).

Effects of iodothyronines. In Table 2 the K_i values of various iodothyronines for the 5′-deiodination of rT_3 and 3′,5′-T_2 are shown. All inhibitions are competitive and K_i values of these analogs are similar for both deiodinations. In addition diiodotyrosine was tested. It can be seen (Table 2) that this compound is about 100 times less active than T_4 and even two times less active than thyronine.

Effects of radiographic agents. Figure 1 shows the effect of increasing concentrations of various radiographic agents and T_4 on the conversion of rT_3 into 3,3′-T_2. The K_i values of these compounds, except for diatrizoic acid, are given in Table 2. At the same time the K_i values for the 5′-deiodination of 3′,5′-T_2 are shown. All inhibitions with these agents are competitive with respect to substrate. An example of the type of inhibition of iopanoic acid and ipodic acid is shown in Fig. 2. As can be extracted from Fig. 1 and Table 2, 5′-deiodination of rT_3 and 3′,5′-T_2 were inhibited best by iopanoic acid, ipodic acid and iophenoxic acid and to a somewhat lesser extent by T_4. There was no significant difference between the inhibitory potency of these three radiographic agents. The radiographic agents amidotrizoic acid, iotroxic acid, iodamide, metrizoic acid and acetrizoic acid inhibited the 5′-deiodination of rT_3

Table 1. K_m, V_{max} and K_i values for rT_3 and 3′,5′-T_2 5′-deiodinations catalyzed by rat liver microsomal fraction

Substrate	Inhibitor	pH	K_m (μM)	K_i (μM)	V_{max} (nmoles/min/mg protein)
rT_3		6.5	0.063 ± 0.011		0.271 ± 0.113
	3′,5′-T_2			0.42 ± 0.14	
3′,5′-T_2		6.5	0.36 ± 0.07		0.155 ± 0.040
	rT_3			0.033 ± 0.007	
rT_3		7.2	0.103 ± 0.046		0.380 ± 0.107
	3′,5′-T_2			1.05 ± 0.21	
3′,5′-T_2		7.2	0.77 ± 0.41		0.270 ± 0.055
	rT_3			0.134 ± 0.040	

Values are means ± S.E. (n = 6).

Table 2. K_i values of various competitive inhibitors for the 5′-deiodination of rT_3 and 3′,5′-T_3 by rat liver microsomal fraction

Inhibitor	K_i of inhibitor (μM)	
	rT_3-5′-deiodination	3′,5′-T_2-5′-deiodination
rT_3	—	0.13 ± 0.04
3′,5′-T_2	1.0 ± 0.3	—
T_4 (▼)	2.7 ± 1.0	2.4 ± 0.6
T_3	17.3 ± 9.5	19.6 ± 6.1
3,5-T_2	9.6 ± 1.0	10.1 ± 2.3
3-T_1	—*	12.3 ± 2.3
Thyronine	103 ± 46	90 ± 16
Diiodotyrosine	191 ± 107	177 ± 29
ANS	4.7 ± 1.1	4.3 ± 1.2
Salicylic acid	476 ± 191	302 ± 54
Sulfosalicylic acid	114 ± 16	142 ± 30
Diiodosalicylic acid	0.3 ± 0.1	0.4 ± 0.2
Iopanoic acid (○)	1.8 ± 0.5	1.5 ± 0.4
Ipodic acid (●)	2.2 ± 0.8	2.0 ± 0.3
Iophenoxic acid (△)	2.1 ± 0.5	1.8 ± 0.7
Tyropanoic acid (▲)	27.9 ± 6.4	21.5 ± 14.2
Ioglycamic acid (□)	107 ± 26	24.9 ± 9.2
Iodipamide (■)	25.2 ± 5.6	16.2 ± 3.1

Conversion studies were done at pH 7.2.
Values are means ± S.E. ($n = 4$).
Symbols refer to Fig. 1.
* Could not be determined due to experimental problems.

and 3′,5′-T_2 for less than 20% at a concentration of 100 μM, while iocetamic acid at this concentration inhibited these reactions for approximately 50% (results not shown).

