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ORIGINAL ARTICLE

Positive correlation between type 1 and 2 iodothyronine deiodinases activities in human goiters

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Abstract Type 1 (D1) and 2 (D2) iodothyronine deiodinases are selenocysteine-containing enzymes that catalyze the deiodination of T4 to T3 in the thyroid and in peripheral tissues. Despite their importance to the plasma T3 pool in human beings, there are few studies about their behavior in human thyroids. In order to better understand iodothyronine deiodinase regulation in the thyroid gland, we studied thyroid tissue samples from follicular adenoma (AD, $n = 5$), toxic diffuse goiter (TDG, $n = 6$), nontoxic multinodular goiter (NMG, $n = 40$), papillary thyroid carcinoma (PTC, $n = 8$), and surrounding normal tissues (NT, $n = 7$) from 36 patients submitted to elective thyroidectomy. D1 and D2 activities were determined by quantification of the radioiodine released by ^{125}I-rT3 or ^{125}I-T4 under standardized conditions, and expressed as pmol rT3 deiodinated per minute and mg protein (pmol rT3 min^{-1} mg^{-1} ptn) and fmol T4 deiodinated per minute and mg protein (fmol T4 min^{-1} mg^{-1} ptn), respectively. D1 activity detected in TDG and AD tissues were significantly higher than in NT, PTC or NMG samples. D2 activity was also significantly higher in TDG and AD samples than in PTC, NMG, or NT. There was great variability in D1 and D2 enzymatic activities from distinct patients as well as from different areas from the same goiter. There was a positive correlation ($P < 0{,}0001$, $r = 0.4942$) between D1 and D2 activities when all samples were taken into account, suggesting that—in the thyroid—these two iodothyronine deiodinases may have related regulatory mechanisms, even if conditioned by other as yet unknown factors.

Keywords Human goiters · Type 1 iodothyronine deiodinase · Type 2 iodothyronine deiodinase · Positive correlation

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Introduction

Type 1 iodothyronine deiodinase (D1) and type 2 iodothyronine deiodinase (D2) catalyze T4 activation, and both isoforms are present in human thyroid tissue [1]. Liver and kidney D1 activity is known to decrease in hypothyroidism while, in contrast, D2 increases in pituitary, brain and brown adipose tissue. Thyroid function is normally unaltered in patients with thyroid carcinomas but in most differentiated thyroid carcinomas there is a decrease in function compared to normal thyroid tissue, although they continue to produce thyroglobulin (Tg), which can be iodinated and form T_3 and T_4. The human thyroid gland is morphologically and functionally heterogenous, and even when exposed to the same amounts of thyrotropin (TSH) follicular cells behave differently. D2 is highly expressed in Graves' disease thyroid tissue and in some adenomas (ADs) in which it may contribute to the relatively high circulating T3 serum level [2]. A high activity of D2 has

also been described in the human mesothelioma cell line MSTO-211H, whereas cells derived from normal mesothelium do not express D2 activity [3]. Therefore, D2 seems to be induced by neoplastic transformation in some tissues, but this cannot be extrapolated to all tissues; for example, in human thyroid papillary carcinoma D2 is decreased when compared to normal tissues [2]. A decrease in D1 activity in thyroid tumors, as compared to normal tissue, has also been described [4]. In order to better understand iodothyronine deiodinase regulation in the thyroid gland, we studied D1 and D2 activities in 66 thyroid tissue samples from follicular AD ($n = 5$), toxic diffuse goiter (TDG, $n = 6$), nontoxic multinodular goiter (NMG, $n = 40$), papillary thyroid carcinoma (PTC, $n = 8$), and surrounding normal tissues (NT, $n = 7$) from a total of 36 patients submitted to elective thyroidectomy.

