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CASE REPORT

Thyroid over-expression of type 1 and type 2 deiodinase may account for the syndrome of low thyroxine and increasing triiodothyronine during propylthiouracil treatment

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Abstract

Although propylthiouracil inhibits type 1 deiodinase, leading to a more rapid fall in triiodothyronine (T_3) than thyroxine (T_4) levels in patients treated for hyperthyroidism, we report a patient with Graves' disease whose free T_3 paradoxically rose during such treatment, despite low free T_4 levels and increasing doses of propylthiouracil. A similar response has previously been associated with high levels of thyroid stimulating antibodies, but it has been unclear why there should be a dichotomy in the circulating thyroid hormone profile. Thyroid tissue from our patient contained very high levels of type 1 and, especially, type 2 deiodinase, in contrast to other patients treated with Graves' disease, which were most likely secondary to high levels of thyroid stimulating antibodies. This unusual response to propylthiouracil is important to recognise therapeutically, and represents a further situation in which abnormal expression of deiodinase enzymes has clinical significance.

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Introduction

Thionamide antithyroid drugs are widely used as first line treatment for Graves' disease. They reduce thyroid hormone synthesis by inhibiting the iodination and coupling actions of thyroid peroxidase (1) and they also have an immunomodulatory activity which accounts for the long-term remission seen in 40–50% of treated patients (2). Propylthiouracil differs from carbimazole and its active metabolite, methimazole, by having an additional inhibitory effect on type 1 deiodinase (D1), reducing the conversion of thyroxine (T_4) to triiodothyronine (T_3) (3, 4).

A paradoxical response to propylthiouracil was first reported in a minority of patents in whom there was a high ratio of T_3 to T_4, together with increasing goitre, undetectable thyrotrophin (TSH) and a normal or slightly elevated basic metabolic rate (5). In a later study, Hegedüs and colleagues (6) identified seven Graves' patients, out of 50 consecutively treated with propylthiouracil, in whom there was initial normalisation of serum T_3 and T_4 levels and then a rise of T_3 levels into the thyrotoxic range, together with undetectable TSH levels and lower than normal T_4 levels. Goitre size increased in these patients by 50%. A partial explanation for this unexplained biochemical response was the finding that these patients had persistent elevation of thyroid stimulating antibodies, whereas these declined in the other 43 patients during propylthiouracil treatment. However, this observation alone cannot account for the sustained elevation of T_3 while the T_4 levels are low.

We have recently seen a patient who had a similarly anomalous response to propylthiouracil, in whom analysis of the thyroid tissue revealed unusually high levels of D1 and particularly type 2 deiodinase (D2) activity, suggesting that an abnormal pattern of deiodinase expression accounts for this clinical and biochemical profile.

Case report

A 30-year-old travel agent presented to her GP at the beginning of 2001 with a history of a goitre of 6 months duration, together with hot sweats, tremors and irritability. The symptoms had worsened over this time and more recently had been accompanied by diarrhoea, weight loss and increased appetite. Her mother had primary hypothyroidism. On examination there were obvious features of thyrotoxicosis and a large firm diffuse goitre, lid lag and exophthalmos but not

ophthalmoplegia. At presentation, the TSH levels were suppressed, the free T_4 levels were 64.5 pmol/l (reference range 11–26 pmol/l) and the free T_3 levels were >25 pmol/l (reference range 3.3–7.5 pmol/l).

Carbimazole, 20 mg a day, was given but within 2 weeks the patient had developed a rash and the treatment was changed to propylthiouracil, 100 mg three times a day (tds). Four weeks after starting antithyroid drugs, the free T_4 levels were 4.3 pmol/l (free T_3 was not measured at this time). Propylthiouracil was reduced to 50 mg tds and at the beginning of March the free T_4 levels were 3.6 pmol/l but the free T_3 levels remained high at 11.2 pmol/l, while the TSH levels were <0.1 mU/l. Propylthiouracil was increased to 100 mg two times a day and a month later the thyroid function tests showed a free T_4 level of 6.3 pmol/l and a free T_3 level of 19.1 pmol/l. The goitre was enlarging clinically. This pattern persisted for the next 6 months, with low free T_4, elevated free T_3 and undetectable TSH levels, and the patient continued to have symptoms of thyrotoxicosis, including emotional lability, tremor and sweaty palms, together with light periods, despite taking propranolol. The patient was taking no other medication throughout this time.

