Stimulation of thyroidal iodothyronine 5'-monodeiodinase by long-acting thyroid stimulator (LATS)

Sing-Yung Wu, R. Reggio, W. Florsheim, I. J. Chopra and D. H. Solomon

Abstract. To evaluate the effect of long-acting thyroid stimulator (LATS) on thyroid iodothyronine monodeiodinating activity, we have studied the in vitro conversion of T4 to T3 by mouse thyroid homogenate comparing tissue from LATS treated (0.1 ml LATS (+) serum, ip, for 3 days) with tissues from LATS(-) Graves' disease patients' serum or normal serum treated controls. Five out of seven LATS (+) sera were shown to stimulate the T4 5'-deiodinase significantly in mouse thyroid. There was no significant correlation between LATS titre and deiodinase activities in the different sera tested. To compare the effect of LATS and TSH (0.2 IU, ip daily), studies were carried out from 12 to 72 h. LATS had a similar latency of 12 h on the stimulation of thyroid deiodinase compared to TSH as reported earlier. However, the conversion activities reached a plateau by 12 h after LATS treatment, while it continued to rise upon daily TSH injection from 24 to 72 h. In addition, TSH caused a marked reduction of thyroid protein and an early peaking in serum T3 and T4 at 12 h, whereas LATS caused no detectable change in thyroid protein and a gradual rise in circulating T3 and T4. The kinetic analysis indicated that LATS-mediated stimulation of T4 5'-deiodinase was, similar to TSH, associated with an increase in maximum velocity (V_max were 139, 208 and 505 pmol/mg protein/30 min respectively in control, LATS and TSH-treated animals) without a demonstrable change in the apparent K_m (approximately 2.0 μM for T4). The present study demonstrated that some LATS-rich sera stimulate thyroid T4 to T3 conversion in mouse. It provides an insight into the mechanism of increased T3 secretion from Graves thyroid glands.

Thyrotropin-inducible thyroid hormone inner and outer ring monodeiodinases are present in thyroid gland in the dog (Wu 1983) and in man (Ishii et al. 1983). Recently, we have characterized mouse thyroid iodothyronine monodeiodinases which are found to be extremely sensitive to TSH stimulation (Wu et al. 1985). It is known that the thyroidal secretion of T3 is a much larger fraction of total T3 production in patients with Graves' hyperthyroidism than in euthyroid individuals (Woeb et al. 1970; Inada et al. 1975; Maguire et al. 1976; Faber et al. 1982). Moreover, enhanced iodothyronine monodeiodinating activity is found in Graves' thyroid tissues (Ishii et al. 1981). However, the mechanism responsible for the increased conversion in the stimulated thyroid gland in Graves' disease patients is not clear. The present study was undertaken to examine whether or not that serum from LATS-positive Graves' patients can stimulate thyroidal T4 to T3 converting enzyme in mice.

Materials and Methods

Patients and LATS assay
Sera were obtained from seven Graves' disease patients who were LATS-positive and had been rendered euthyroid by prior radioiodine therapy and thyroid hormone...
replacement; three LATS-negative Graves' disease patients who were either euthyroid (patient MA, Exp. 11, Table 1, serum T4 142 nmol/l normal 65–155) following propylthiouracil treatment or hyperthyroid (patient RO Exp I, Table 1, serum T4 187 nmol/l; and patient A, Exp II, serum T4 161 nmol/l and serum T3 4.93 nmol/l normal 0.77–3.23) and seven healthy euthyroid normal subjects. LATS was measured in 0.5 ml aliquots of undiluted serum by a modification of the McKenzie bioassay in mice (Chopra et al. 1970). In two patients (patient F and B), thyroid stimulating activity was measured in IgG from 150 µl of serum using an in vitro assay (Maayan et al. 1978) employing mouse thyroid lobes. T3 released in the medium during 4 h of incubation of thyroid lobes with patients' IgG was compared to that released in the presence of an equivalent amount of IgG from a pooled serum of normal subjects. IgG was prepared by precipitation with polyethylene glycol in a final concentration of 12.5%. The precipitate was washed and dissolved in phosphate buffered saline in a volume equal to the original sample volume.