Subjects and methods

Patients

Samples ($n = 66$) from different areas of human thyroid (PTC, $n = 8$; AD, $n = 5$; TDG, $n = 6$; NMG, $n = 40$; and NT, $n = 7$) were collected from 36 patients attending the Endocrine or the Head and Neck Surgery Divisions at Clementino Fraga Filho University Hospital. NTs were obtained from five distinct areas of three patients with PTC, from one area of a patient diagnosed with follicular carcinoma, and one area of a patient submitted to elective parathyroidectomy and no thyroid disease associated (Table 1). All TDG patients used the anti-thyroid drug Tapazol® (methimazole) until 1 week before the surgery, when iodine treatment with Lugol® started. Only one TDG patient (number 15) used propylthiouracil (PTU) treatment, nevertheless it was discontinued 42 days before surgery, when Tapazol® and then iodine treatment took place, excluding, therefore, D1 activity interference due to pharmacological interventions.

All patients were euthyroid at the moment of the surgery, which was indicated independently by attending physicians based on clinical indications. Different thyroid diseases were diagnosed after the surgery procedure according to histopathologically criteria by analysis of two pathologist physicians and only after their final written report. Tumors were histologically classified according to World Health Organization (WHO) recommendations [5]. Pathological classification of malignant thyroid tumors (pTNM) was determined by the tumor/node/metastases (TNM) system [6]. Clinical data, image exams, and serum hormone levels were retrospectively reviewed in medical records. The study protocol was approved by the Institutional Human Research and Ethics Committee of the Clementino Fraga Filho University Hospital (no. 195/04).The information obtained from the study did not influence or affect the patients' diagnosis or treatment and all patients gave their informed consent.

Thyroid samples processing

Fresh thyroid tissue samples were collected from the surgery room, immediately frozen in liquid nitrogen and stored at −80°C until analysis. Samples were cleaned of fibrous tissue or hemorrhagic areas, minced, and homogenized using an Ultra-Turrax homogenizer (IKA, Staufen, Germany) in an ice bath. The homogenate was centrifuged at $9{,}300 \times g$ for 6 min at 4°C, and the supernatant was stored at −80°C until assayed.

Type 1 iodothyronine deiodinase activity (D1)

D1 activity was determined using methods previously published [7]. In short, 25 mg from each tissue analyzed were homogenized in 0.1 M sodium phosphate buffer containing 1 mM EDTA, 0.25 M sucrose, and 10 mM dithiothreitol (DTT), pH 6.9. Homogenates (100 µg protein) were incubated, in duplicate, for 1 h at 37°C, with 1 µM rT3 (Sigma-Aldrich, USA), freshly purified tracer ^{125}I-rT3 (Perkin-Elmer Life Sciences, Boston, MA, USA), and 10 mM DTT in 0.1 M sodium phosphate buffer containing 1 mM EDTA, pH 6.9. Total reaction volume was 300 µl. In our assay conditions, only D1 activity is measured since deiodinase activities were completely blocked in the presence of 100 mM PTU, a specific D1 inhibitor. D1 final activity was expressed as the difference between the sample activity and its respective background.

Type 2 iodothyronine deiodinase activity (D2)

D2 activity was determined using methods previously published [8]. In short, 25 mg from each tissue analyzed were homogenized in 0.1 M sodium phosphate buffer containing 1 mM EDTA, 0.25 M sucrose, and 20 mM DTT, pH 6.9. Homogenates (200 µg protein) were incubated, in duplicate, for 2 h at 37°C with 1nM T4 (Sigma-Aldrich, USA), freshly purified tracer ^{125}I-T4 (Perkin-Elmer Life Sciences, Boston, MA, USA), and 20 mM DTT in 0.1 M sodium phosphate buffer containing 1 mM EDTA, pH 6.9. Total reaction volume was 300 µl. In our assay conditions, only D2 activity is measured since deiodinase activities were completely blocked in the presence of T4 in excess (100 nM). D2 final activity was expressed as the difference between the sample activity and its respective background.

Protein was measured by the Bradford method, [9] after incubation of homogenates with NaOH (2.5 M) for 30 min at room temperature.

