Effects of intrathyroidal metabolism of thyroxine on thyroid hormone secretion: increased degradation of thyroxine in mouse thyroids stimulated chronically with thyrotrophin

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Abstract. Mice were infused continuously with graded doses of bovine TSH (bTSH) and changes in plasma concentrations of bTSH and T₄ were measured. Then mice infused with 100 mU TSH per day were sacrificed on days 0, 1, 3 and 5 and their thyroids were excised to determine in vitro secretion of T₄, T₃ and rT₃ during 3 h of incubation. At the end of the incubation, thyroidal contents of T₄, T₃ and rT₃ were also determined after pronase digestion. Plasma bTSH levels were increased on day 1 to a level of 110 µU/ml and remained unchanged thereafter. Plasma T₄ concentrations increased approximately 2-fold on day 1, but decreased to initial levels on days 3 and 5. Changes in T₄ secretion in vitro paralleled those in plasma T₄ concentrations; T₄ secretion increased 2-fold on day 1, and decreased to the pre-TSH levels on days 3 and 5. In contrast, T₃ secretion increased throughout the experimental period. The T₃/T₄ ratio in thyroidal secretion in vitro was the same as that in thyroidal contents on days 0 and 1 of TSH infusion, but the former was significantly greater than the latter on days 3 and 5. PTU (5.9 x 10⁻⁵ M), a known inhibitor of T₄ deiodination, added to the incubation media did not affect T₄, T₃ and rT₃ secretion in vitro. When [¹²⁵I]T₄ was added to the media and incubated with mouse thyroid, no labelled products of T₄-deiodination were observed to appear in the media even in mice infused with TSH for 5 days. These results suggest that intrathyroidal metabolism of T₄ has physiological significance in controlling thyroid hormone secretion at least in TSH-stimulated thyroids.

Thyroid hormone metabolism has been extensively studied by many investigators in various extrathyroidal tissues (Oppenheimer & Surks 1971; Middlesworth 1974; Chopra et al. 1978). Deiodination of T₄ in thyroid glands was also reported using thyroid lobes of rats (Haibach 1971; Green 1978) and cultured human thyroid cells (Bidey et al. 1976). Recently, 5'- or 5-deiodinating activity was observed in the particulate fraction of thyroids of rats (Erickson et al. 1981, 1982) and man (Ishii et al 1981, 1982), and these activities were increased in TSH-stimulated thyroids in rats and in thyroids in patients with Graves' disease. However, there is little evidence that intrathyroidal metabolism of thyroid hormones plays a significant role in regulating their secretion in vivo. The present experiments were carried out to investigate whether such intrathyroidal metabolism affects hormone secretion in thyroids of mice stimulated chronically with TSH.
Material and Methods

Experiments were performed in male DDY mice (30–40 g body weight) fed with a regular diet. Bovine TSH (bTSH) was from Sigma Chemical Co., St. Louis, Mo, USA and pronase (10⁶ tyrosine units/g) was from Kaken Science Co., Tokyo, Japan. Other reagents were purchased from commercial sources.

Continuous infusion of bTSH

A tail vein of the mouse was cannulated with a polyethylene tube (0.3 mm inner diameter) connected at the other end through a tuberculin needle to a 1 ml syringe mounted on an infusion pump (Showa Science Co., Tokyo, Japan). Saline was infused for more than 2 h to allow the mouse to become accustomed to these new conditions. With a fresh syringe, bTSH in saline containing 0.1% bovine serum albumin (BSA) was infused continuously at a rate of 1 ml/day for 1 to 5 days. Syringes were changed every 24 h.

