PITUITARY THYROTROPH FUNCTION IN HYPOTHYROID RATS

By

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ABSTRACT

The suggestion has been made that the substantial increases in pituitary thyrotrophin (TSH) concentration reported in hypothyroid rats by some workers might be attributable to incomplete surgical thyroidectomy or partial goitrogen block. An attempt, therefore, was made to correlate the severity of hypothyroidism with the storage and release rate of TSH. Female and male Long-Evans rats were made hypothyroid either by surgical thyroidectomy or by administration of propylthiouracil (PTU). Rats were also surgically or chemically thyroidectomized and injected daily with subnormal doses of thyroxine (T₄) so as to ameliorate the hypothyroidism. Pituitary and serum levels of TSH were quantified, both by bioassay (stasis tadpole), and radioimmunoassay. The results showed TSH reduced in the pituitary of the severely hypothyroid rat. In both females and males, amelioration of the hypothyroidism with small chronic doses of T₄ did not increase bioassayable or immunoassayable TSH stores. Paradoxically, bioassay showed serum TSH titers increased in females inversely proportional to the apparent severity of the hypothyroidism. However, radioimmunoassay of the sera of males, whose hypothyroidism was ameliorated by T₄, revealed no significant increases in TSH over thyroidectomy controls. Therefore, the results

a) Deceased.
were unclear as to whether subnormal quantities of T₄ increase TSH release from the pituitary. The differences in serum TSH concentration between hypothyroid-ameliorated females and males are not attributable to sex or to differences in the reliability between the bioassay and immunoassay. However, bioassayable increases in TSH stores may perhaps reflect an influence exerted by pituitary gonadotrophins or sex steroids.

There is considerable evidence that circulating titers of thyrotrophin (TSH) increase in the rat after surgical or chemical thyroidectomy (Bakke & Lawrence 1964; D'Angelo 1961, 1969; Wilbur & Utiger 1967; van Rees 1966). Disagreement still persists, however, regarding TSH concentration in the pituitary of the hypothyroid rat (Fig. 1). D'Angelo (1961, 1969) reported that pituitary TSH concentration fell to essentially zero after goitrogen administration, whereas Bakke & Lawrence (1964) found marked and sustained increases in hormone stores. The suggestion was made that the rebound in TSH stores after thyroidectomy may be attributable to the presence of small quantities of thyroid hormones (D'Angelo 1969). In view of the existing controversy, an experiment was performed which utilized complete and incomplete surgical and chemical thyroidectomy, and both bioassay and immunoassay to quantify TSH in the pituitary and blood.

Fig. 1.
Disagreement regarding pituitary thyrotrophin concentration in hypothyroid rats.
Animals and treatments. – Long-Evans female and male rats, 26 to 28 days of age at experimental onset, were used in this study. Females were either surgically thyroidectomized, fed propylthiouracil (PTU, 0.05 % or 0.1 %) mixed in a standard laboratory diet (Evans et al. 1964), or left intact as normal controls. Some were also given PTU (0.1 %) and injected daily (ip) with 0.05–0.1 μg thyroxine (T₄) per 100 g body weight. Growth, as indicated by gain in body weight, was followed each week. Ten or more representative females in the control and each of the 4 treated groups were sacrificed at experimental intervals of 15, 30, 60, and 90 days. Some males were accorded the same treatment as females except that surgical thyroidectomy and the lower dose of PTU was omitted. Furthermore, a higher daily dose of T₄ (0.2 μg/100 g body weight) was maintained throughout the experiment in the replacement therapy group. Three animals in each of the 3 groups were sacrificed at weekly intervals for 13 weeks. Other males were treated in a similar manner except that, this time, only the lower dose of PTU was administered and via the drinking water. Furthermore, the T₄ was given subcutaneously (nuchal region). In addition, individuals were surgically thyroidectomized with T₄ replacement given to some. After 8 weeks, 8 to 9 males in each of the 5 groups were killed. The males in these groups were fed pelleted Purina rat chow.

Autopsy. – Female rats were exsanguinated during autopsy while under ether anaesthesia. The anterior pituitaries of representative females were weighed, pooled, and homogenized in 1 ml 0.01 n NaOH. The extract was then diluted 10-fold by the addition of phosphate-buffered saline (0.01 M PO₄ + 0.15 M NaCl) containing 1 % bovine serum albumin (BSA), pH 7.5, and 6000 kallikrein-inactivating units of Trasylol®. After centrifugation, small aliquots of the clear extracts were stored at −20°C. Sera were also pooled and stored at −20°C.