Table 1 Goiter nodular areas are specified, when available

Patient no.	Sex/age (years)	TSH	Goiter area	Size (cm)	Histology	Stage	Invaded organ
PTC (Papillary thyroid carcinoma)							
1	F/38	0.12	Right lobe nodule	2.5	Classic type	pT2NxMx	No
1			Isthmus				
2	M/24	1.21	Right lobe nodule	2.0	Classic type	pT1NxMx	No
2			Left lobe nodule				
3	F/64	0.66	Right lobe nodule	4.0	Follicular variant	pT2NxMx	No
3			Right lobe nodule	3.5			
4	M/27	3.02	Left lobe nodule	5.0	Diffuse sclerosing variant	pT3NxMx	No
5	F/65	1.21	Right lobe nodule	1.0	Classic type	pT2NxMx	No
AD (follicular adenoma)							
6	F/42	1.54	Left lobe nodule	1.0			
6			Left lobe nodule	0.6			
7	F/43		Left lobe nodule	3.5			
8	F/55	2.76	Left lobe nodule	1.7			
9	F/28		Left lobe nodule	1.3			
TDG (toxic diffuse goiter)							
10	F/27	0.80	Left lobe				
11	F/49	1.88	Left lobe				
12	M/44	1.22	Right lobe				
13	F/33	0.97	Right lobe				
14	F/22	0.89	Right lobe				
15	F/29	1.34	Left lobe				
NMG (nontoxic multinodular goiter)							
16	F/46	1.22	Isthmus nodule	3.5			
16			Isthmus				
16			Right lobe				
17	M/39	0.67	Right lobe nodule	2.0			
17			Right lobe				
18	M/54		Right lobe nodule	1.1			
18			Left lobe nodule	1.5			
19	F/33	1.61	Left lobe nodule	5.4			
20	M/55		Isthmus				
20			Right lobe				
20			Right lobe nodule	2.8			
21	F/59	0.45	Right lobe nodule	3.5			
21			Right lobe				
21			Left lobe nodule	2.0			
21			Left lobe				
22	F/50		Left lobe nodule	0.8			
23	F/71		Right lobe nodule	2.0			
24	F/42		Right lobe				
25	M/64	0.74	Right lobe nodule	2.5			
25			Right lobe				
26	F/52	0.16	Right lobe nodule	1.5			
26			Right lobe				
27	F/13	1.15	Isthmus				
27			Right lobe nodule	0.6			
27			Right lobe				

Table 1 continued

Patient no.	Sex/age (years)	TSH	Goiter area	Size (cm)	Histology	Stage	Invaded organ
27			Left lobe				
28	F/47	0.92	Left lobe nodule	1.0			
29	F/47	2.07	Isthmus				
29			Isthmus nodule	2.0			
29			Isthmus				
29			Left lobe nodule	1.3			
29			Left lobe				
29			Right lobe nodule	2.0			
29			Right lobe				
30	F/71	0.40	Right lobe nodule	0.4			
30			Right lobe				
31	F/56		Left lobe				
32	F/50		Right lobe				
33	F/62		Left lobe				
34	F/54		Right lobe				
NT (surrounding normal thyroid tissue)							
35	M/24	0.67	Left lobe		Normal thyroid tissue surrounding parathyroid		
3	F/64	3.02	Right lobe				
4	M/27	0.92	Left lobe				
36	F/47	1.21	Right lobe		From follicular carcinoma		
5	F/65	1.21	Right lobe				
5			Right lobe				
5			Left lobe				

TSH reference: 0.4–4.0 μUI/ml, Stage according to pTNM system. Goiter nodular areas are specified, when available

Statistical analysis

D1 and D2 activities values were analyzed using One-Way ANOVA, results are presented as mean ± SEM. Post hoc multiple comparisons (Student–Newman–Keuls) were used when ANOVA indicated significant differences among groups. The results of serum TSH, which does not have a normal distribution, were analyzed by non-parametric ANOVA (Kruskal–Wallis test), and presented as median [maximum–minimum]. Analysis of correlation between D1 and D2 activities was done using the Pearson's correlation coefficient. All analyses were performed using the Graph-pad Prism software (version 4 for Macintosh). A value of $P \leq 0.05$ was considered statistically significant.