In vitro thyroid hormone secretion

After infusion of TSH, mice were bled and killed by a cervical dislocation, and tracheae with adhering thyroids were excised. These were incubated each in a separate test tube (12 × 75 mm) in 0.25 ml of Krebs-Ringer bicarbonate buffer containing 0.2% BSA and 0.1% glucose. Following pre-incubation in a shaking water bath at 37°C under 95% O₂–5% CO₂ for 30 min, the tissue was transferred to fresh media, incubated for 3 h and then the thyroid lobes were removed from the trachea, weighed and frozen for determination of thyroidal hormone contents. The media were collected for analysis of T₄, T₃ and rT₃. In experiments studying effects of antithyroid drugs on thyroid hormone secretion in vitro, propylthiouracil (PTU, 5.9 × 10⁻⁵ M = 10 μg/ml) or methimazole (MMI, 10⁻³ M) was added to the incubation media.

Thyroid hormone contents

The thyroid homogenates were digested with pronase according to the method of Inoue & Taurog (1967) in rats. Briefly, each thyroid was homogenized in a glass homogenizer in 0.5 ml of cold Tris buffered saline (0.03 M Tris-HCl–0.11 M NaCl, pH 8.5) containing 0.02 M MMI. The homogenate was centrifuged at 1000 × g for 15 min at 4°C. A quarter ml of the buffer containing pronase (1.75 mg/tube) and then a drop of toluene was added to the supernate (0.25 ml). After anaerobic digestion at 37°C for 18 h, the iodothyronines were extracted with methanol:2 N ammonia (99:1). For measurements of T₄, T₃ and rT₃, the extract was evaporated to dryness and the residue was re-dissolved with 0.1 M sodium phosphate buffer (pH 7.4) containing 0.1% BSA. In mice, our previous study (Chiu et al. 1983) showed that homogenates of the thyroid pre-labelled with ¹²⁵I were digested satisfactorily with pronase.

Measurements

Plasma bTSH concentrations in bTSH-infused mice were measured by RIA developed in this laboratory. Purified bTSH and anti-bTSH antisera were kindly supplied by Dr. Rees Smith, Cardiff, UK. T₄ and T₃ in plasma, incubation media and pronase-digests were measured by specific RIAs (Gamma Coat T₄ and T₃, Clinical Assays, Cambridge, Mass, USA). Reverse T₃ was measured with RIA kits (Dainabot Radiosotope Lab., Matsudo, Chiba, Japan). In these RIAs, cross-reactivities of the antisera with other iodothyronines were as follows: 4.8% with T₃ and 1.0% with rT₃ for T₄ RIA, 0.21% with T₄ and < 0.01% with rT₃ for T₃ RIA, and 0.009% with T₄ and < 0.0001% with T₃ for rT₃ RIA, respectively. In vitro thyroidal secretion and thyroidal content of each iodothyronine were expressed as pmol/mouse.

Degradation of [¹²⁵I]T₄ added to the incubation media

[3,5⁻¹²⁵I]T₄ (80 μCi/ml; 698 μCi/μg) was obtained from Dainabot Radiosotope Lab. (Matsudo, Chiba, Japan) in 50% propylene glycol and purified by dialysis according to the method of Schussler & Plager (1967) with a modification by adding 1% BSA instead of human serum. The labelled T₄ was added to the media (0.1 μCi/tube) and incubated with mouse thyroids under the same conditions as described above.

Thin layer chromatography

After the incubation, iodoamino acids in the media were extracted with 2 vol of methanol:2 N ammonia (99:1) containing 10⁻⁴ M of unlabelled T₄, T₃ and rT₃ as carriers. The extract was converted by evaporation and an aliquot was applied to a silica gel plate for thin layer chromatography (Eastman Kodak Co., Rochester, NY, USA) and chromatographed by an ascending system in ethyl acetate:methanol:2 N ammonia (5:2:3) solvent (West et al. 1965). Inorganic iodide, T₄, T₃ and rT₃ could be separated satisfactorily in this system. The areas of the chromatogram corresponding to the bands on its radioautogram or to the bands of iodothyronine markers stained by diazotized sulphanilic acid were excised and counted by an automatic γ-counter. The degradation of [¹²⁵I]T₄ during incubation was determined by subtracting the value in tissue free incubation.