Male rats were sacrificed by decapitation and trunk blood collected individually. The sera were stored at −20°C. In the experiment of 8 weeks, anterior pituitaries were weighed, and homogenized individually in 1 ml 0.05 m phosphate buffer containing 1 % BSA, pH 7.5. In the experiment of 13 weeks, pituitaries were individually homogenized in the same reagents used for females (total of 1 ml). After centrifugation, the clear pituitary supernatants were stored at −20°C.

Hormone assays. – Growth hormone (GH) in the pituitaries of female rats was quantified by the complement fixation immunoassay (Wasserman & Levine 1961; Tashjian et al. 1968) using an antiserum to rat GH obtained from Rhesus monkeys. Pituitary GH in male rats was quantified by radioimmunoassay (Schalch & Reichlin 1966).

Pituitary and serum TSH concentration in females was measured by the original stasis tadpole bioassay of D'Angelo et al. (1942). In males, pituitary and serum TSH was quantified by radioimmunoassay (Reichlin et al. 1970).

Protein-bound iodine determination. – Protein-bound iodine (PBI) was quantified in the serum of females by the method of La Roche et al. (1964).

Pituitary morphology. – Eight anterior pituitaries obtained from some of the surgically thyroidectomized and normal control females were prepared for histological examination (Schooley et al. 1966). Differential cell counts, obtained according to the published method (Schooley et al. 1966), were expressed as per cent of pituitary volume.
Selected anterior pituitaries were processed for electron microscopy by perfusing 60-days-post-thyroidectomized or normal control females with chilled (4°C) 3.25% glutaraldehyde which was adjusted to pH 7.4 with Sorensen's phosphate buffer (Hayat 1970). After 5 min, the pituitaries were dissected free and placed in the chilled fixative for an additional 10 min. Small blocks (0.5 mm³) of tissue, removed from the central region of the pituitary, were post-fixed 30 min in 2% osmium tetroxide buffered to pH 7.4 with 0.67 M s-collidine (Hayat 1970). The tissue blocks were then dehydrated conventionally through a series of alcohols and propylene oxide, embedded in Epon 812 (Shell Chemical Corp.), sectioned at 600 to 800 Å, and mounted on carbon-stabilized formvar-coated grids. Sections were then stained with lead citrate, and examined and photographed in an electron microscope at 6000 magnifications.

**RESULTS**

_Completeness of thyroidectomy._ – Completeness of thyroidectomy was confirmed by the stasis of body growth (Figs. 2 and 3; Table 1), and the virtual disappearance of GH from the pituitary (Figs. 2 and 4; Table 1). As expected, daily injections of subnormal doses of T₄, to female rats fed PTU, maintained pituitary GH concentration, although subnormal, over most of the experiment and led to a slight increment in growth (Fig. 2). While T₄ did not always maintain pituitary GH concentration in thyroidectomized male rats (Fig. 4; Table 1), it did cause increased growth (Fig. 3; Table 1).

Completeness of surgical thyroidectomy was further substantiated in females by the absence of ¹³¹I accumulation in the thyroid region (Evans et al. 1964). In addition, the extent of hypothyroidism in all females was evaluated by

_table 1._

Growth, pituitary growth hormone (GH) and thyrotrophin (TSH) concentration, and serum TSH concentration in male rats. Either surgically thyroidectomized (T), thyroidectomized while injected with thyroxine (T₄, 0.2 μg/100 g body weight/day), administered propylthiouracil (PTU, 0.05% in the drinking water), or administered PTU while injected with T₄ as compared to normal (NC). Rats were sacrificed 8 weeks after experimental onset.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Body weight (g)</th>
<th>Pituitary GH (μg/mg)</th>
<th>Pituitary TSH (mU/mg)</th>
<th>Serum TSH (mU/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>9</td>
<td>356 ± 15</td>
<td>40.6 ± 4.5</td>
<td>8.7 ± 0.5</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>T</td>
<td>8</td>
<td>211 ± 6</td>
<td>-0-</td>
<td>4.1 ± 0.3</td>
<td>41.8 ± 3.2</td>
</tr>
<tr>
<td>T + T₄</td>
<td>8</td>
<td>247 ± 5</td>
<td>2.7 ± 1.5</td>
<td>3.5 ± 0.3</td>
<td>36.9 ± 2.7</td>
</tr>
<tr>
<td>PTU</td>
<td>9</td>
<td>177 ± 5</td>
<td>0.4 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>11.1 ± 0.8</td>
</tr>
<tr>
<td>PTU + T₄</td>
<td>9</td>
<td>225 ± 6</td>
<td>1.2 ± 0.5</td>
<td>4.5 ± 0.4</td>
<td>10.9 ± 0.7</td>
</tr>
</tbody>
</table>

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Decreased body growth (left) and disappearance of immunoassayable growth hormone (right) in pituitaries of female rats either surgically thyroidectomized, fed propylthiouracil (PTU), or fed PTU while injected with thyroxine ($T_4$) as compared to normal. The dose of $T_4$ was reduced 50% 30 days after experimental onset because, at that time, it appeared growth was proceeding too rapidly.