Results

Patients

Table 1 shows the clinical and pathological characteristics of all patients enrolled in this study. Thyroid samples covered patients aged 13–71 years, gender proportion incidence of 1M:3F and different thyroid diseases diagnoses, including benign and malignant thyroid tumors, NMGs, as well as autoimmune thyroid pathologies (Graves' disease). D1 and D2 activities were also measured in different areas of the thyroid gland, such as right and/or left lobes, isthmus and nodular and non nodular goiter areas, as specified in Table 1. D1 activity was measured in 8 PTC samples (cases 1–5); 5 AD samples (cases 6–9); 6 DTG samples (cases 10–15), 40 MNG samples (cases 16–34), and 7 NT samples (cases 3, 4, 5, 35, and 36), used as control group. D2 activity was also measured in the same samples, exception being cases 14, 29 (right lobe nodule area), 35, and 72 because there was not enough thyroid tissue to run assays. All thyroid cancer patients had no evidence of metastases according to clinical, cervical lymph nodes biopsy and image examination, when available.

D1 and D2 activities in human goiters

There was no statistical difference in serum TSH among the patients with PTC [1.21 μUI/ml (0.122–3.02 μUI/ml)], AD [1.54 μUI/ml (1.33–2.76 μUI/ml)], TDG [1.095 μUI/ml

(0.80–1.81 μUI/ml)], or NMG [0.74 μUI/ml (0.16–2.07 μUI/ml)] compared with the NT [1.065 μUI/ml (0.67–3.02 μUI/ml)] group, and all were within the normal range.

D1 activity was significantly higher in AD (24.8 ± 5.5 pmol min^{-1} mg^{-1} ptn, $n = 5$) and TDG (27.6 ± 5.2 pmol min^{-1} mg^{-1} ptn, $n = 6$) tissues, than in the NT (7.86 ± 1.9 pmol min^{-1} mg^{-1} ptn, $n = 7$), PTC (5.24 ± 1.1 pmol min^{-1} mg^{-1} ptn, $n = 8$), and NMG (13.9 ± 1.7 pmol min^{-1} mg^{-1} ptn, $n = 40$) (Fig. 1).

D2 activity was also significantly higher in AD (0.97 ± 0.09 fmoles.min^{-1} mg^{-1} ptn, $n = 5$) and TDG (1.55 ± 0.35 fmoles min^{-1} mg^{-1} ptn, $n = 5$) samples than in PTC (0.42 ± 0.12 fmoles min^{-1} mg^{-1} ptn, $n = 8$), but only TDG D2 activity was significantly increased in relation to NT (0.75 ± 0.04 fmoles min^{-1} mg^{-1} ptn, $n = 6$) or NMG (0.59 ± 0.05 fmoles min^{-1} mg^{-1} ptn, $n = 40$) (Fig. 2).

There was a great variability in D1 and D2 enzymatic activities from distinct patients, as can be seen in Figs. 1 and 2. Different areas within the same NMG also showed a great deal of variability that was at least as great as among tissues from different patients within a given group (results not shown). In ten patients, we were able to evaluate both nodular and paranodular (normal, NT) tissue samples: in six the D1 activity in the nodules was two times (or more) greater than in the paranodular tissues, and in two this activity was decreased by 20% or more in relation to the "normal" tissue. In relation to the D2 activity, the same comparison showed completely different results: in one patient the D2 activity in the paranodular tissue was more than three times that of the nodular tissue, while in another two the nodule was twice as active as the paranodular

