CLINICAL STUDY

Type 1 and type 2 iodothyronine deiodinases in the thyroid gland of patients with 3,5,3'-triiodothyronine-predominant Graves' disease

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Abstract

Objective: 3,5,3'-triiodothyronine-predominant Graves' disease (T₃-P-GD) is characterized by a persistently high serum T₃ level and normal or even lower serum thyroxine (T₄) level during antithyroid drug therapy. The source of this high serum T₃ level has not been clarified. Our objective was to evaluate the contribution of type 1 and type 2 iodothyronine deiodinase (D1 (or DIO1) and D2 (or DIO2) respectively) in the thyroid gland to the high serum T₃ level in T₃-P-GD.

Methods: We measured the activity and mRNA level of both D1 and D2 in the thyroid tissues of patients with T₃-P-GD ($n=13$) and common-type GD (CT-GD) ($n=18$) who had been treated with methimazole up until thyroidectomy.

Results: Thyroidal D1 activity in patients with T₃-P-GD (492.7 ± 201.3 pmol/mg prot per h) was significantly higher ($P<0.05$) than that in patients with CT-GD (320.7 ± 151.9 pmol/mg prot per h). On the other hand, thyroidal D2 activity in patients with T₃-P-GD (823.9 ± 596.4 fmol/mg prot per h) was markedly higher ($P<0.005$) than that in patients with CT-GD (194.8 ± 131.6 fmol/mg prot per h). There was a significant correlation between the thyroidal D1 activity in patients with T₃-P-GD and CT-GD and the serum FT₃-to-FT₄ ratio ($r=0.370$, $P<0.05$). Moreover, there was a strong correlation between the thyroidal D2 activity in those patients and the serum FT₃-to-FT₄ ratio ($r=0.676$, $P<0.001$).

Conclusions: Our results suggest that the increment of thyroidal deiodinase activity, namely D1 and especially D2 activities, may be responsible for the higher serum FT₃-to-FT₄ ratio in T₃-P-GD.

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Introduction

The monodeiodination of thyroxine (T₄) to 3,5,3'-triiodothyronine (T₃) activates the major secretory product of the iodine-sufficient human thyroid gland, producing ~80% of the circulating T₃ in humans (1). Type 1 and type 2 iodothyronine deiodinase (D1 (or DIO1) and D2 (or DIO2) respectively) catalyze this reaction (2–4). The roles of D1 and D2 in the production of circulating T₃ in humans are unknown. Both D1 and D2 activities are demonstrated in the human thyroid, and Salvatore *et al.* (5) reported that intrathyroidal T₄ to T₃ conversion by D2 may contribute to the relative increase in thyroidal T₃ production in patients with Graves' disease (GD). On the other hand, Laurberg *et al.* (6) estimated by an indirect method using propylthiouracil (PTU) that D1-generated T₃ in the thyroid gland is the major source of plasma T₃ in hyperthyroid humans. Recently, some cases of relatively high serum T₃ levels were reported in patients with follicular

thyroid carcinoma (7), GD during PTU treatment (8), thyroglobulin (*Tg*) gene mutations (9), and McCune–Albright syndrome (10). In these cases, D2 activity in the thyroid tissues was increased, and the T₄ to T₃ conversion catalyzed by D2 was assumed to be responsible for the T₃ toxicosis.

In most patients with hyperthyroid GD, the elevated serum T₄ and T₃ levels decrease to within their respective normal ranges after appropriate antithyroid drug therapy is initiated. However, we (11) and other investigators (12, 13) have noted that in ~12% of patients with GD, serum T₃ levels remain raised, while serum T₄ levels become normal or even low. We have termed this phenomenon T₃-predominant GD (T₃-P-GD) (11). Previously, we reported that the T₄ 5'-deiodinating activity in the thyroid tissues of patients with T₃-P-GD was higher than that in patients with common-type GD (CT-GD) (11). In our previous study, we used 5 μM T₄ as a substrate, an amount appropriate for human D1, but 100- to 1000-fold higher than that

for D2, thus obscuring the contributions of the D2 pathway to T₃ production. Therefore, to investigate the relative roles of thyroidal D1 and D2 in the establishment of a higher serum free T₃ (FT₃) relative to serum free T₄ (FT₄) in patients with T₃-P-GD, we evaluated thyroidal activities and mRNA levels of both D1 and D2 in patients with T₃-P-GD and CT-GD.