Decreased body growth of male rats fed propylthiouracil (PTU, 0.1%) or fed PTU while injected (ip) with thyroxine ($T_4$, 0.2 µg/100 g body weight/day) as compared to normal. SEM too small for illustration are omitted.
Table 2.
Protein-bound iodine (PBI) in the serum of female rats at sequential intervals after either surgical thyroidectomy (T), propylthiouracil (PTU) feeding, or PTU feeding plus thyroxine (T₄) injection (0.05–0.1 µg/100 g body weight/day) as compared to normal. There were 10 rats at each interval in the several groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PBI (µg/100 ml)</th>
<th>Day of experiment</th>
<th>Average 15–90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>T</td>
<td>0.31</td>
<td>0.35</td>
<td>0.12</td>
</tr>
<tr>
<td>0.05 % PTU</td>
<td>0.26</td>
<td>0.27</td>
<td>0.15</td>
</tr>
<tr>
<td>0.1 % PTU</td>
<td>0.81</td>
<td>—</td>
<td>0.57</td>
</tr>
<tr>
<td>0.1 % PTU + T₄</td>
<td>0.36</td>
<td>0.88</td>
<td>—</td>
</tr>
<tr>
<td>Normal</td>
<td>3.00</td>
<td>2.70</td>
<td>3.20</td>
</tr>
</tbody>
</table>

* Insufficient serum for analysis.

Fig. 4.
Disappearance of radioimmunoassayable growth hormone in pituitaries of male rats fed propylthiouracil (PTU, 0.1 %) or fed PTU while injected (ip) with thyroxine (T₄, 0.2 µg/100 g body weight/day) as compared to normal. Three rats per point. SEM too small for illustration are omitted.
Fig. 5.
Decreased thyrotrophin concentration in the pituitaries of female rats at sequential intervals after either surgical thyroidectomy (T), propylthiouracil (PTU) feeding, or PTU feeding plus thyroxine (T₄) injection as compared to normal.

Fig. 6.
Elevated thyrotrophin concentration in the sera of female rats at sequential intervals after either surgical thyroidectomy (T), propylthiouracil (PTU) feeding, or PTU feeding plus thyroxine (T₄) injection as compared to normal.
serum PBI concentration (Table 2). The serum PBI levels of all hypothyroid females were markedly subnormal. However, those whose hypothyroidism was ameliorated by T₄ displayed slightly elevated serum PBI concentrations.

**Pituitary and serum TSH.** — Both bioassay and radioimmunoassay revealed subnormal concentrations of TSH in the pituitaries of all hypothyroid rats. Prolonged hypothyroidism (beyond 8 weeks) resulted in a 50% reduction in pituitary TSH concentration (Fig. 5; Table 1). However, amelioration of the hypothyroidism with small chronic doses of T₄ did not increase pituitary stores of TSH.

As expected, serum titers of TSH in all normal rats were relatively low, ranging between 0 and 8 mU/100 ml when measured by radioimmunoassay (Fig. 7; Table 1) and 100 mU/100 ml by bioassay (Fig. 6). Furthermore, in agreement with previous reports, circulating TSH levels were markedly elevated (3 to 12 times over normal) following surgical thyroidectomy or goitrogen block. Paradoxically, bioassay showed serum TSH titers increased in females, in inverse relationship to the apparent severity of the hypothyroid-
ism being 2, 5, and 13 times normal in surgically thyroidectomized, PTU-fed, and PTU and T4-treated animals, respectively (Fig. 6). However, radio-immunoassay of the sera of males, whose hypothyroidism was ameliorated by T4, revealed no significant increases in TSH over thyroidectomy controls (Fig. 7; Table 1).

**Anterior pituitary morphology.** – Differential cell counting, on 90-day post-thyroidectomized females, showed a 6-fold increase in the pituitary basophils which are considered to be thyrotrophs together with a reduction in chromophobes (Fig. 8). The reduction in acidophil volume was precipitous, reaching essentially zero 30 days after thyroidectomy, which is in agreement with the equally precipitous decline in pituitary GH alluded to earlier.