Statistical analysis

The significance of difference was determined by Student’s t-test.

Results

Effects of bTSH infusion on plasma bTSH and T₄ concentrations

In the first series of experiments, mice were infused with graded doses (0–500 mU/day) of bTSH
Effects of TSH infusion on in vitro T₄ and T₃ secretion

At the end of the 5-day infusion period, T₄ and T₃ secretion rates in vitro were determined (Fig. 2). T₄ secreted from thyroid lobes into the media was unchanged by infusion of TSH (20–500 mU/day) for 5 days, but T₃ secretion was increased with a dose of TSH 50 mU or more per day.

In the second series of experiments, mice were infused with 100 mU/day of TSH and sacrificed on days 0, 1, 3 and 5 of infusion to determine possible changes in rates of T₄ and T₃ secretion in vitro (Fig. 3, open columns). T₄ secretion was twice normal on day 1 but decreased to pre-TSH levels on days 3 and 5. On the other hand, T₃ secretion was 3 times normal on day 1 and remained high thereafter.

![Fig. 1.](image)

Plasma bTSH and T₄ concentrations in mice during a 5 day infusion of bTSH. Mice were infused with graded doses of bTSH for 5 days and were bled on days 0, 1, 3 and 5 of infusion. ND, plasma bTSH concentrations were not detectable (<10 µU/ml). Results shown are the means ± SE of values for 6 mice. One determination was made for each mouse.

![Fig. 2.](image)

Effects of graded doses of TSH infusion for 5 days on T₄ and T₃ secretion in vitro in mice. After the 5 day infusion of TSH, secretion of T₄ and T₃ in vitro was determined. Results shown are the means ± SE of values for 6 mice. One determination was made for each mouse. **P < 0.01 vs control (0 mU/day).
Effects of PTU on in vitro T₄ and T₃ secretion in TSH-infused mice

PTU added to the incubation media of thyroids of mice infused with 100 mU TSH per day did not affect T₄ secretion on days 0 and 1, but increased on days 3 and 5 to the level of day 1 (Fig. 3, shaded columns), thus preventing the decrease (from the high level of day 1) in T₄ secretion seen on days 3 and 5 in the absence of PTU. T₃ secretion was, however, not affected by PTU throughout the experimental period.

**Fig. 3.**

T₄ and T₃ secretion in vitro with and without PTU in the media. Mice were infused with 100 mU/day of TSH for 5 days. Thyroids were excised on days 0, 1, 3 and 5, and T₄ and T₃ secretion in vitro was determined with or without 5.9 x 10⁻⁵ M PTU in the incubation media. Results shown are the means ± se of values for 5 mice. One determination was made for each mouse. **P < 0.01; *P < 0.05 vs control (day 0).**

Effects of TSH infusion on thyroidal content of T₄ and T₃

Changes in thyroidal content of T₄ and T₃ during TSH infusion are shown in Fig. 4. Thyroidal T₄ was decreased during TSH infusion, but T₃ was increased significantly only on day 5. PTU added to the incubation media had no effect on thyroidal hormone content at the end of the incubation period and data pertaining to the two groups (± PTU) were combined. Weights of thyroids were not changed on day 1 of TSH infusion, but increased about 2-fold on days 3 and 5.

**Fig. 4.**

T₄ and T₃ contents in thyroids. Mice were infused with 100 mU/day of TSH for 5 days and were sacrificed on days 0, 1, 3 and 5 of infusion. At the end of the incubation for measurements of thyroid hormone secretion in vitro, thyroidal contents of T₄ and T₃ were determined after pronase digestion. Results shown are the means ± se of values for 10 mice. One determination was made for each mouse. **P < 0.01; *P < 0.05 vs control (day 0).**

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**Effects of PTU on T₃/T₄ ratio in in vitro secretion from thyroids of TSH-infused mice**

Molar T₃/T₄ ratios in thyroidal secretion in the presence or absence of PTU and in thyroid glands were compared (Fig. 5). The data were taken from Figs. 3 and 4. T₃/T₄ in thyroids of mice infused with 100 mU TSH per day increased gradually from 0.1 to 0.2 during 5 days of TSH infusion. T₃/T₄ in thyroidal secretion was the same as that in thyroids on days 0 and 1 of infusion, but the former was significantly greater than the latter on days 3 and 5. In the presence of PTU in the incubation media, T₃/T₄ ratios in thyroidal secretion and in thyroids were the same throughout the experimental period.