Materials

Dithiothreitol (DTT), polyethylene glycol (carbowax 6000), 3,5-diiodothyronine (3,5-T2), T3 and T4 were purchased from Sigma Chemical Co, St. Louis, MO. [125I]T3 was prepared by radiiodination (sp act ≥ 400 mCi/mg) of 3.5-T2 by a method described previously (Nakamura et al. 1977). Bovine TSH (Thytropar) was obtained commercially from Armour Pharmaceutical Co, Phoenix, AZ.

Animal preparation

Female Swiss Webster Albino mice (20–25 g) were purchased from Hill Top Laboratory, Chatsworth, CA. Animals were maintained on regular animal chow which was removed 12 h prior to sacrifice. Patients' serum (0.1 ml) or TSH (0.2 IU) was given ip for one to three consecutive days as specified in each experiment. Control animals received either saline or serum from healthy individuals without history of thyroid disease and with normal thyroid functions.

Enzyme preparation and the study of conversion of thyroid hormone in vitro

Mice were sacrificed by cervical dislocation, and thyroid glands were quickly removed and placed on chilled ice. The thyroid glands from three mice were pooled and were homogenized in cold motor driven glass-glass homogenizer in 99 vol ice-cold 0.14 M phosphate buffer, pH 7.4. The in vitro conversion of T4 to T3 by tissue homogenates was studied according to the method reported previously (Wu et al. 1985). The results were expressed as picomoles T3 produced per mg protein or per mg wet tissue in 30 min. The statistical analysis by the two-tailed Student's t-test on unpaired data was used in comparing control and animals treated with LATS-positive serum or TSH. P-values were corrected in multicomparisons by Bonferroni t-test (Glantz 1981). The correlation coefficient (r) between LATS titres and deiodinase activities was calculated by the method of Pearson product-moment correlation coefficient (Glantz 1981). Tissue protein was determined by a modification of the method of Lowry et al. (Hartree 1972).

Results

Effect of LATS on thyroidal iodothyronine monodeiodinating activity

As shown in Table 1, we have studied seven LATS-positive (LATS(+)) sera. Significant increase in T4 to T3 monodeiodinating activity in mouse thyroid were found in groups of mice treated with five LATS(+) patient's sera (patients F, B, GL, GI and R) as compared to either saline, normal sera or LATS-negative (LATS(−)) Graves' disease patients' sera treated controls. Two patients' sera with lowest LATS content (patients M and GR) did not cause a significant change in the monodeiodinating activity. One LATS(−) patient serum (experiment II) appeared able to stimulate the monodeiodination to a modest but significant degree as compared to normal serum control. There was no significant correlation between LATS titre and deiodinase activities in the different sera studied (r = 0.37, N = 6, Exp II–VII).

Latency of effect of LATS treatment on thyroidal, hepatic and renal iodothyronine monodeiodinating activities

Mice were divided into five groups, and each group was treated with either daily injection of either saline or LATS (from patient F, 0.1 ml of serum, ip) and sacrificed at 12, 24, 48 and 72 h after the first injection. Results are shown in Fig. 1. By 12 h, a significant increase in outer ring monodeiodinase (43.5% increase from baseline, P < 0.005) was found in thyroid. There were little changes thereafter.

Treatment with LATS caused a slight increase (not statistically significant) in monodeiodination as seen in the liver at 24 to 72 h (a 20% increase above baseline). LATS treatment had little effect on renal monodeiodination of iodothyronine from 12 to 72 h (Fig. 1).
Changes of thyroidal protein content and serum T₃, T₄ concentration following LATS or TSH treatment