Materials, subjects, and methods

Materials

[¹²⁵I]T₄ and [¹²⁵I]T₃ (reverse T₃ or rT₃) were purchased from Perkin Elmer (Boston, MA, USA). Sephadex LH-20 was purchased from Pharmacia Biotech. All other chemicals were of the highest quality and were obtained from Sigma Chemical Co. or Nakalai Tesque (Kyoto, Japan) unless otherwise indicated.

Subjects

We studied 13 patients with T₃-P-GD and 18 patients with CT-GD who had undergone thyroidectomy between January, 2007 and October, 2007 at Kuma Hospital. GD was diagnosed on the basis of clinical findings and laboratory tests showing high serum FT₄ and FT₃ levels, low TSH concentrations, increased anti-TSH receptor antibody (TRAb) titer, and a high radioactive iodine uptake. At the time of surgery, while the serum FT₄ levels in the patients with T₃-P-GD declined to normal or low during methimazole (MMI) treatment, their serum FT₃ levels remained high or relatively high for more than 3 months. Their serum FT₃-to-FT₄ ratios were all above the normal range. In the patients with CT-GD, MMI treatment resulted in a decline in both serum FT₄ and FT₃ levels to within the normal range and a decline in FT₃-to-FT₄ ratios to within the normal range. The mean dose of MMI was 25 ± 10 mg/day in the patients with T₃-P-GD and 7 ± 3 mg/day in the patients with CT-GD. This study was approved by the ethics committee at Kuma Hospital, and all the patients gave informed consent.

Thyroid function tests

Serum concentrations of TSH, FT₄, and FT₃ were measured with a chemiluminescent immunoassay (ARCHTEC i2000; Abbott Japan). Serum TRAb titer levels were measured using a human radioreceptor assay (DYNO test; Yamasa Co., Tokyo, Japan) (14) with a reference range of <1.0 IU/l. Thyroid-stimulating antibody (TSAb) titer levels were measured in terms of the amount of cAMP produced in cultured porcine thyroid cells (Yamasa Co.) with a reference range of <180% (15). The volume of the thyroid gland was measured by ultrasonography as reported previously (16).

5' deiodinase assays

Human thyroid tissues were homogenized, and a microsomal fraction was prepared as described previously (5). D1 and D2 activities were assayed as described previously (5). In brief, the reactions contained microsomal protein, 0.1 nM [¹²⁵I]T₄ purified by LH-20 chromatography, 2 nM cold T₄, 20 mM dithiothreitol (DTT), 1 mM PTU in 0.1 M potassium phosphate, and 1 mM EDTA, pH 6.9 (D2 conditions) or 0.2 nM [¹²⁵I]rT₃ purified by LH-20 chromatography, 1 μM rT₃, and 10 mM DTT in the presence or absence of 1 mM PTU (D1 conditions). Incubations were for 120 min (D2 conditions) or 60 min (D1 conditions) at 37 °C. ¹²⁵I⁻ was separated from unreacted substrate or iodothyronine products by trichloroacetic acid precipitation. Separated ¹²⁵I⁻ was counted with a gamma-counter. The deiodinating activity was expressed in picomoles (D1) or femtomoles (D2) of I⁻ released per mg protein/h. Deiodination of T₄ and rT₃ produced equimolar concentrations of labeled I⁻ and T₃ (from T₄) or 3,3'-diiodothyronine (from rT₃) as assessed by paper chromatographic separation of the reaction products (17).

RNA preparation and real-time quantitative PCR

Total RNA from thyroid tissues was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Real-time quantitative PCR assays were performed using an Opticon 2 apparatus (Bio-Rad Lab.). Briefly, 1 μg total RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad Lab.) according to the manufacturer's instructions. Human D1, D2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were analyzed using the iQ SYBR Green Super MIX (Bio-Rad Lab.). The primers were as follows: 5'-TTAGTTCATAGCAGATTTTCTTGTC-3' (sense) and 5'-CTGATGTCCATGTTGTTCTTAAAGC-3' (antisense) amplify the human D1 cDNA; 5'-TCA-TTCTGCTCAAGCACGTG-3' (sense) and 5'-ACCATTC-CACTGTTGTAC-3' (antisense) amplify the human D2 cDNA; and 5'-GCACGTCAAGGCTGAGAAC-3' (sense) and 5'-TGGTGAAGACGCCAGTGGA-3' (antisense) amplify the human GAPDH cDNA. Real-time PCR experiments were performed in triplicate, and mRNA levels were expressed as arbitrary units after correction for GAPDH mRNA level.

