Effects of propylthiouracil and methylmercaptoimidazole on thyroglobulin synthesis

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Abstract. The effect of 6-propyl-2-thiouracil (PTU), and 1-methyl-2-mercaptopimidazole (MMI) on thyroglobulin (Tg) biosynthesis has been studied in vivo and in vitro. In vivo experiments were performed in rats treated for 20 days with PTU or MMI, analyzing soluble and particulate, cold and $^{125}$I-labelled Tg. Thyroglobulin biosynthesis was also investigated by in vitro experiments, incubating thyroid tissue with labelled amino acid and carbohydrate in the presence of antithyroid compounds. It has been found that in vivo antithyroid agents decrease the amount of soluble Tg and increase the proportion of particulate Tg. Tg from treated animals is poorly iodinated being mainly represented by its 12S subunit. In vitro studies demonstrate that PTU and MMI inhibit Tg biosynthesis which is impaired in the polypeptide chain and as in carbohydrate chains addition. Thus the inhibition of the hormonogenetic processes induced by antithyroid treatment leading to a depressed iodinating activity also appears to be related to a significant impairment of the production of the Tg molecule, the specific iodine acceptor.

Thionamide drugs decrease the formation of thyroid hormones by inhibiting the incorporation of iodide into tyrosyl residues of thyroglobulin. This effect is due to the inhibition of the initial oxidation of inorganic iodide and its subsequent binding to tyrosyl residues in thyroglobulin (Mallof & Soodak 1965; Morris & Hager 1966); in addition propylthiouracil is bound to thyroglobulin in the thyroid gland of rats. This binding could affect thyroglobulin structure by inhibiting the coupling reaction (Papapetrou et al. 1975). Furthermore, binding of both PTU and MMI to thyroglobulin has been observed in a model incubation system, containing purified thyroid peroxidase (Taurog 1976). PTU also inhibits the peripheral deiodination of iodothyronines, thus reducing the amount of thyroid hormones degraded in vivo (Morreale de Escobar & Escobar del Rey 1967).

The possible role of antithyroid compounds on the biosynthesis and functional properties of the specific iodine acceptor, i.e. thyroglobulin, has not been extensively investigated.

The specific aim of the present study was to establish whether the decreased hormone formation produced by the antithyroid drugs, PTU and MMI, could be in part related to a decrease of thyroglobulin synthesis. For this purpose experiments were performed both in vivo by treating rats with antithyroid compounds, and in vitro by studying in thyroid tissue the effects of PTU and MMI on the amino acid and carbohydrate incorporation into Tg.

Material and Methods

**In vivo experiments**

Male Wistar rats were fed a standard diet and received drinking water ad libitum. In each of two separate experiments three groups of 20 animals were used: the control group received tap water, the PTU group received water containing 0.1% PTU, and the MMI group water containing 0.05% MMI. From the amount of water drunk by the animals it was established that each rat of
the PTU group ingested 16 mg of drug/day, and that of each of the MMI group, 5 mg/day. After 20 days of treatment the animals were killed by exsanguination, and the thyroids were removed, washed, weighed and pooled for each group. Four animals of each group were injected ip, 24 h before sacrifice, with 100 μCi \(^{125}\)I. Soluble and solubilized proteins were extracted as previously reported (Monaco & Robbins 1972). Briefly, thyroid glands were taken, washed and homogenized in 0.1 M Tris-HCl, 0.25 M sucrose, pH 7.4, in a Potter-Elvehjem homogenizer with a teflon pestle at 1100 r.p.m., three strokes in ice. The homogenate was centrifuged for 1 h at 105 000 × g and the supernatant (containing the soluble proteins) was dialyzed at 4°C against Tris-HCl buffer, changed several times. The washed pellet was re-homogenized in 2% digitonin, 10 strokes at 2400 r.p.m. in ice, and re-centrifuged at 105 000 × g for 1 h at 4°C. The supernatant, containing the solubilized proteins, was dialyzed against Tris-HCl buffer. One hundred mg of soluble proteins per gram of wet tissue was obtained from the control animals by this procedure, 75–80% consisting of 19S Tg; this amount corresponded to 46 mg of Tg per 100 mg of total protein, measured on the homogenate by the method of Lowry et al. (1951). By digitonin extraction about 6 mg of solubilized proteins per gram of wet tissue was extracted, 70% consisting of 19S Tg. Thus, by the extraction procedure from normal thyroid rat 50 mg of total Tg was obtained (soluble and solubilized) per 100 mg of total protein in the homogenate. By this procedure 103 μg of \(^{125}\)I was extracted out of 118 μg determined per 100 mg of wet tissue.

Thyroglobulin was isolated from soluble and solubilized proteins by ammonium sulphate fractionation followed by density gradient centrifugation at 20°C in 10–40% sucrose in a Beckman L5-65 B ultracentrifuge (Monaco & Robbins 1972).