Treatment of mice with LATS resulted in no significant change in thyroid protein content whereas that with TSH caused a 19.8% decrease at 12 h (P < 0.0015 cf baseline value; P < 0.015 cf LATS-treated group at 12 h) (Fig. 2). The decrease of thyroid protein content at 12 h after TSH treatment corresponded to a maximal rise in serum T₄ (a 197% increase from baseline value of 42.6 ± 3.9 nmol/l P < 0.02) and serum T₃ (a 101% increase from baseline value of 1.15 ± 0.08 nmol/l P < 0.02) (Fig. 3). Serum concentration of T₃ and T₄ gradually returned to near baseline values at 48 h despite daily TSH injection; and then serum T₃, but not T₄, resurged at 72 h. LATS treatment, on the other hand, resulted in a significant increase in serum T₃ and T₄ concentration at 12 h (cf baseline P < 0.02 in both cases), and the increase continues to a plateau between 24 and 72 h (at 24 h, a 84% rise from baseline value for T₃, P < 0.02; a 71% rise from baseline value for T₄, P < 0.02).

Table 1.
Effect of LATS on thyroidal iodothyronine monodeiodinating activity.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Graves' disease patients</th>
<th>Treatment</th>
<th>Groups mice (N)</th>
<th>T₄ to T₃ conversion¹ (pmol T₃/mg tissue (mean ± SEM))</th>
<th>P-value</th>
<th>LATS bioassay² response index (%)</th>
<th>LATS in vitro³ assay (ng T₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline</td>
<td>12</td>
<td>27.5 ± 3.4</td>
<td>n. s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RO</td>
<td>LATS (-)</td>
<td>6</td>
<td>30.4 ± 2.5</td>
<td>n. s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>LATS (+)</td>
<td>6</td>
<td>42.0 ± 2.9</td>
<td>&lt; 0.005</td>
<td>0.42 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>NL.serum</td>
<td>6</td>
<td>25.7 ± 2.4</td>
<td>&lt; 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>LATS (-)</td>
<td>6</td>
<td>32.9 ± 1.0</td>
<td></td>
<td></td>
<td>3542</td>
<td>0.39 ± 0.07</td>
</tr>
<tr>
<td>B</td>
<td>LATS (+)</td>
<td>6</td>
<td>43.1 ± 2.1</td>
<td>&lt; 0.0005</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>NL.serum</td>
<td>6</td>
<td>30.1 ± 2.6</td>
<td>n. s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>LATS (-)</td>
<td>6</td>
<td>32.2 ± 1.5</td>
<td></td>
<td></td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>LATS (+)</td>
<td>6</td>
<td>35.7 ± 1.4</td>
<td>n. s.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>NL.serum</td>
<td>6</td>
<td>26.8 ± 0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G L</td>
<td>LATS (+)</td>
<td>6</td>
<td>31.4 ± 1.4</td>
<td>&lt; 0.02</td>
<td>1527</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>NL.serum</td>
<td>6</td>
<td>27.4 ± 1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G R</td>
<td>LATS (+)</td>
<td>6</td>
<td>28.0 ± 1.0</td>
<td></td>
<td></td>
<td>751</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>NL.serum</td>
<td>6</td>
<td>29.6 ± 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LATS (+)</td>
<td>6</td>
<td>38.0 ± 1.5</td>
<td>&lt; 0.025</td>
<td></td>
<td>956</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>NL.serum</td>
<td>6</td>
<td>32.2 ± 1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>LATS (+)</td>
<td>6</td>
<td>48.8 ± 4.2</td>
<td>&lt; 0.005</td>
<td>1157</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ The converting activity was measured in a mixture containing 0.5 mg equivalent of tissue homogenate, 4 mM DTT, 2.5 mM T₄ and 120 mM phosphate buffer (pH 7.0). The reactions were carried out at 37°C for 30 min, after which 2 volumes of 95% ethanol were added with mixing. T₃ was measured in supernatant by radioimmunoassay. P-values represent comparisons with NL (normal) serum of LATS-negative Graves' patient serum (when available).

² LATS bioassay was performed using 0.5 ml of serum as reported previously (Chopra et al. 1970).

³ In vitro LATS assay was carried out by a modified method of Maayan et al. (1978). IgG from 150 µl of normal serum added in the incubation mixture resulted in 0.22 ± 0.04 ng T₃ discharge from thyroid tissue into 100 µl of medium in 4 h of incubation.