Statistical analysis

Group data were expressed as means ± s.d., and statistical significance was analyzed by the unpaired *t*-test or the Mann-Whitney *U* test, as appropriate. Correlations were analyzed by Pearson's correlation coefficient test. *P* values <0.05 were considered to indicate a significant difference.

Results

Clinical findings

Some basic characteristics of the 13 patients with T₃-P-GD and the 18 patients with CT-GD who completed the study are listed in Table 1. These measurements were made at the time of the thyroidectomy. The differences in the serum levels of TSH and FT₄ were not statistically significant. As expected, the patients with T₃-P-GD had a higher mean serum FT₃ level and FT₃-to-FT₄ ratio than the patients with CT-GD. The mean TRAb and TSAb levels were greater in the patients with T₃-P-GD. The mean volume of the thyroid gland was approximately seven times higher in the patients with T₃-P-GD. After thyroidectomy and an appropriate dose of L-T₄ administration, serum FT₄ and FT₃ levels in the patients with T₃-P-GD changed to within the normal range (1.21 ± 0.15 ng/dl (FT₄) and 2.12 ± 0.27 (FT₃) respectively), and the FT₃-to-FT₄ ratio declined to 1.78 ± 0.33 .

D1 and D2 activities in thyroid tissues

The D1 activity in thyroid tissues of the patients with T₃-P-GD (492.7 ± 201.3 pmol/mg prot per h) was significantly higher ($P < 0.05$) than that in the patients with CT-GD (320.7 ± 151.9 pmol/mg prot per h; Fig. 1). The D2 activity in thyroid tissues of the patients with T₃-P-GD (823.9 ± 596.4 fmol/mg prot per h) was markedly higher ($P < 0.005$) than that in the patients with CT-GD (194.8 ± 131.6 fmol/mg prot per h; Fig. 1).

To investigate whether thyroidal D1 and D2 contribute to the FT₃-to-FT₄ ratio, we investigated the correlation between the serum FT₃-to-FT₄ ratio and the corresponding thyroidal D1 and D2 activities. As shown in Fig. 2A, the D1 activity of patients with T₃-P-GD and CT-GD significantly correlated with the

Figure 1 D1 and D2 activities in thyroid tissues. Open circles represent CT-GD patients; closed circles represent T₃-P-GD patients; solid squares represent mean value levels.

serum FT₃-to-FT₄ ratio ($r = 0.370$, $P < 0.05$). Meanwhile, the D2 activity of those patients strongly correlated with the serum FT₃-to-FT₄ ratio ($r = 0.676$, $P < 0.001$; Fig. 2B).

Furthermore, we investigated the correlation between the serum TRAb titer level and the corresponding thyroidal D1 and D2 activities. The thyroidal D1 activity of patients with T₃-P-GD and CT-GD significantly correlated with the serum TRAb titer level ($r = 0.502$, $P < 0.01$). The thyroidal D2 activity of those patients also significantly correlated with the serum TRAb titer level ($r = 0.502$, $P < 0.01$).

D1 and D2 mRNA in thyroid tissues

We investigated the thyroidal D1 and D2 mRNA. The thyroidal D1 mRNA level in the patients with T₃-P-GD (0.028 ± 0.015 arbitrary unit) was significantly higher than that in the patients with CT-GD (0.016 ± 0.014 arbitrary unit; Fig. 3). On the other hand, there was no significant difference between the thyroidal D2 mRNA level in the patients with T₃-P-GD (0.545 ± 0.276 arbitrary unit) and that in the patients with CT-GD (0.494 ± 0.234 arbitrary unit; Fig. 3).

Next, we examined whether D1 and D2 activities correlated with the corresponding mRNA level. There was a significant correlation between the D1 activity and the D1 mRNA level in the thyroid tissues from T₃-P-GD and CT-GD patients ($r = 0.502$, $P < 0.01$). On the other hand, there was no significant correlation between the D2 activity and the D2 mRNA level in the thyroid tissues from those patients ($r = 0.362$, $P = 0.076$).