The typical thyrotroph was vacuolated and depleted of specific granules 2 months after thyroidectomy (thyroidectomy cell). Electron microscopy showed the vacuoles represent expanded cisternae of the granular endoplasmic reticulum (Fig. 9).

![Graph showing volume percentages of anterior pituitary components at sequential intervals after surgical thyroidectomy (T) as compared to normal (N). Eight pituitaries per point.](image)
DISCUSSION

Because D'Angelo (1969) postulated that increased pituitary TSH concentrations in hypothyroid rats may reflect a state of incomplete thyroidectomy, rigorous criteria, previously established by Evans et al. (1964), and serum PBI concentration were used to validate the completeness of surgical or chemical thyroidectomy in female rats in this experiment. The criteria used to evaluate severity of the hypothyroidism in males were not as numerous; they consisted of measurement of body growth and of pituitary GH concentration. Nevertheless, the stasis of body growth and the precipitous decline in pituitary GH concentration of surgically or chemically thyroidectomized males were comparable to those of females subjected to similar experimental manipulation. Therefore, surgical or chemical thyroidectomy was presumed to be as complete in males as in females. However, it must be recognized that the slight differences in the experimental protocol preclude any valid comparison between male and females.

Whereas bioassay revealed circulating TSH titers to be markedly elevated in females whose hypothyroidism was ameliorated by $T_4$, no such elevation
was detected with radioimmunoassay in males treated in a similar manner. In most other respects, however, the relative changes in serum and pituitary TSH concentration, of animals subjected to similar experimental conditions, were in agreement. For this reason, the disagreement in serum TSH titers in the hypothyroid-ameliorated groups, alluded to above, is perplexing since they are not attributable to sex differences or to gross differences in the reliability between the bioassay and the immunoassay method.

As intimated previously, the pituitary and serum TSH levels in both radioimmunoassay trials were confirmatory. The quantification of TSH by radioimmunoassay, therefore, is regarded as more reliable than the stasis tadpole bioassay in which crude pituitary extracts are employed. A modest concentration of purified rat luteinizing hormone (LH) was shown recently to stimulate tadpole metamorphosis (Ching 1973). Thus, it probably would have been prudent to quantify TSH in the hypothyroid-ameliorated female by radioimmunoassay. Furthermore, the stages of the oestrous cycle were not followed, and it is difficult to ascertain whether the peak of bioassayable pituitary TSH seen in normal females at puberty (30 days of experimentation, 56 days of age) reflect an actual increase in TSH during pro-oestrous (Newcomer & Brown-Grant 1971), was an erroneous finding due to a surge in LH stores (Bradshaw & Critchlow 1966), or reflect an increase in both TSH and LH. It is conceivable that the prevailing controversy regarding pituitary TSH concentration during various hypothyroid states may, in part, reflect differences in the reliability of the assay methods employed and/or fluctuations in TSH stores evoked by changing levels of pituitary gonadotrophins or sex steroids (Yamada et al. 1966).

Pituitary morphology appeared commensurate with the assayable levels of stored hormones. There was a rapid degranulation and decline in number of GH-producing acidophils after thyroidectomy. Thyrotroph mass increased after thyroidectomy (thyroidectomy cells) but, like the GH-producing acidophils, these cells, in agreement with earlier reports (Farquhar & Rinehart 1954; Barnes 1963), were virtually devoid of their characteristic specific granules. Nevertheless, as indicated above, TSH titers persisted in the pituitary of the severely hypothyroid rat and, moreover, increased in the circulation. Thus, some TSH is evidently synthesized and readily released by the thyroidectomy cell. The hormone is apparently stored in non-packaged form, presumably in the widely dilated cisternae of the granular endoplasmic reticulum.

ACKNOWLEDGMENTS

The authors thank Dr. John G. Pierce of the University of California at Los Angeles for the gift of purified beef TSH, which was used in the radioimmunoassay. Thanks are also extended to Dr. Seymour Reichlin of Tufts University, Boston, for providing
the antibody to beef TSH used in the radioimmunoassay. The TSH standard used in the stasis tadpole bioassay was the NIH-B4 sample. The purified rat GH used as the standard and also to generate antibody for the complement fixation immunoassay was kindly supplied by Dr. Stanley Ellis of the Ames Research Laboratory, Moffett Field, California. This research was supported by USPHS Grants 5-S01-RR-05403, AM 03150, and 5-T01-HD-00101.

REFERENCES

Bakke J. & Lawrence N.: Acta endocr. (Kbh.) 46 (1964) 111.

Received on June 27th, 1973.