Effects of other inhibitors. Salicylic acid and ANS, agents known to displace T_4 from serum binding proteins, were competitive inhibitors, the latter being about 100 times more active than salicylic acid (see Table 2). Two derivatives of salicylic acid, viz. sulfosalicylic acid and especially diiodosalicylic acid, have a greater inhibitory activity than salicylic acid (Table 2). Addition of D,L-propranolol, a β-adrenergic blocker commonly used in treating hyperthyroid patients, was found to inhibit both 5′-deiodinations noncompetitively with K_i values of 0.4–0.7 mM.

From the data in Table 2 it can be deduced that the K_i values of the various inhibitors for the 5′-deiodination of rT_3 correlate positively with those for the 5′-deiodination of 3′,5′-T_2 ($r = 0.97$).

Figure 3 shows the effect of increasing concentrations of 2,6-diiodophenol derivatives on the conversion of rT_3 into 3,3′-T_2. The inhibitory activity is strongly dependent on the substituent at C_4, iodine being the best of the substituents tested. The order of activity was I ⩾ OH > NO_2 > COOH ⩾ alanine. Unfortunately, 2,6-diiodophenol was not available.

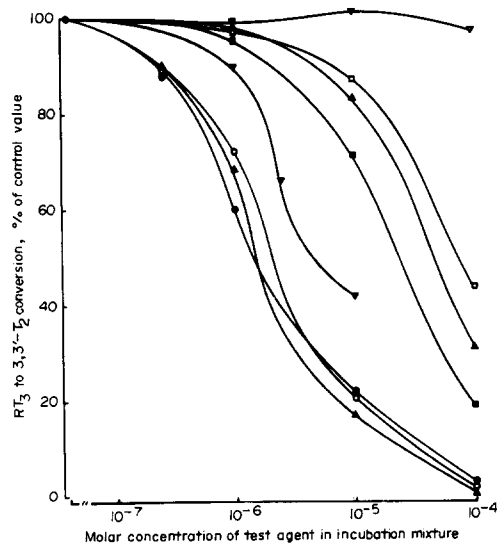

Fig. 1. Inhibition of the conversion of rT_3 into 3,3′-T_2 by increasing concentrations of radiographic agents and T_4 (▼). For explanation of symbols see Table 2; in addition, the effect of diatrizoic acid is shown (△). Conversion studies were done with 0.1 μM rT_3 at pH 7.2. Data are mean of two closely agreeing experiments performed in duplicate.

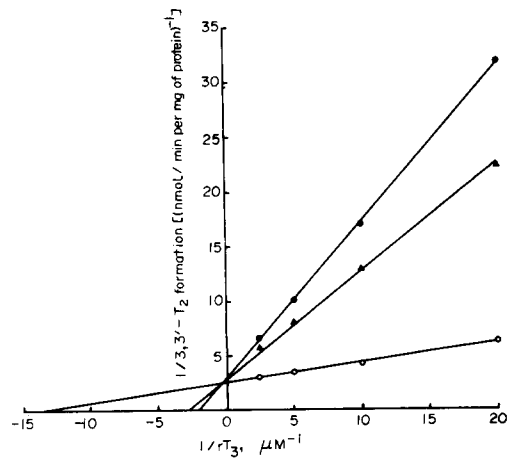

Fig. 2. Lineweaver–Burk plot of the conversion of rT_3 into 3,3′-T_2 at pH 7.2 in the absence (○) and presence of 10 μM iopanoic acid (●) and 10 μM ipodic acid (△). Results are mean of two experiments performed in duplicate.