Table 1 Basic characteristics of patients with T₃-P-GD and CT-GD who completed the study. The normal ranges were 0.3–5.0 μ IU/ml for TSH, 0.7–1.6 ng/dl for free thyroxine (FT₄), 1.7–3.7 pg/ml for free T₃ (FT₃), and 1.8–3.3 ((pg/ml)/(ng/dl)) for the FT₃-to-FT₄ ratio. A TSH concentration < 0.003 μ IU/ml was regarded as 0, for the purpose of statistical calculation. Values shown are means $\pm$ s.d. Values in patients with T₃-P-GD and CT-GD were compared using the unpaired *t*-test or the Mann–Whitney *U* test, as appropriate.

	T ₃ -P-GD (n=13)	CT-GD (n=18)	P value
Age	36 $\pm$ 12	49 $\pm$ 16	0.008
Gender (F/M)	10/3	15/3	0.676
Dose of MMI (mg)	25 $\pm$ 10	7 $\pm$ 3	< 0.001
TSH (μ IU/ml)	2.50 $\pm$ 5.05	2.03 $\pm$ 2.05	0.754
FT ₄ (ng/dl)	0.89 $\pm$ 0.79	0.87 $\pm$ 0.15	0.919
FT ₃ (pg/ml)	4.72 $\pm$ 3.65	2.35 $\pm$ 0.42	0.038
FT ₃ /FT ₄	6.6 $\pm$ 3.0	2.7 $\pm$ 0.5	0.001
TRAb (U/l)	206 $\pm$ 276	5.04 $\pm$ 4.68	< 0.001
TSAb (%)	1124 $\pm$ 582	350 $\pm$ 338	< 0.001
Thyroid volume (ml)	227 $\pm$ 106	32 $\pm$ 23	< 0.001

Figure 2 Correlation between thyroidal D1 (A) and D2 (B) activities and FT₃-to-FT₄ ratio. Open circles represent CT-GD patients; closed circles represent T₃-P-GD patients.

Furthermore, we investigated the correlation between the serum TRAb titer level and the corresponding thyroidal D1 and D2 mRNA level. There was a significant correlation between the thyroidal D1 mRNA level and the serum TRAb titer level in the patients with T₃-P-GD and CT-GD ($r=0.651$, $P<0.01$). On the other hand, there was a significant but weak correlation between the thyroidal D2 mRNA level and the serum TRAb titer level in those patients ($r=0.489$, $P<0.05$).

Discussion

The serum FT₄ and FT₃ levels and FT₃-to-FT₄ ratio in the patients with T₃-P-GD changed to within the almost normal range after thyroidectomy and administration of the appropriate dose of L-T₄. Therefore, it was suggested that T₃ production from the thyroid mainly contributed to the elevated serum T₃ level in T₃-P-GD. T₃ production from the thyroid is thought to originate from deiodination of T₄ in the thyroid and from the hydrolysis of Tg. It is thought that the thyroid gland deiodinates both T₄ released from Tg and T₄ taken up from the vascular bed (18).

Figure 3 D1 and D2 mRNA in thyroid tissues. Open circles represent CT-GD patients; closed circles represent T₃-P-GD patients; solid squares represent mean value levels.

We demonstrated that the deiodinase activities of D1 and especially D2 in the thyroid tissues of T₃-P-GD were significantly higher than those of CT-GD. Increased D2 activity of the thyroid tissues was also observed in some cases of T₃ thyrotoxicosis such as follicular thyroid carcinoma, GD during PTU treatment, TG gene mutations, and McCune–Albright syndrome (7–10). Laurberg *et al.* suggested that D1-generated T₃ in the thyroid was a major part of the total T₃ production in untreated GD (6). However, in T₃-P-GD patients who were treated with MMI, the serum FT₄ level was within the normal range, while the serum FT₃ level was elevated. Therefore, we suggest that the mechanism(s) by which elevated serum FT₃ levels are maintained in the patients with T₃-P-GD probably differ from those in the patients with untreated GD. Although we have neither direct nor indirect results to indicate which deiodinase is responsible for the elevated serum T₃ level in T₃-P-GD, the closer correlation between thyroidal D2 activity and serum FT₃-to-FT₄ ratio favors thyroidal D2 as the cause, but this is not definitive.