(N) number of groups of mice (usually 3 mice per group): animals were treated with patient’s serum (0.1 ml, ip, daily for 3 days) or with saline.
Latency of effect of LATS treatment on thyroidal, hepatic and renal iodothyronine monodiodinating activities. All animal groups received one dose of LATS (0.1 ml serum ip) daily and received the last dose at 24 h prior to sacrifice except one group that was sacrificed 12 h after the first injection. The reaction mixture is the same as in the legend of Table 1. The results are expressed as pmol T₃ produced per mg tissue in 30 min at 37°C. Values are mean ± SEM. The numbers in parentheses represent number of groups of mice (usually 3 mice in each group) for each data point.

Change of thyroidal protein content following LATS or TSH treatment. The animal groups treated with LATS were described in the legend of Fig. 1. The reaction mixture is the same as in the legend of Table 1. Thyroidal protein was determined by a modification of the method of Lowry et al. (Hartree 1972). The results were expressed as mg protein/g thyroid tissue (mean ± SEM). The numbers in parentheses represent the number of groups of mice (usually 3 mice in each group) for each data point.
Fig. 3.
Change of serum T₃ and T₄ concentration following LATS or TSH treatment. The animal groups treated with LATS or TSH were described in the legend of Fig. 2. Serum T₃ and T₄ were determined by radioimmunoassay. The results were expressed as % change from baseline value (mean ± SEM).

Fig. 4.
Lineweaver-Burk plot of T₄-to-T₃ conversion activities in thyroid from LATS-, TSH-, or saline-treated mice. The production rate of T₃ is presented as pmol per mg protein in 30 min at 37°C. Points are the mean of two determinations.
Kinetic studies

Fig. 4 shows the data on the effect of substrate concentration on the rate of thyroidal conversion of T₄ to T₃ in saline-, TSH- and LATS-treated (patient F serum) animals. Kinetic analysis of the data indicated that LATS-mediated stimulation of outer ring monodeiodination was similar to TSH, associated with increases in the maximum velocity (Vₘₐₓ was 139, 208 and 505 pmoles respectively in saline-, LATS- and TSH-treated animals), without a demonstrable change in the apparent Kₘ (approximately 2.0 µM for T₄).

Discussion

Our recent report (Wu et al. 1985) has shown that mouse thyroid iodothyronine mono-deiodinating activity is extremely sensitive to exogenous bovine TSH stimulation. The increase in deiodinase activity after TSH administration is dose dependent. It has also been shown that enhanced conversion of T₄ to T₃ is found in thyroid gland from Graves' disease patients (Ishii et al. 1981). In the present study, we found that five out of seven LATS(+) serum significantly stimulated the T₄ 5'-monodeiodinating activity in mouse thyroid (two were compared with LATS-negative Graves' patient serum, and three were compared with normal serum control). However, there was no significant correlation between LATS titre and deiodinase activity in the different sera. We studied two sera of one patient (patient F), first from 1981 when he was LATS(+) and second from 1982 when he had become LATS(-) following prolonged treatment with corticosteroids for thyroid acropachy (Parker et al. 1982). The first serum caused a marked stimulation of the T₄ to T₃ conversion in the mouse thyroid (experiment 1, Table I). However, his second serum had little effect on the thyroid deiodinase or serum T₃ or T₄ concentration.

Hamada et al. (1980), who studied a cultured human thyroid slice system, found that IgG samples from most of untreated Graves' patients did not stimulate release of thyroid hormones from normal human thyroid tissue. However, a slight but significant increase in T₃ release was observed when slices were incubated with a very active pooled IgG (adenylate cyclase stimulating activity ≥ 500% of control). Their results may explain partly that not all LATS(+) sera are capable of stimulating T₄ to T₃ conversion. The mechanism for the variability in LATS(+) serum to stimulate thyroid deiodinase is not clear. It may be partly related to the amount of LATS in the patient's serum (as demonstrated in patient F), to the variable affinity of the polyclonal LATS immunoglobulins to thyroid receptors (Eisen 1974) and/or to the sensitivity of our assay.