The patient agreed to have a total thyroidectomy which was performed in October. The thyroid weighed 117 g and was composed of hyperplasic follicles with scanty colloid, with background lymphoid and plasma cell infiltration, lymphoid follicle formation and Hürthle cell changes, appearances consistent with treated Graves' disease. She was commenced on thyroxine immediately after surgery. Three weeks after the operation, her free T_4 was normal at 25.6 pmol/l as was the free T_3 at 6.2 pmol/l; the patient at this time was taking 75 μg thyroxine a day.

Methods

Homogenate preparation

The patient's (FAS) thyroid sample was compared with 6 other Graves' samples, all of which were from patients who had been treated with carbimazole for a minimum of 3 months prior to surgery. Thyroid gland samples were homogenised in 10 volumes PE buffer (0.1 mol/l phosphate (pH 7.2), 2 mmol/l EDTA), and 1 mmol/l dithiothreitol (DTT), using a polytron (Kinematica, Lucerne, Switzerland) and a motor driven potter (Ika Labortechnik, Staufen, Germany). The homogenates were stored at −80 °C until further analysis. Protein concentrations were measured by the method of Bradford (7), using bovine serum albumin (BSA) as a standard.

Deiodinase assays

Deiodinase activities were analysed either by quantitation of radioiodide released by outer ring deiodination of [3′,5′-^{125}I]reverse (r) T_3 (D1) or [3′,5′-^{125}I]T_4 (D2), or by analysis of radioactive diiodothyronine (3,3′-T_2) generated by inner ring deiodination of [3′-^{125}I]T_3 (type 3 deiodinase, D3) by high performance liquid chromatography (HPLC).

D1 assay

D1 activities were measured by incubation of 100 nmol/l (10^5 c.p.m.) [^{125}I]rT_3 for 30 min at 37 °C with the indicated amounts of tissue homogenates. Samples were incubated in duplicate in the absence or presence of 0.1 mmol/l 6-n-propyl-2-thiouracil (PTU) in 0.1 ml PE buffer containing 10 mmol/l DTT (PED10). Blank incubations were carried out in the absence of enzyme. Reactions were stopped by the addition of 0.1 ml 5% (wt/vol) BSA in water followed by the addition of 0.5 ml 10% (wt/vol) trichloroacetic acid in water. After pelleting of the precipitated [^{125}I]iodothyronines, [^{125}I]iodide was further isolated from the supernatant on LH-20 mini-columns, equilibrated and eluted with 0.1 mol/l HCl. Deiodinase activities in tissue preparations were corrected for non-enzymatic deiodination observed in the blanks.

D2 assay

D2 activities were measured by incubation of 1 nmol/l (10^5 c.p.m.) [^{125}I]T_4 for 60 min at 37 °C with the indicated amounts of tissue homogenates. Samples were incubated in duplicate in the presence of 100 nmol/l unlabelled T_3 to block D3, and in the absence or presence of 100 nmol/l unlabelled T_4 to saturate D2, in 0.1 ml PED10. Release of $^{125}I^-$ was determined and corrected for non-enzymatic deiodination as described above. The difference in fractional deiodination between incubations with 1 and 100 nmol/l T_4 represented low K_m D2 activity.

D3 assay

D3 activities were measured by incubation of 1 nmol/l (10^5 c.p.m.) [^{125}I]T_3 for 60 min at 37 °C with tissue homogenate at a concentration of 1 mg/ml. Samples were incubated in duplicate in the absence or presence of 100 nmol/l unlabelled T_3 to saturate D3, in 0.1 ml PED10. The reactions were stopped with the addition of 0.1 ml ice-cold MeOH. After centrifugation, 0.1 ml of the supernatant was mixed with 0.1 ml 0.02 mol/l ammonium acetate (pH 4.0), and 0.1 ml of the mixture was applied to a 250 × 4.6 mm Symmetry C18 column connected to an Alliance HPLC system (Waters, Etten-Leur, The Netherlands), and eluted with a gradient of acetonitrile in 0.02 mol/l ammonium acetate (pH 4.0) at a flow rate of 1.2 ml/min. Radioactivity in the eluate was monitored on-line using a Radiometric A-500 flow scintillation detector (Packard, Meriden, CT, USA). Conversion of labelled T_3 to radioactive

3,3′-T_2 and, eventually, to 3′-T_1 was corrected for non-enzymatic deiodination as observed in blanks incubated in the absence or presence of enzyme. The difference in fractional deiodination between incubations with 1 and 100 nmol/l T_3 represented low K_m D3 activity.

Results

Both D1 and D2 activities were detected in homogenates of thyroid tissue from Graves' disease patients (Fig. 1). There is a wide scatter in both deiodinase activities in these samples, with a marked correlation between the D1 and D2 activities. However, thyroid tissue from patient FAS showed a disproportionately increased D2 activity relative to the D1 activity. D3 activities were not detected in these thyroid tissues. Further experiments were carried out comparing deiodinase activities in sample FAS to sample #176, the sample with the second highest D1 and D2 activities.