**In vitro experiments**

Thyroids from untreated animals were removed, cleared of connective tissue, pooled and 8 thyroid hemilobes were pre-incubated in each incubation flask in Eagle’s solution for 2 and 4 h, as described by Monaco & Robbins (1973), in the presence of 2 mM PTU or 1 mM of MMI, and then incubated for 2 h with 50 μCi of \(^{3}H\)galactose. Control experiments were carried out in the same way but without PTU-MMI. Then the thyroids were homogenized, and labelled soluble and solubilized thyroglobulin were extracted as above and analyzed by density gradient centrifugation at 20°C as described previously (Monaco & Robbins 1973).

The total protein content of thyroid homogenate was measured by the method of Lowry et al. (1951).

Thyroglobulin determination was carried out on the purified protein using an extinction coefficient \(E_{1\text{cm} 1\%}\) at 280 nm of 10.4. Iodine content of thyroglobulin was determined by the method of Zak et al. (1952).

Proteolytic activity was determined, after thyroid homogenization, by measuring the amount of tyrosine produced from exogenous thyroglobulin as described by Griescu & Lemarchand-Béraud (1973).

Male Wistar rats weighing 450 ± 50 g were from Morini Laboratory, Modena, Italy; the standard diet, containing a normal iodine level, was the MIL type B from Morini. PTU and MMI were from K & K-ICN Pharmaceut, Inc., N.Y.; digitonin was from Fisher Scientific Co. and was prepared as described by Monaco & Robbins (1972). \[^{3}H\]4.5-L-leucine (S.A. 30 000 mCi/mmole) and \[^{3}H\]D-galactose (S.A. 10 000 mCi/mmole) were from Amersham-Searle. \(^{125}\)I-carrier-free was from Sorin, Saluggia. Unisolve I was from Koch Light Laboratory Ltd. \(^{125}\)I was estimated in a well-type scintillation counter (LKB ultragamma). \(^{3}H\)-labels were counted in a Nuclear Chicago Mark 1 liquid scintillation in 10 ml of Unisolve I. Eagle’s medium without leucine was from Burroughs Wellcome Co. All the other chemicals were of reagent grade.

**Results**

After 20 days of treatment with PTU and MMI the weight of the gland was increased 2–3 times as compared to controls (Table 1). In the goitrous gland the concentration of proteins on the whole as well as that of soluble Tg was less than in the control thyroids, both as expressed as mg/g of wet weight as well as when expressed as mg Tg/100 mg

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rats (No.)</th>
<th>Thyroid Weight (mg)</th>
<th>Thyroid proteins mg/g wet tissue</th>
<th>Thyroglobulin mg/100 mg protein</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Tg</td>
</tr>
<tr>
<td>Controls</td>
<td>40</td>
<td>31 ± 3</td>
<td>96</td>
<td>72</td>
</tr>
<tr>
<td>PTU</td>
<td>40</td>
<td>94 ± 8</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>MMI</td>
<td>40</td>
<td>75 ± 7</td>
<td>48</td>
<td>20</td>
</tr>
</tbody>
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Table 1.

Effects of goitrogens on thyroid weight, on soluble proteins and on soluble and on solubilized Tg.
The percentage of radioactivity present in the soluble and solubilized Tg fraction referred to total Tg extracted is indicated between brackets.

of protein. However the particulate protein solubilized by digitonin was slightly increased in the treated gland.

By studying the radiiodine incorporation it was observed that antithyroid compounds, reduced the amount of iodinated soluble Tg (Table 2). PTU being more effective. However, when analyzing the distribution of iodinated Tg between the soluble and solubilized fraction it was shown that in the MMI treated thyroids the relative proportion of iodinated solubilized Tg was higher than in the control. It must be noted that the reduction in soluble Tg is due not only to the reduction on iodination, but also to the expected depletion of colloid; the solubilized Tg shows a relative increase and thus raises the question that thyroglobulin secretion could be abnormal. In addition soluble thyroglobulin isolated from thyroids after 20 days of treatment with antithyroid drugs contained 0.01% of stable iodine in the PTU group and 0.02% in the MMI group compared to 0.25% in Tg from control animal.