The response of mouse thyroidal iodothyronine monodeiodinating activity to LATS stimulation was similar to TSH in latency study with a significant increase in enzyme activity by 12 h after the treatment. However, there were some differences in the response of mice to LATS and TSH in our assay system. The conversion activity reached a plateau by 12 h after LATS treatment, while it continued to rise after TSH injection from 24 to 72 h (Fig. 1 and Wu et al. 1985). The protein content in mouse thyroid gland remained essentially unchanged after LATS treatment, while it decreased significantly at 12 h after TSH treatment. The pattern of change in serum T₃ and T₄ concentration after LATS treatment was different from that seen after TSH treatment (vide supra). We do not know of a clear explanation for these differences. The difference and degree of saturation in the binding of the receptors of thyroid membrane may be involved.

The TSH induced reduction in thyroid protein is presumably due to increased resorption, hydrolysis and secretion of thyroglobulin and thyroid hormones, and corresponds to the early peaking of T₄ and T₃ level in serum at 12 h. The late surge of serum T₃ (without that of T₄) at 72 h may be a result of increased secretion of T₃ from thyroid gland where T₄ outer-ring monodeiodinating activity increased continuously from 12 to 72 h after TSH treatment. It is unclear why a similar change or a delayed response in thyroid protein content was not observed after LATS treatment. It is possible that a high dose of LATS may show changes similar to those observed after TSH.

The present study demonstrated that thyroid T₄ to T₃ conversion can be stimulated following the administration of LATS-rich sera in mouse. Even though elevated serum levels of T₃ and T₄, as also occur after LATS treatment, are known to induce hepatic monodeiodinase (Grussendorf et al. 1977), we recently have shown that they actually suppressed monodeiodinase activity in thyroid
(Wu 1985). Hence, it is likely that the enhanced monodeiodinase activity in thyroid following LATS treatment could not be caused by the elevated serum concentration of T₃ and T₄ and rather be caused by a direct effect of LATS. The stimulated thyroidal monodeiodinating activity after LATS provides an insight into the mechanism of increased T₃ secretion from Graves' thyroid glands. Other possible mechanisms may include change in T₃-T₄ content in the thyroglobulin (Abrams & Larsen 1973), heterogeneity of thyroglobulin, a differential release rate of T₃ and T₄ (Laurberg 1979), or increased monodeiodination of T₄ from extrathyroidal pool (Laurberg 1986). More work is needed to further clarify the relative importance of these mechanisms.

In our previous studies (Wu 1983; Wu et al. 1985), we have postulated that the TSH-mediated induction of thyroid iodothyronine monodeiodinating activity is due mainly to an increased synthesis of the enzyme rather than increased affinity. The present findings of an increased Vₘₐₓ with no significant change in apparent Kₘ as well as a 12–24 h latency for the thyroid response to LATS are consistent with the possibility of new enzyme synthesis.

Acknowledgment

This work was supported in part by grants from the Veterans Administration and USPHS Grants.

References


Faber J, Lumnkoltz I B, Kirkegaard C, Siersback-Nielsen K & Friis T (1982): Metabolic clearance and production rates of 3,3′-diiodothyronine, 3′,5′-diiodothyro-
nine and 3′-monoiodothyronine in hyper- and hypothyroidism. Endocrinology 16: 199–206.


Maguire S B, Dennehy A & Cullen M J (1976): The effect of thyrotoxicosis and hypothyroidism on the extrathyroidal conversion of thyroxine to triiodothyro-


Wu S Y (1985): Effect of thyroid hormones, food deprivation and cold exposure on thyroid T4-outer-ring monodeiodinating activity (5'-DIA) in mice, p 160 Abstr. Prog 9th Int Thyroid Congr, September, Sao Paulo, Brazil.

Received June 26th, 1985.
Accepted August 4th, 1986.

Dr Sing-Yung Wu, Nuclear Medicine Service, VA Medical Center, 5901 East Seventh Street, Long Beach, CA 90822, USA.