The effect of protein concentration on D1 and D2 activities in thyroid homogenates from samples FAS and #176 was measured by incubation of protein in the range of 0.01–0.1 mg/ml for the D1 assay (Fig. 2A) and 0.1–1 mg/ml for the D2 assay (Fig. 2B). D1 activity was linear with protein up to 0.05 mg/ml, and D2 activity was linear with protein up to 0.25 mg/ml. Deviation from linearity above these protein concentrations appeared to be due largely to significant substrate depletion.

The identity of the deiodinase activity measured in the D1 assay was confirmed by the finding that the addition of PTU resulted in a >90% inhibition of rT_3 deiodination. The nature of the deiodinase activity determined in the D2 assay was further investigated by testing the effects of the addition of increasing concentrations of unlabelled T_4, rT_3 or T_3 on the deiodination of radioactive T_4. Figure 2C shows that the inhibitory potency of these unlabelled iodothyronines decreases in the order $T_4 > rT_3 > T_3$, which is characteristic for D2. The K_m value determined by Lineweaver-Burk analysis of the concentraton-dependent deiodination of T_4 amounted to 1.5 nmol/l (Fig. 2D), which is also in agreement with previous data.

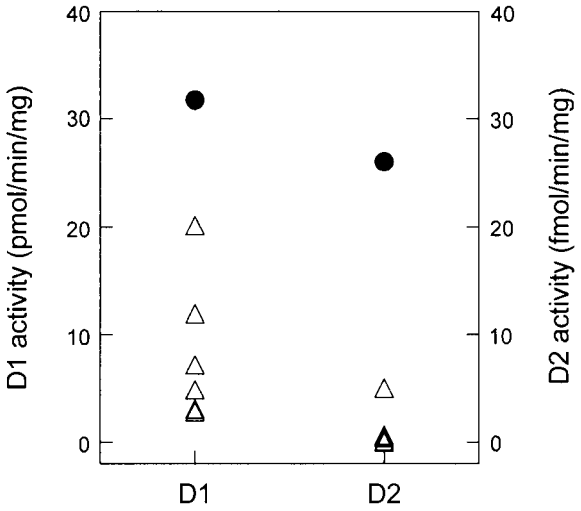

Figure 1 D1 and D2 activities in the individual thyroid gland tissue homogenates. The six Graves' patients treated with carbimazole are identified by triangles while patient FAS, treated with propylthiouracil, is shown as a solid circle.

Discussion

This patient's clinical course closely resembled that previously described in patients with a paradoxical biochemical response to propylthiouracil, with an elevation of circulating free T_3 levels, low free T_4 levels and undetectable TSH levels, despite increasing doses of propylthiouracil, accompanied by an enlarging goitre and symptomatic thyrotoxicosis. This biochemical picture led to uncertainty about the best way to deal with her thyrotoxicosis. Thyroidectomy was finally used to control her Graves' disease, and the subsequently normal biochemistry on replacement thyroxine rules out any systemic, general abnormality in thyroid hormone metabolism.

Normal thyroid tissue expresses both D1 and D2, which are responsible for catalysing deiodination of T_4 to T_3, thus contributing to the plasma pool of T_3. The third deiodinase enzyme, D3, is not expressed in the thyroid and catalyses the conversion of T_4 to reverse T_3 and T_3 to 3,3′-diiodothyronine, thus inactivating these hormones.

Analysis of iodothyronine deiodinase activity in this patient's thyroid was undertaken because the only likely explanation for these unusual results appeared to be an abnormal pattern of enzyme expression. This was prompted by the description of D3 over-expression in infantile haemangiomas as a cause of 'consumptive hypothyroidism' (8). If these tumours are large enough, their inactivation of both T_3 and T_4 by D3 can exceed the rate of thyroid hormone synthesis despite maximal endogenous TSH secretion. We have also recently identified three patients in whom over-expression of D2 in extensive follicular carcinoma deposits led to an unusual biochemical pattern with very low free T_4 levels, despite normal free T_3 and TSH levels (9). The results of the analysis of the present patient's thyroid revealed very high levels of D1 and especially D2 activity, which contrasted with the activities seen in another six contemporary patients treated with carbimazole.