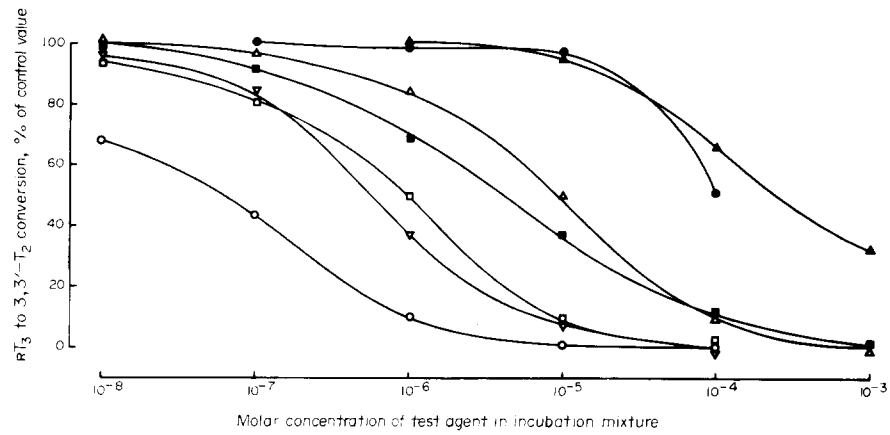

Fig. 3. Inhibition of the conversion of rT_3 into 3,3'-T_2 by increasing concentrations of 2,6-diiodophenol derivatives. Deiodination studies were performed at pH 6.5 using 0.1 μM rT_3. ○, 2,4,6-Triiodophenol; ●, 2,6-diisopropylphenol; ▼, 3,5-diiodosalicylic acid; △, 3,5-diiodo-4-hydroxybenzoic acid; ▲, 3,5-diiodotyrosine; □, 2,6-diiodohydroquinone; ■, 2,6-diiodo-4-nitrophenol. All points represent the mean of three separate determinations.

Instead, 2,6-diisopropylphenol was tested, the activity of which was found to be similar to that of diiodotyrosine (see Fig. 3).

DISCUSSION

In vitro studies of the enzymatic reductive deiodination of iodothyronines in rat liver and kidney have led to the hypothesis that T_4 is sequentially deiodinated by two enzymes, viz. an iodothyronine 5- and 5'-deiodinase [8, 10, 11]. The fact that T_4 and rT_3 are 5'-monodeiodinated by the same enzyme has been confirmed by several investigators [10, 11, 16, 17]. Recently, Chopra [18] showed that 3',5'-T_2 and 3,3'-T_2 are similarly substrates for a common 5'-deiodinase, and the involvement of a single enzyme in the 5'-deiodination of rT_3 and 3',5'-T_2 has also been suggested by Visser [15]. In this study a similarity of the K_m and K_i for rT_3, and the K_m and K_i for 3',5'-T_2 at two pH values was found. This is a strong indication that a single hepatic enzyme catalyzes 5'-monodeiodination of both rT_3 and 3',5'-T_2. When taking these data together one could conclude that the 5'- (or 3'-) monodeiodinations of T_4, rT_3, 3',5'-T_2 and of 3,3'-T_2 in rat liver are all mediated by one enzyme. This is strengthened by the fact that the 5'-monodeiodination of rT_3 and 3',5'-T_2 are inhibited in a similar manner by a number of iodothyronines, several radiographic agents and various other substances as tested in this study. We have chosen radiographic agents, because these iodine-containing compounds have been reported to influence thyroid hormone deiodination *in vivo* [19] and, in addition, some of these agents are potent inhibitors of the liver system converting T_4 to T_3 [2, 10].

We found that iopanoic acid, ipodic acid and iophenoxic acid are very strong competitive inhibitors of the 5'-deiodination of rT_3 and 3',5'-T_2 with K_i values of approx. 2 μM irrespective of the agent or reaction tested. The inhibitory activity of these compounds was similar to that of T_4 (Table 2). The structure of iophenoxic acid differs from that of iopanoic acid (Fig. 4) in that it contains an amino instead of a hydroxyl group in the *meta* position to the side chain. Clearly this does not affect inhibitory potency. The structural differences between iopanoic acid and iopdic acid are much more pronounced, though this is not reflected by their activity (Fig. 4 and Table 2). Comparing the structures of iopanoic acid, tyropanoic acid and ipodic acid (Fig. 4), one