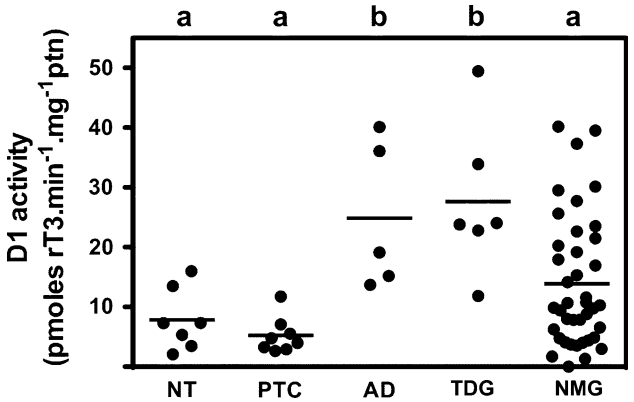

Fig. 1 Type 1 iodothyronine deiodinase activity in human thyroid tissues. *NT* normal tissue, *PTC* papillary thyroid carcinoma, *AD* adenoma, *TDG* toxic diffuse goiter, *NMG* nontoxic multinodular goiter. Results are presented as mean ± SEM. Post-hoc multiple comparisons (Student–Newman–Keuls) were used when ANOVA indicated significant differences among groups. *Different letters* indicate statistically significant differences between groups ($P < 0.01$: TDG vs. PTC, NT, and NMG; AD vs. PTC. $P < 0.05$: AD vs. NT and NMG)

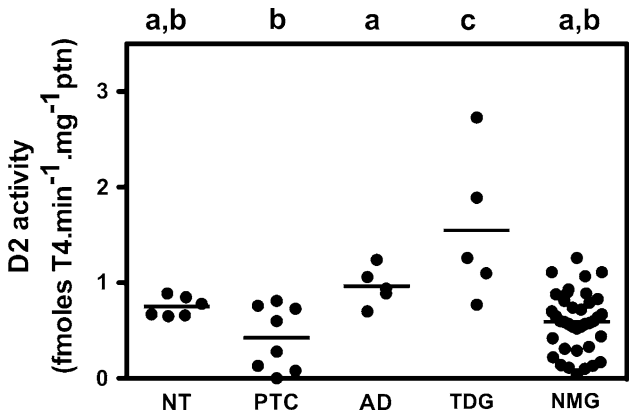

Fig. 2 Type 2 iodothyronine deiodinase activity in human thyroid tissues. *NT* normal tissue, *PTC* papillary thyroid carcinoma, *AD* adenoma, *TDG* toxic diffuse goiter, *NMG* nontoxic multinodular goiter. Results are presented as mean ± SEM. Post-hoc multiple comparisons (Student–Newman–Keuls) were used when ANOVA indicated significant differences among groups. *Different letters* indicate statistically significant differences between groups ($P < 0.01$: TDG vs. PTC, NT, and NMG. $P < 0.05$: AD vs. PTC and TDG)

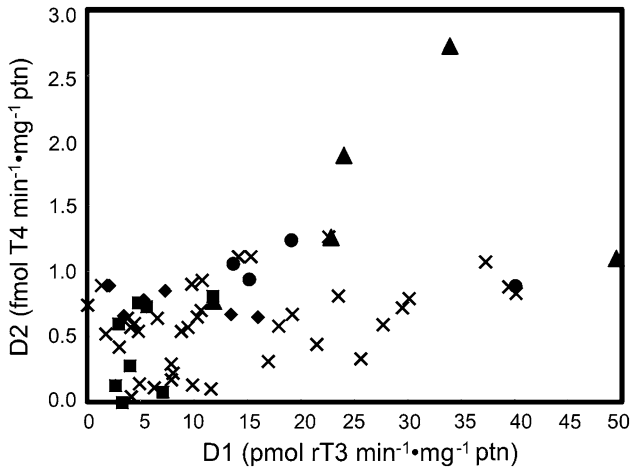

Fig. 3 Positive correlation between D1 and D2 activities in human thyroid tissues; $r = 0.4942$, $P < 0{,}0001$. *Filled diamond* normal tissue (NT), *filled square* papillary thyroid carcinoma (PTC), *filled circle* adenoma (AD), *filled triangle* toxic diffuse goiter (TDG), *times symbol* nontoxic multinodular goiter (NMG)

tissue. The activities of the two kinds of thyroid tissues were almost equal in the remaining patients.

There was a very significant positive correlation ($r = 0.4942$, $P < 0{,}0001$) between D1 and D2 activities when all samples studied were taken into account (Fig. 3).

