Tonic effect of endogenous TSH on the in vitro thyroid cAMP response to TSH

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Abstract. The present study was undertaken to compare the effects of 3,5,3'-triiodothyronine (T3) alone and T3 plus bovine thyrotrophin (bTSH) given chronically in vivo on the TSH-stimulated cyclic adenosine 3',5'-monophosphate (cAMP) production in a mouse thyroid in vitro. Mice were given T3 (5 μg/ml) in drinking water for 4 days. The thyroid cAMP concentrations after an incubation with 10 mU/ml of TSH for 10 min were decreased by 50% in T3-treated mice as compared to the control. In the second experiment, mice were given T3 alone or T3 plus 0.5 mU of bTSH ip daily for 4 days. The combined treatment with T3 and TSH partially restored the reduction of cAMP response to TSH that was induced by T3 alone. In the third experiment, mice were given T3 alone for 7 days, or T3 for 7 days plus TSH for the last 3 days. The reduced cAMP response to TSH induced by T3 alone was again partially restored by the concomitant treatment with TSH.

These results indicate 1) that the capacity of the thyroid cAMP to respond to TSH is regulated, at least in part, by a trophic effect of endogenous TSH and 2) that the impaired capacity caused by a loss of tonic effect of endogenous TSH is reversible.

It has been well documented that in vivo administration of thyroid hormone to animals causes a decrease in the capacity of the thyroid gland to respond to thyrotrophin (TSH) (Croxson et al. 1977; Florsheim et al. 1970; Friedman et al. 1977, 1979; Gafni et al. 1975; Pisarev et al. 1976; Rapoport et al. 1976; Shimizu & Shishiba 1975; Shishiba et al. 1974, 1975; Takasu et al. 1974; Yu et al. 1976). The parameters of thyroid function reported to be affected by the thyroid hormone treatment are as follows: thyroid hormone secretion (Florsheim et al. 1970; Shishiba et al. 1975), colloid droplet formation (Shimizu & Shishiba 1975; Shishiba et al. 1974, 1975), ornithine-decarboxylase activity (Friedman et al. 1979; Yu et al. 1976) and RNA synthesis (Pisarev et al. 1976), adenylyl cyclase activity and cAMP formation (Friedman et al. 1977, 1979; Gafni et al. 1975; Takasu et al. 1974; Yu et al. 1976).

However, it is still unknown whether the suppressive effects of thyroid hormones on these parameters are due to their direct effects on thyroid glands (short-loop negative feedback) or to the withdrawal of a tonic effect of endogenous TSH resulting from elevated thyroid hormone levels.

Recently, Gafni et al. (1975), Croxson et al. (1977) and Friedman et al. (1979) postulated that the loss of responsiveness to TSH after a T3 pre-treatment is probably primarily due to a prolonged reduction of circulating TSH levels, rather than to elevated serum T3 concentrations.

The present study was undertaken to answer the question as to whether, or not, the inhibitory effects of T3 given in vivo on the responsiveness of thyroid cAMP to TSH in vitro are due to increases in the circulating thyroid hormone levels per se (short-loop negative feedback) or to a loss of tonic effects of endogenous TSH.

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Materials and Methods

Animals used were DDY male mice weighing 25 g, and they were fed a low iodine diet for 2 to 4 weeks. In Experiment 1 and 2, the mice were injected sc with 3,5,3'-triiodothyronine (T3) (1 µg/0.2 ml) dissolved with 50% propylene glycol and diluted with distilled water, and were then given T3 (5 µg/ml) in drinking water ad libitum for 4 days. In Experiment 2, a group of mice was injected ip with 0.5 mU of bovine thyrotropin (bTSH) (Thytoparan, Armour), dissolved in 0.9% saline with 0.2% bovine serum albumin (BSA) every 24 h for 4 days together with T3 treatment. In Experiment 3, mice were given T3 for 7 days. Groups of mice were injected with vehicle (0.2% BSA) or 0.5 mU of TSH every 24 h for the last 3 days during the T3 treatment.