**Effects of TSH infusion on in vitro rT₃ secretion and on thyroidal rT₃**

Reverse T₃ secretion in vitro was increased significantly on day 5 and PTU augmented rT₃ secretion on days 3 and 5 of infusion of 100 mU TSH per day (Fig. 6). Thyroidal rT₃ was increased 3-fold on day 3 and 6-fold on day 5.

**Effects of MMI on in vitro hormone secretion from thyroids of TSH-infused mice**

MMI (10⁻³ M) added to the incubation media had no significant effects on secretion of T₄, T₃ and rT₃ in vitro during 5 day infusion of TSH (Table 1).

**Degradation of [¹²⁵I]T₄ added to the incubation media**

The fate of [¹²⁵I]T₄ added to the incubation media...
was incubation. By subtracting values in tissue free incubation, no radiolabelled products of deiodination of T₄ (inorganic iodide, T₃ and rT₃) were observed in the media even after incubation with thyroids of TSH-infused mice, while, in experiment done in tandem, in vitro T₄ secretion in mice infused with TSH for 5 days was increased by PTU in the same way as shown in Fig. 3.

Table 1.
Effects of MMI in the media on T₄, T₃ and rT₃ secretion in vitro in mice infused with TSH in vivo.

<table>
<thead>
<tr>
<th>Days of infusion</th>
<th>T₄ secretion (pmoles/mouse)</th>
<th>T₃ secretion (pmoles/mouse)</th>
<th>rT₃ secretion (pmoles/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMI (-)</td>
<td>MMI (+)</td>
<td>MMI (-)</td>
</tr>
<tr>
<td>0</td>
<td>4.31 ± 0.32</td>
<td>4.73 ± 0.58</td>
<td>0.45 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.30 ± 0.06</td>
<td>0.34 ± 0.05</td>
</tr>
<tr>
<td>1</td>
<td>10.58 ± 2.24*</td>
<td>7.42 ± 0.53*</td>
<td>1.40 ± 0.28**</td>
</tr>
<tr>
<td></td>
<td>1.35 ± 0.11**</td>
<td>0.30 ± 0.03</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>4.13 ± 0.19</td>
<td>4.91 ± 0.69</td>
<td>1.81 ± 0.17**</td>
</tr>
<tr>
<td></td>
<td>1.85 ± 0.22**</td>
<td>0.48 ± 0.07</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>4.31 ± 0.27</td>
<td>3.85 ± 0.05</td>
<td>1.34 ± 0.22**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.69 ± 0.08**</td>
<td>0.67 ± 0.07**</td>
</tr>
</tbody>
</table>

Mice were infused with 100 mU/day of TSH and T₄, T₃ and rT₃ secretion from excised thyroids was determined on days 0, 1, 3 and 5 of infusion with or without 10⁻³ M MMI in the incubation media. Results shown are the means ± SE of values for 5 mice. One determination was made for each mouse. ** P < 0.01; * P < 0.05 vs control (day 0).

Radioactivity was taken up in part by thyroid lobes (5–7%) at the end of the incubation. More than 95% of the radioactivity in methanol extracts of the media was detected as T₄. By subtracting values in tissue free incubation, no radiolabelled products of deiodination of T₄ (inorganic iodide, T₃ and rT₃) were observed in the media even after incubation with thyroids of TSH-infused mice, while, in experiment done in tandem, in vitro T₄ secretion in mice infused with TSH for 5 days was increased by PTU in the same way as shown in Fig. 3.