Discussion

Normal human thyroid tissue expresses both D1 and D2. D1 expression is regulated by numerous factors, perhaps

the most important of which in human pathophysiology are the thyroid hormones. T3 induces D1 expression in liver and other tissues contributing to the T3 excess commonly found in hyperthyroidism. In addition to being induced by T3, in the thyroid gland D1 activity is also induced by TSH [10]. Herein we show that there can be significant differences in thyroid D1 activity among different diseases, even when there are no significant alterations in serum TSH. Furthermore, we also detected a rather heterogeneous distribution of D1 activity within any given multinodular goiter suggesting that besides TSH others systemic and/or local modulators can regulate the thyroid gland D1 activity.

The finding of D2 activity in the human mesothelioma cell line MSTO-21H suggests that D2 can be induced by neoplastic transformations since cells derived from normal mesothelium do not express D2 activity [3], but these findings cannot be extrapolated to all tissues nor to the other iodothyronine deiodinases. In fact, in several human tissues such as liver, kidney and thyroid the enzymatic activity of D1 is decreased when the tissues undergo neoplastic transformation, [4, 11] and a decreased D2 activity has been reported in PTC [2]. Our data show that at least some of the PTC samples examined had low D1 and D2 activities in relation to the paranodular (normal, NT) tissues. The small number of PTC and NT samples studied, and a possible intrinsic variability within the groups, may preclude this difference to reach statistical significance.

Graves' disease patients generally have a high serum T3/T4 ratio. The cause of this relative increase in circulating T3 has been evaluated but is not clear yet. According to Lauberg et al., the thyroid itself is an important or major source of T3 production in Graves' disease and toxic diffuse goiter patients, while normally it contributes relatively little to serum T3. From their data they conclude that—in hyperthyroid patients—the major part of serum T3 is originated by intrathyroidal deiodination of T4, rather than from an increase of the Tg T3:T4 ratio, and suggest this would be mainly due an increased type 1 deiodinase activity [12]. In a previous study, Salvatore et al. [13] have reported that a very increased expression and activity of D2 in 2 human hyperactive thyroids (Graves' disease and hyperthyroidism secondary to a TSH-producing pituitary tumor), and suggested that this could be the principal cause of the increased serum T3 in Graves' disease and toxic ADs of the thyroid. Our data shows significant increases in D1 and D2 activity in the diffuse toxic goiters, and of D1 in the thyroid ADs, showing that both iodothyronine deiodinase isoforms can contribute to T3 generation, at least in Graves' disease. Furthermore, in spite of the small number of TDG analyzed we found a significantly positive correlation between the two deiodinase isoforms (data not shown). Thus, it would seem that the predominance of one or the other deiodinase activity in the hyperactive thyroid is within the range of biological variability, rather than a systematic effect responsible for the increased serum T3 in hyperthyroid patients.

D2 levels are reported to be low in papillary carcinoma of thyroid [2]. In fact, our data shows higher D2 activity in TDG: $P < 0.01$ versus NT, and $P < 0.001$ versus PTC. D2 activity detected in AD samples also was also higher than the enzymatic activity present in PTC samples ($P < 0.05$) but the activity was not significantly different from the NT samples.

In spite of the great variability in D1 and D2 enzymatic activities from distinct patients, as well as from different areas of the same goiter, we found a highly significant positive correlation between D1 and D2 activities when all samples were taken into account. While this may suggest similar or related regulatory mechanisms for both deiodinases isoforms in the human thyroid, this may be an oversimplification considering that:

- A certain proportion of the NMG samples have D1 activity levels comparable to those of the TDG/AD samples, but the same is not true for the D2 activity, which is not increased in AD or in NMG samples.
- In paired NT:AD samples, the D1 activity was higher in the AD than in the respective paranodular (NT) tissue, while D2 activity in both was much better matched.

Understanding deiodinase isoforms regulation among different thyroid diseases, specially concerning to nodule pattern variations and prognosis outcomes, could be useful to improve clinical management in the future. Further studies are still necessary to elucidate the mechanisms involved in the regulation of the differing iodothyronine deiodinase isoforms in the human thyroid gland.

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