After completion of the in vivo treatment, the mice were bled by venipuncture from the orbital plexus to determine serum T4 concentrations by radioimmunoassay using RIA-MAT T4 (Daiichi Radioisotope Co., Tokyo, Japan) and were terminated by cervical dislocation.

The in vitro study was performed according to the method described earlier by Ikeda et al. (1980) with minor modifications. The thyroid lobes from each mouse were detached from the trachea and placed into a cold Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4). They were pre-incubated in a KRB buffer with 0.2% BSA, 0.1% glucose and 10 mM theophyllin for 30 min at 37°C under a 95% O2-5% CO2 atmosphere. They were then incubated in a fresh KRB buffer with 10 mM theophyllin and 10 mU/ml of TSH for 10 min at 37°C under 95% O2-5% CO2. After incubation with TSH, the thyroid lobes were quickly weighed and homogenized in glass homogenizers, and the homogenates were then centrifuged at 10,000 g for 15 min at 4°C and the supernatant was evaporated in a water-bath at 50°C under a stream of N2 gas. The sediments were re-suspended with 500 µl of acetate buffer (pH 6.2). Aliquots of the buffer were used for determination of the cAMP concentrations by radioimmunoassay using commercial kits (Mitsutoatsu Co., Tokyo, Japan).

In this in vitro incubation system, 10 mU/ml of TSH elicited a maximal cAMP response.

Thyroid cAMP concentrations were calculated per mg wet weight in each experiment. Values for thyroid cAMP concentrations of each group were expressed as a percent of the control group.

Data were analysed using Student's t-test.

Results

Serum T4 concentrations in mice after in vivo treatment

Data are shown in Table 1. Serum T4 levels were decreased by T3 treatment when the mice had been fed a low iodine diet for 14 days. When mice had been fed a low iodine diet for 28 days, the effects of T3 treatment on serum T4 concentrations were not significant. TSH given for the last 3 days significantly increased serum T4 levels during T3 treatment in the third experiment.

Effects of T3 given for 4 days on thyroid cAMP response to TSH (Experiment 1)

Data are shown in Fig. 1. Thyroid cAMP concentrations after an incubation with 10 mU/ml of TSH were reduced in T3-treated mice by 50% as compared to the controls. Basal thyroid cAMP levels after incubation with only 10 mM theophyllin for 30 min were not significantly different between T3-treated mice and the controls (0.79 ± 0.07 vs 0.76 ± 0.09 pmoles/mg wet weight, mean ± SEM).

Table 1. Serum T4 concentrations in mice.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Duration of low iodine diet (days)</th>
<th>Group</th>
<th>Serum T4 (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>Control</td>
<td>3.6 ± 0.2 a (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3</td>
<td>2.2 ± 0.4 b (5)</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>Control</td>
<td>2.9 ± 0.3 (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3</td>
<td>1.4 ± 0.3 c (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3 + TSH</td>
<td>1.3 ± 0.1 d (4)</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>Control</td>
<td>1.4 ± 0.3 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3</td>
<td>1.2 ± 0.4 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3 + BSA</td>
<td>2.0 ± 0.4 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3 + TSH</td>
<td>2.9 ± 0.6 e (5)</td>
</tr>
</tbody>
</table>

In Experiment 1 and 2, T3-treated mice were given T3 (5 µg/ml) in drinking water for 4 days. T3 plus TSH-treated mice were given T3 in drinking water and injected ip with 0.5 mU of TSH for 4 days. In Experiment 3, T3-treated mice were given T3 for 7 days. T3 plus TSH-treated mice were injected with 0.5 mU of TSH for the last 3 days during T3 treatment. T3 plus BSA-treated mice were injected with 0.2% BSA for 3 days in place of TSH.

a: mean ± SE.

Significantly different from value for control:

b: P < 0.02;
c: P < 0.01;
d: P < 0.001;
e: P < 0.05.