Table 2.
Degradation of [¹²⁵I]T₄ added to the incubation media.

<table>
<thead>
<tr>
<th></th>
<th>PTU in the media</th>
<th>Radioactivity in the media (%)</th>
<th>Radioactivity in thyroids (%)</th>
<th>T₄ in extracts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue free¹</td>
<td>(−)</td>
<td>(100)</td>
<td>−</td>
<td>93.7</td>
</tr>
<tr>
<td>Saline-infused²</td>
<td>(−)</td>
<td>80.6</td>
<td>5.6</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>80.1</td>
<td>5.4</td>
<td>95.3</td>
</tr>
<tr>
<td>TSH-infused³</td>
<td>(−)</td>
<td>76.9</td>
<td>7.1</td>
<td>96.2</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>75.3</td>
<td>6.8</td>
<td>95.4</td>
</tr>
</tbody>
</table>

Mouse thyroids were incubated in vitro for 3 h with [¹²⁵I]T₄ with or without PTU (5.9 × 10⁻⁵ M) in the media. After incubation, iodoamino acids in the media were extracted with methanol-2 N ammonia and analyzed by thin layer chromatography (see Material and Methods). About 6% loss of label in going from media to methanol-extracts was observed in all groups including 'tissue free'. Data are the means of duplicate determinations.

¹ Labelled T₄ was incubated without mouse thyroids.
² Mice were infused with saline for 5 days as normal control.
³ Mice were infused with 100 mU TSH per day for 5 days.
⁴ Per cent radioactivity in the media at the end of the incubation.
⁵ Per cent radioactivity in the thyroid lobes at the end of the incubation. Residual radioactivity was found in surrounding tissues.
⁶ Per cent radioactivity of band of T₄ on the thin layer chromatogram.
Discussion

Chiu et al. (1983) have previously reported that plasma T4 concentrations increased only transiently in mice even if a large injected dose of TSH was repeated. In the present study, this effect was also observed in TSH-infused mice. Several explanations are possible. First, infused TSH may be inactivated by production of the antibody against bTSH. However, plasma bTSH concentrations measured by RIA were elevated and remained so during 5 days of infusion of the highest levels of infused TSH. Second, the decrease in the transiently high plasma T4 may be attributable to an actual decreased thyroidal secretion of the hormone. Third, increase in clearance of T4 in extra-thyroidal tissues is another possibility. The clearance rate of T4 in blood is increased in patients with thyrotoxicosis (Sterling & Chodos 1956) and in thyrotoxic rats (Cullen et al. 1973).

The present study examined the second possibility by measuring in vitro secretion of T4 after TSH stimulation. In mice infused chronically with TSH, increase in in vitro thyroidal secretion of T4 was transient. In vitro release of thyroid hormones from excised thyroids may not reflect in vivo secretion. However, the changes in thyroidal secretion of T4 measured here in vitro are consistent with those found in plasma levels of the hormone. As for T3, plasma concentration of the hormone determined at the time of sacrifice was significantly higher than the initial level after 5 day infusion of TSH as was in vitro T3 secretion (data are not shown). Moreover, it has recently been demonstrated that TSH added to the incubation media increased both free intrathyroidal and released thyroid hormones proportionally (Kubota et al. 1982). These results suggest that the changes in thyroid hormone secretion measured in vitro reflect similar changes in in vivo secretion rates indicating that the decrease in thyroidal secretion of T4 is one of the causes for the decrease in plasma T4 concentration.

Although refractoriness to stimulation by TSH of thyroid hormone synthesis and secretion has been described (Field et al. 1979), our previous report showed that refractoriness to TSH is not the cause of the decrease after the initial rise in plasma T4 concentrations (Chiu et al. 1983). Furthermore, the present study shows that T3 secretion in vitro was continuously increased by infusion of TSH for 5 days.