It is predicted that D1 activity in the liver and kidney might be increased in T₃-P-GD, as D1 is positively regulated by T₃ (19). Maia *et al.* (21) estimated that D2 is the major contributor of extrathyroidal T₃ production in euthyroid subjects, and peripheral T₃ production may switch from D2 to D1 dependency in thyrotoxic patients, since the D2 activity decreases due to the posttranslational substrate-induced inactivation of D2 (20, 21). In this study, since the serum FT₄ level in the patients with T₃-P-GD was within the normal range, we considered that D2-generated T₃ mainly contributes to the extrathyroidal T₃ production in the patients with T₃-P-GD. Therefore, it is suggested that any change in D1 activity in the liver and kidney of T₃-P-GD patients would contribute little to the peripheral T₃ production, even if D1 activity is increased in T₃-P-GD.

D2 mRNA is positively regulated by cAMP via cAMP-responsive element in the human D2 gene (22, 23) and negatively regulated by T₃ at pretranslational level (24, 25). In this study, the correlation between the thyroidal D2 mRNA level and the TRAb titer in the patients with T₃-P-GD and CT-GD was weak. These results suggest that not only positive regulation by cAMP, which is produced in the thyroid cells by TRAb stimulation, but also negative regulation by T₃ may also regulate the thyroidal D2 mRNA level in those patients. Further investigations are necessary to clarify the mechanism(s) by which thyroidal D2 mRNA is regulated.

Although the thyroidal D2 activity in the patients with T₃-P-GD was significantly higher than that in the patients with CT-GD, there was no significant difference between the thyroidal D2 mRNA level in the patients with T₃-P-GD and that in the patients with CT-GD. Furthermore, there was no significant correlation between the thyroidal D2 activity and the D2 mRNA level in those patients. It is well known that D2 activity is negatively regulated at the posttranslational level by its preferred substrate T₄ via the stimulation of the ubiquitin-mediated proteasome degradation of the enzyme (20). There was no significant difference between the serum FT₄ level in the patients with T₃-P-GD and that in the patients with CT-GD in this study. Therefore, it is suggested that translational and/or posttranslational mechanism(s), which are not induced by T₄, may be involved in the higher thyroidal D2 activity in the patients with T₃-P-GD.

Interestingly, the correlation between the TRAb titer level and the thyroidal D2 activity was stronger than that between the TRAb titer level and the thyroidal D2 mRNA level in the patients with T₃-P-GD and CT-GD. These results suggest that TRAb may regulate the D2 activity not only at pretranslational level, but also at translational and/or posttranslational level(s).

Of note is the fact that the volume of the thyroid gland was greater in the patients with T₃-P-GD. It is likely that the large goiter size is related to stimulation by higher TRAb in the patients with T₃-P-GD. Interestingly, higher thyroidal D2 activities have been observed in some large goitrous thyroid diseases such as TG gene mutations or McCune–Albright syndrome (9, 10). These findings suggest that a large goiter itself or any stimulating factor(s) that enlarge the thyroid volume may induce higher thyroidal D2 activity in the patients with T₃-P-GD. Further investigations are necessary to clarify the mechanism(s) by which higher thyroidal D2 activity is induced in the patients with T₃-P-GD.

Both the thyroidal D1 activity and the D1 mRNA level in the patients with T₃-P-GD were significantly higher than those in the patients with CT-GD. Furthermore, there was a significant correlation between the thyroidal D1 activity and the D1 mRNA level in the patients with T₃-P-GD and CT-GD. Significant positive correlation between the thyroidal D1 mRNA level and the serum TRAb titer level was present in those patients.

These results suggest that the thyroidal D1 activity may be mainly regulated at pretranslational level by cAMP, which is produced in the thyroid cells by TRAb stimulation.

On the other hand, in our previous study, both TG and the iodine content in thyroid tissues of patients with T₃-P-GD were lower than those of patients with CT-GD (26). Therefore, an enhanced iodine metabolism and possibly a higher rate of TG hydrolysis with prompt release of thyroid hormones, mostly T₃, may also contribute to the higher serum FT₃-to-FT₄ ratio in the patients with T₃-P-GD.

In conclusion, this study suggests that both thyroidal D1 and, especially, D2 may at least partly contribute to the higher serum FT₃-to-FT₄ ratio in the patients with T₃-P-GD. Further studies are needed to clarify the mechanism(s) by which the higher serum FT₃-to-FT₄ ratio and higher thyroidal D1 and D2 activities are induced in the patients with T₃-P-GD.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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