It is known that the production rate of T_3 is double that of T_4 in hyperthyroidism and this is due to D1 over-expression that can be inhibited acutely by propylthiouracil (10–12). In Graves' thyroid tissue, D2 mRNA is over-expressed, despite the high levels of T_3 which, under normal circumstances, would inhibit

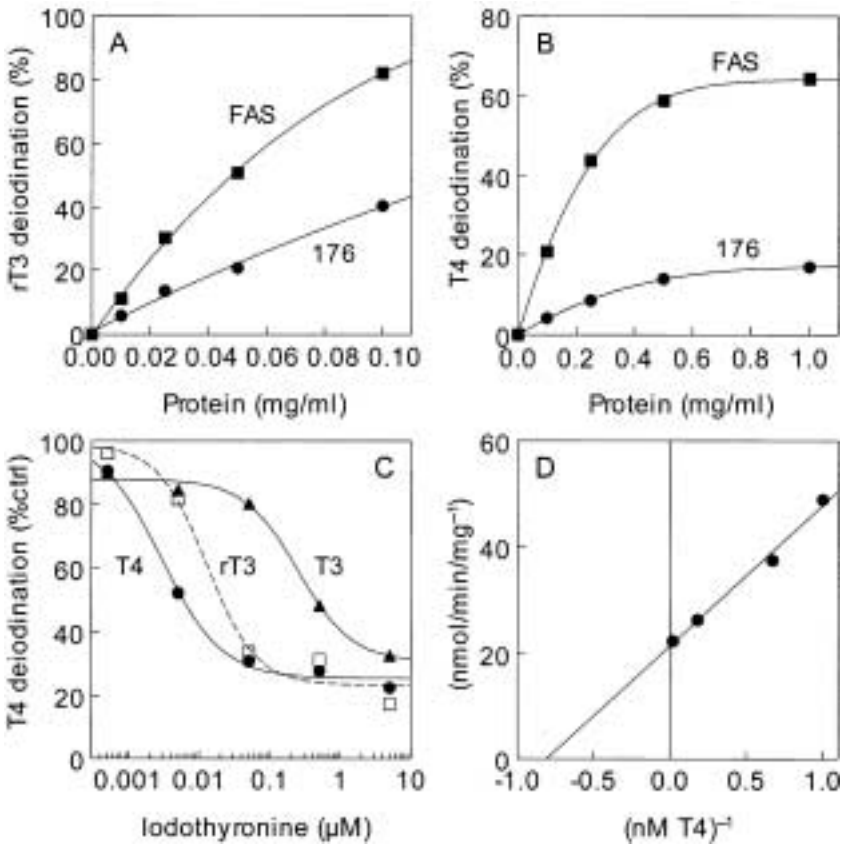

Figure 2 (A) Effect of protein concentration on D1 activity in thyroid tissue homogenates from patients #176 and FAS. (B) Effect of protein concentration on D2 activity in thyroid tissue homogenates from patients #176 and FAS. (C) Effects of increasing concentrations (0.5 nmol/l–5 μmol/l) of unlabelled T_4, rT_3 and T_3 on the outer ring deiodination of [^{125}I]T_4 by thyroid homogenate from patient FAS (0.1 mg protein/ml). (D) Lineweaver-Burk plot of the concentration-dependent deiodination of T_4, using the data depicted in C.

expression (13). It has been suggested that thyroid stimulating antibodies activate the cyclic AMP-dependent promoter which overwhelms the negative transcriptional effect of T_3 (14). It is clear from an earlier study that, in contrast to the normal pattern of a fall in the levels of these antibodies during antithyroid drug treatment, the levels paradoxically rise in association with the abnormal biochemical response in patients like ours (6). Although serum was not available retrospectively to analyse in our patient, the persistence of thyroid stimulating antibodies can be inferred by the histological findings, enlarging goitre and sustained thyrotoxicosis.

Together with the findings on D1 and D2 expression, we suggest that this syndrome is caused by a paradoxical rise in thyroid stimulating antibodies after propylthiouracil treatment that stimulates D2 over-expression in the thyroid, increasing the ratio of T_3 to T_4. We presume that the D1 over-expression which we also found, despite continued propylthiouracil treatment, is secondary to T_3-mediated upregulation of D1 synthesis, which, in turn, will exacerbate the over-production of T_3 and the consumption of T_4. Finally, the enlarging goitre, secondary to unopposed thyroid stimulating antibodies, may increase the overall production of T_3 at the expense of T_4 simply by allowing larger amounts of D1 and D2 to be synthesised. What remains unexplained is why thyroid stimulating antibodies rise rather than fall in these patients, and understanding this aspect may shed new light on the immunoregulatory properties of antithyroid drugs. Finally, this case illustrates an unusual pattern of response to propylthiouracil, which may not be familiar to those who do not use this as the antithyroid drug of first choice and whose early recognition may spare patients a prolonged and fruitless course of medication before definitive treatment is instituted.

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