T3 secretion in vitro was higher than normal throughout the 5 day period of TSH infusion in contrast to that of T4, and the T3/T4 ratio in thyroidal secretion was significantly greater than that in the thyroids on days 3 and 5. Since the thyroidal T3/T4 ratio is determined principally by the ratio of these amino acids in thyroglobulin, the discrepancy in this ratio in secreted vs thyroidal hormone suggests that either preferential release of T3 from thyroglobulin and/or selective degradation of T4 occur in the gland. With regard to the former, heterogeneous metabolism of thyroid iodine pools (Schneider 1964) might lead to preferential hydrolysis of a small pool of T3-rich thyroglobulin with a higher T3/T4 ratio than would be expected on the basis of the average T3/T4 determined for total thyroidal thyroglobulin. The results of the experiments suggest that TSH-stimulated deiodination of T4 to T3 in the thyroid was at least partly responsible for the high T3/T4 ratio in the secreted material.

In the present study, a large increase in thyroidal rT3 was observed in mice infused with TSH for 3 or 5 days. In control mice, percentage of rT3 in total thyroidal iodothyrones was very similar to that in dogs determined by RIA (Lauberg 1978a). Taurog et al. (1976) demonstrated that, when compared with T4, more thyroidal rT3 was formed in rats with lower iodine intake. It is not clear at present why rT3 increases in chronically stimulated thyroids in mice, but it might be possible that rT3 could be increased in chronically stimulated thyroids.

Thus, PTU present in the incubated media in concentrations that inhibit the conversion of T4 to T3 in rat liver homogenates (Chopra 1977) and in a particulate fraction of thyroids in rats (Erickson et al. 1981) and man (Ishii et al. 1981) prevented the decrease in T4 secreted in vitro on days 3 and 5 of the in vivo TSH infusion after the initial rise on day 1 causing a significant increase in secreted T3/T4 over the control value on days 3 and 5 and over thyroidal T3/T4. MMI which does not inhibit hepatic and thyroidal deiodinations was without effect. Our study with addition of [125I]T4 to the incubation media appears to indicate that deiodination of T4 re-entered to thyroids from the media described by Green (1978), if any, does not affect secretion rates of thyroid hormones measured in vitro under the present experimental conditions, and that the effect of PTU on in vitro secretion of T4 on days 3 and 5 of TSH infusion is mainly due
to inhibition of the deiodination of T₄ prior to release of the hormone from the gland.

In perfused canine thyroids, PTU in the perfusate decreased secretion of T₃ and rT₃ without increase in T₄ secretion (Laurberg 1978b). However, in our results, the increase in T₄ secretion in the presence of PTU was not accompanied by a decrease in T₃ and rT₃ secretion. TSH-stimulated thyroids were used in our studies, which may explain the difference of the effect of PTU. PTU increased rT₃ secretion in vitro on days 3 and 5 of TSH infusion, probably because it inhibited deiodination of rT₃ in addition to that of T₄. In Graves' thyroid tissue which is presumed to be chronically stimulated, the sequential deiodinations of T₄ enhanced and PTU inhibits all steps of the metabolism (Ishii et al. 1982).

Gerber et al. (1981) demonstrated that when organic binding of iodine was severely depressed by NaClO₄, the parital resistance of endocytosis to chronic TSH stimulation in rats was abolished and T₄ release was increased. However, our results indicate that both 1 μM MMI and 10 nM NaClO₄ (unpublished data) have failed to reproduce the effect of PTU. In rat thyroid homogenates, T₄ degradation mediated by thyroid peroxidase was demonstrated (Dawber et al. 1971) and the degradation was blocked by both MMI and PTU. However, a lack of effect of MMI on T₄ secretion suggest that the peroxidatic degradation of T₄ is unlikely in our system.

Our results suggest that PTU augments T₄ secretion from chronically TSH-stimulated thyroids because it is an inhibitor of monodeiodination of iodothyronines. The results indicate further that intrathyroidal metabolism of T₄ has physiological significance in controlling thyroid hormone secretion, at least in TSH-stimulated thyroids.

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