Numbers in parenthesis indicate numbers of determination.
Effects of T₃ plus TSH given concomitantly for 4 days on thyroid cAMP response to TSH (Experiment 2)

Data are shown in Fig. 2. Treatment with T₃ alone caused a 75% decrease in a thyroid cAMP response to TSH in this experiment. TSH (0.5 mU/day) given ip daily for 4 days together with T₃ partially restored the reduction induced by T₃ alone. Basal thyroid cAMP concentrations were not affected by administration of TSH (0.5 mU/day) alone for a 4 day period (data not shown).

Effects of TSH given for the last 3 days during T₃ treatment for 7 days (Experiment 3)

Data are shown in Fig. 3. Treatment with T₃ alone for 7 days reduced the thyroid cAMP response to TSH by 80%. TSH given for the last 3 days during T₃ treatment, again restored in part the suppression induced by T₃ alone, while BSA did not.

Discussion

The present study demonstrates for the first time that a prolonged T₃ treatment in vivo caused a marked decrease in a thyroid cAMP response to TSH in vitro. Gafni et al. (1975) reported that T₃ treatment for 10 days reduced the thyroid cAMP formation after an iv injection with TSH in vivo.

Furthermore, our data indicates for the first time that the concomitant treatment of TSH together with T₃ partially restored the impaired cAMP response to TSH induced by T₃ treatment alone. These findings strongly suggest that the loss of responsiveness caused by the T₃ treatment is, at least in part, due to the withdrawal of a tonic effect of endogenous TSH.

Our conclusions are in accordance with those of previous investigators (Croxson et al. 1977; Friedman et al. 1979; Gafni et al. 1975). Gafni et al. (1975) reported that the impaired responsiveness of thyroid hormone secretion to TSH induced by prolonged T₃ treatment was completely restored with concomitant treatment of TSH with T₃ or by withdrawal of T₃. Croxson et al. (1977) reported that T₃ treatment failed to inhibit the TSH-mediated thyroid hormone release in human beings during daily injection of bovine TSH despite a 4-fold elevation in serum T₃ concentrations. Fur-
thermore, Friedman et al. (1979) demonstrated that the impaired ornithine-decarboxylase activity resulting from hypophysectomy or T₃ pre-treatment was restored by combined treatment of TSH with hypophysectomy or a T₃ pre-treatment.

In the third experiment, it was shown that the loss of thyroid cAMP response to TSH caused by T₃ treatment was partially restored by the concomitant administration of TSH for the last 3 days during the T₃ treatment for 7 days. These findings indicate that the process in the impairment of thyroid cAMP response resulting from a loss of tonic effects of endogenous TSH is reversible. Similar phenomena were observed in previous experiments. The loss of responsiveness of thyroid hormone release to TSH caused by T₃ pre-treatment was restored by a withdrawal of T₃ for 3 days (Gafni et al. 1975). Furthermore, the impaired thyroid ornithine-decarboxylase activity resulting from hypophysectomy was restored by treatment of TSH injected more than 2 days after the hypophysectomy (Friedman et al. 1979).

It is well known that chronic TSH treatment causes thyroid desensitization to subsequent TSH stimulation in vivo as well as in vitro (Rapoport & Adams 1976; Field et al. 1979; Zakarija & McKenzie 1975; Holmes et al. 1980). In the present study, TSH was injected daily for 3–4 days. Therefore, it is possible that in vitro thyroid cAMP response to TSH could have been blunted in the animals which were given TSH injection, as a result of thyroid desensitization to TSH. Nevertheless, the results show that the concomitant TSH administration with T₃ for 3–4 days did increase thyroid cAMP response to TSH as compared to the thyroid cAMP response of animals given T₃ alone. Thus, taken together, it would be reasonable to conclude that in these experimental conditions, repeated TSH administration restores thyroid cAMP response to TSH in mice treated with T₃, although the restoration could have been underestimated by a possible thyroid desensitization to TSH.