The experiments performed in vitro (Table 3) clearly demonstrate that PTU and MMI inhibit the incorporation into soluble Tg of both tritiated

<table>
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<tr>
<th>Pre-incubation (h)</th>
<th>CPM $\times 10^{-3}$/mg</th>
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<tbody>
<tr>
<td></td>
<td>Soluble protein</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Leucine 2</td>
<td>53.6</td>
</tr>
<tr>
<td>Leucine 4</td>
<td>80.6</td>
</tr>
<tr>
<td>Galactose 2</td>
<td>21.4</td>
</tr>
<tr>
<td>Galactose 4</td>
<td>3.2</td>
</tr>
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</table>

Each figure refers to 4 pooled glands. Data are expressed as CPM incorporated per mg of soluble protein and are the mean of duplicate experiments. The SEM was equal to $\pm$ 7.
leucine and galactose. The most striking inhibition was observed with galactose. After 4 h pre-incubation with antithyroid compound the incorporation of [3H]galactose was 23% of the incorporation into the untreated tissue. Similar results were obtained with MMI, which reduced labelled leucine and galactose incorporation to 32% and 27%, respectively, of the incorporation obtained in control tissue. When the data are expressed as cpm incorporated per gram of wet tissue similar findings are obtained.

The sedimentation properties of Tg isolated from MMI-treated animals showed (Fig. 1) that both soluble and solubilized Tg consisted of a higher proportion of its 12S subunit which represents more than 50% of the total 125I-radioactivity precipitated in 1.4-1.8 M ammonium sulphate compared to 12% of 12S present in Tg isolated under the same condition from untreated animals. Tg from PTU-treated animals contained a higher amount of 12S subunits which accounted for about 70% of the total radioactivity gradient.

The endogenous proteolytic activity of the gland treated with antithyroid agents for 20 days showed 1.45 for PTU and 1.96 for MMI compared to 2.04 for control, measured as µg of tyrosine 10^3 per 100 mg of protein, thus excluding an increase protein catabolism of the treated glands.

Discussion

Since preliminary study showed that thiocyanate, an effective goitrogen, produces an increase of particulate iodoprotein, it seems reasonable to investigate the possibility of a direct action on thyroglobulin synthesis. It also affects the release of iodine by the epithelial thyroid cells more drastically than the uptake (Van Middlesworth 1977).

It therefore appeared logical to assume that antithyroid compounds could also produce an impairment of the synthesis and extracellular migration of thyroglobulin molecule, the specific hormonogenic matrix. The aim of the present study was to establish if antithyroid agents could interfere in the biosynthetic steps of the thyroglobulin molecule either in its polypeptide backbone or in its carbohydrate chains formation.

The studies performed in vivo clearly show that both PTU and MMI drastically inhibit the iodination processes as demonstrated by the low iodine content of Tg. In addition to this well-known inhibiting effect on iodination, it has been demonstrated that both drugs decrease the amount of soluble Tg; furthermore, an increase of the relative proportion of Tg remaining particulate is observed, which in the treated animals accounts for 1/3 of the total Tg extracted, compared to less than 1/10 found in control. In the in vivo experiments performed with radiiodine it was observed that there is a striking decrease of radiiodine incorporated in soluble Tg. The relative increase of the radiiodine contained in the particulate protein could be considered not to be consistent with the widely accepted theory that iodination takes places at the apical border of the cell; but it can be assumed from other evidence (Monaco et al. 1975a) that the iodinating system could be effective, even at a lower rate, at the intracellular level if the specific protein substrate was lacking at its physiological site (Monaco et al. 1975b). Furthermore, from the chemical determination of iodine in the isolated protein, it was demonstrated that antithyroid compounds decrease the level of iodine incorporated in Tg, containing less than 1/10 of the stable iodine found in Tg from untreated animals. The poorly iodinated Tg synthesized by treated animals showed a more unstable subunit structure, since the Tg consisted of a large proportion of its 12S subunit. Thi is in keeping with the findings demonstrating that the stability of the 19S molecule is inversely proportional to the level of iodination (Bürgi et al. 1974; Salvatore & Edelhoch 1973).

All the data obtained in the in vivo experiments indicate that antithyroid compounds, in addition to the well-known inhibitory effects on the iodinating processes, are responsible for the decreased level of iodinated Tg.

The experiments performed in vitro indicate that antithyroid compounds inhibit the amino acid incorporation into the polypeptide back-bone of Tg, thus indirectly confirming the results of the in vivo experiments of the decreased amount of soluble Tg detected in thyroids of treated animals. The scanty amount of soluble Tg must be considered in the light of the fact that in vivo the iodine incorporation is decreased to an extent much more effective than the reduction of Tg. The decrease of soluble iodinated Tg induced also consideration of the possibility that antithyroid compounds could also affect the oligosaccharide chains formation, since this biosynthetic step is a pre-requisite for the iodination of the glycoproteic molecule (Monaco et al. 1975b). The decreased
amount of Tg does not appear to be related to an accelerated catabolic turnover of Tg, since the proteolytic activity of thyroids from treated animals appeared to be equivalent to that shown by normal glands.

Our results demonstrate that antithyroid compounds reduce the formation of the completed thyroglobulin molecule. Thus it can be concluded that the inhibitory effect of antithyroid agents is due not only to the striking inhibition of the iodinating system but also to a significant depression of the Tg synthesis.

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References

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