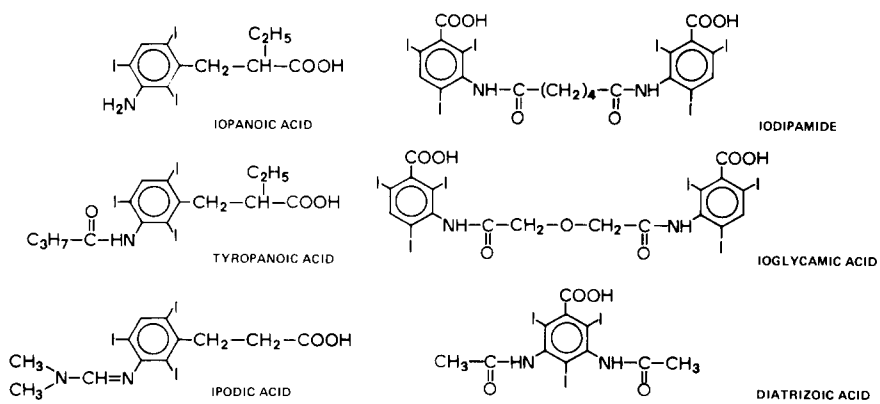

Fig. 4. Structures of some radiographic contrast agents.

should expect tyropanoic acid to be about as active as the other compounds. However, tyropanoic acid appeared to have less than a tenth of the activity of iopanoic acid and ipodic acid (Table 2). As can be gathered from Fig. 4 this difference is situated in the acyl group of tyropanoic acid, which strongly diminishes the electron-donating properties of the amino group. The activities of iodipamide and ioglycamic acid are comparable to that of tyropanoic acid (Table 2). These compounds are also structurally related to tyropanoic acid as they contain two *N*-acyl triiodobenzoic acid groups (Fig. 4). Addition of a second *N*-acetyl amino group to such a ring as in diatrizoate (Fig. 4) results in a virtually inactive structure (Fig. 1). As can be concluded from Table 2 all inhibitors, except for ioglycamic acid, have similar activity towards the 5′-deiodination of rT_3 and 3′,5′-T_2. An explanation for the difference in inhibitory activity of ioglycamic acid cannot be given. Deletion of the side-chain of iophenoxic acid yields 2,4,6-triiodophenol, a compound which is even more inhibitory to the 5′-deiodinase than the most potent radiographic agent (see Figs. 1 and 3). Replacement of the 4-iodine atom by a nitro group, a carboxyl group or by an alanine side-chain (yielding diiodotyrosine) strongly reduces inhibitor activity. Replacement by a hydroxyl group (yielding diiodohydroquinone) affects the inhibitory potency least (Fig. 3). Very interesting is the difference in inhibitory activity between 3,5-diiodo-4-hydroxybenzoic acid and 3,5-diiodosalicylic acid (3,5-diiodo-2-hydroxybenzoic acid), as shown in Fig. 3. The only difference in structure is the position of the hydroxyl group. The reason for the high activity of diiodosalicylic acid is unclear. It does, however, closely resemble the highly potent triiodophenol from which it is derived by substituting COOH for I in one of the *ortho* positions to the OH group.

We found that ANS is a very strong competitive inhibitor (K_i ~4 μM) of iodothyronine 5′-deiodination. This is in discordance with previous reports [20, 21] on 100–1000-fold higher values for the K_i of ANS. However, these studies were performed with homogenate containing an abundance of ANS binding proteins [3], whereas we used low amount of microsomal fraction. On the other hand, our data concerning salicylic acid are in agreement with these other studies [20, 21]. The effects of D,L-propranolol on the 5′-deiodination are contradictory [20, 22, 23]. We found that this compound is a weak noncompetitive inhibitor (K_i 0.4–0.7 mM) of the 5′-deiodination of rT_3 and 3′,5′-T_2 and is therefore of no importance for the *in vivo* inhibition of the deiodination, because the plasma levels of D,L-propranolol during treatment of hyperthyroid patients never exceed a concentration of 0.6 μM [25].

In conclusion, our results strongly suggest that a single hepatic enzyme catalyzes the 5′-deiodination of rT_3 and 3′,5′-T_2. Consequently, all iodothyronines may be 5′-monodeiodinated by one enzyme in rat liver.

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