Since we have not measured serum TSH levels in mice either before or after T₃ treatment, it is not possible to accurately know how T₃ treatment was effective in decreasing TSH levels. However, it is well established by many previous investigators (Florsheim et al. 1970; McKenzie 1967; Shimizu & Shishiba 1975) that T₃ treatment for 3 to 4 days keeps the blood radioactivity at the lowest levels in the mice for the McKenzie bioassay, suggesting that such treatment is sufficient to suppress secretion of

![Cyclic AMP](image_url)

**Fig. 3.**

Effects of TSH (0.5 mU/day) given for the last 3 days during a T₃ treatment for 7 days on thyroid cAMP concentrations after an incubation with 10 mU/ml of TSH for 10 min. Columns and vertical lines indicate mean ± SEM. The numbers in parenthesis indicate numbers of mice. *P < 0.05, **P < 0.01.
endogenous TSH. In the present study (Experiments 1 and 2), mice were treated essentially similar to those for the McKenzie bioassay.

In the present study, mice were injected with 0.5 mU of bTSH daily. The dose of TSH employed in this study is relatively small as compared to the previous studies (Friedman et al. 1979; Gafni et al. 1975) in which 10 mU of bTSH was injected into mice or 0.5 to 1 U of bTSH was injected into rats. It is possible that the recovery was only partial in this experiment because of the relatively small dose of TSH.

Serum T₄ levels increased by TSH injection during T₃ treatment in the third experiment, but not in the second experiment. The reason for it is not known. One possible explanation is as follows. Mice in the third experiment were severely iodine-deficient (serum T₄, 1.4 µg/dl), as compared to mice in the second experiment (serum T₄, 2.9 µg/dl). It might be possible in such conditions as in the third experiment that iodide metabolism from T₃ accumulated in the thyroid gland and then that exogenous TSH stimulated incorporation of the iodide into T₄ and T₄ secretion from the thyroid.

It is well known that iodine inhibits the thyroid adenylate cyclase response to TSH (Burke 1970; Rapoport et al. 1975, 1976; Van Sande et al. 1975). Therefore, the possibility remains that the inhibitory effects of T₃ on thyroid cAMP formation were not due to the hormone but to iodide metabolism from T₃. Under the present experimental conditions it is very difficult to precisely discriminate T₃ effects on thyroid cAMP response from iodide effects on it. Particularly in the third experiment, serum T₄ levels did not significantly decrease following T₃ treatment, but increased by repeated TSH injection despite T₃ treatment. Therefore, as discussed earlier, it is possible that in the third experiment, effects of iodide metabolism from T₃ could have overlapped on the T₃ effects on thyroid cAMP response. However, we believe that the inhibitory effects on thyroid cAMP response were mainly due to T₃ but not to iodide throughout the present experiments because of the following reasons. First, serum T₄ levels decreased following T₃ treatment in the first and second experiment. Second, the inhibitory effects of T₃ were diminished by the combined treatment of TSH and T₃ in the second and third experiment. If the inhibitory effects were due to iodide, they should have been augmented by concomitant administration of TSH (Rapoport et al. 1976).

At present, the mechanism is unknown by which the responsiveness of a thyroid cAMP formation to TSH is impaired by the withdrawal of a tonic effect of endogenous TSH. It is possible that the loss of endogenous TSH may result in: 1 a reduction in TSH receptor, 2) interference in hormone-receptor interaction, 3) the impairment of adenylate cyclase or 4) activation of phosphodiesterase. The first possibility appears to be unlikely, since previous investigators (Witte et al. 1980) found that in vivo T₃ treatment for 5 days did not affect the TSH binding in rat thyroid glands. The fourth possibility seems also to be unlikely, since phosphodiesterase activity has been reported to increase by chronic stimulation with endogenous TSH (Bastomsky et al. 1971). On the other hand, Takasu et al. (1978) reported that adenylate cyclase activity in cultured porcine thyroid cells was decreased when cultured without TSH as compared to when cultured in 5–50 µU/ml of TSH.

In conclusion, the present study indicates 1) that the capacity of the thyroid cAMP to respond to TSH is regulated, at least in part, by a trophic effect of endogenous TSH and 2) that the impaired capacity caused by a loss of tonic effect of endogenous TSH is reversible.

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