Effects of Thyroxine and Cold Exposure on Hypothalamic TRH Levels in Rats with Various Pituitary-Thyroid States

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Synopsis

The hypothalamic content and concentration of thyrotropin-releasing hormone (TRH) were determined by radioimmunoassay in normal, thyroidectomized, hypophysectomized and cold-exposed rats treated with or without thyroxine.

In normal animals, the single administration of thyroxine (1, 5 and 20 μg/100 g B.W.) altered neither the content nor the concentration of TRH in the hypothalamus. However, seven days' administration of this hormone resulted in the dose-dependent increase in the hypothalamic TRH levels. In thyroidectomized rats, the hypothalamic TRH levels were slightly reduced in spite of the marked increase of plasma TSH levels and decrease of pituitary TSH levels. In the animals given thyroxine (10 μg/100 g B.W.) for 7 days in addition to thyroidectomy, however, the TRH levels exceeded that in the animals which underwent thyroidectomy alone. The hypothalamic TRH levels were markedly reduced in hypophysectomized rats. Conversely, in hypophysectomized rats given 7 days' thyroxine (1 and 5 μg/100 g B.W.), the levels were increased dose-dependently. In cold-exposed rats, the plasma TSH levels roughly doubled, but the TRH levels remained unchanged.

These findings strongly suggest that the feedback site of thyroxine extends not only to the pituitary gland but also to the hypothalamus, and that thyroxine has an increasing effect on the hypothalamic TRH level, though the mechanism(s) remain to be clarified.

Although it is well known that thyroid hormone has a direct inhibitory effect on both the secretion and the synthesis of thyrotropin (TSH) at the level of the pituitary, evidence concerning the effects of thyroid hormone on the control of hypothalamic thyrotropin-releasing hormone (TRH) secretion remains contradictory. (Reichlin et al., 1972)

The recent advance in the radioimmunoassay technique has made it possible to determine TRH per se, in various biological fluids and tissues. (Bassiri and Utiger, 1972 and 1974; Jackson and Reichlin, 1974a and 1974b; Oliver et al., 1974a and 1974b; Shambaugh et al., 1975; Utsumi et al., 1975). However, there are yet discrepancies among the findings obtained by radioimmunoassay of TRH on the relationship between TRH and thyroid hormone (Bassiri and Utiger, 1974; Jackson et al., 1974; Montoya et al., 1975; Emerson and Utiger, 1975; Mitsuma et al., 1976).

In an attempt to elucidate the feedback site(s) of thyroid hormone, the fluctuations of radioimmunoassayable hypothalamic TRH levels with special reference to the effects of thyroid hormone and cold exposure in
various conditions affecting the pituitary and plasma TSH levels were determined in the rat.

Materials and Methods

Animal experiments

Male Sprague-Dawley rats weighing 110-380g were used in these experiments. All the rats except those exposed to cold were housed in a constant temperature room, with 12 hr light, 12 hr darkness, and were fed with Oriental Laboratory Chow (Oriental Yeast Industries, Ltd., Tokyo, Japan) and water ad libitum.

Thyroidectomy was carried out surgically by the conventional method and hypophysectomy was performed by the transauditory approach (Tanaka, 1955). L-Thyroxine sodium (T4) was prepared for intraperitoneal injection by solution in a drop of 0.1 N NaOH which was then diluted in 0.9% saline to give the requisite amount in 0.1 ml. Control groups were given 0.9% alkaline saline.

Normal rats were divided into two groups, the first group was given a single injection of T4 in doses of 1, 5 or 20 μg/100 g of body weight 24 hr before decapitation. The second group was treated with T4 in the same doses daily for 7 days and the animals were killed 24 hr after the last injection. In each group of thyroidectomized and hypophysectomized rats, T4 was given daily for 7 days beginning the day after the operation. Normal rats adapted to the circumstance of warm temperature (29±2°C) for 7 days were exposed at 2°C for 30, 60 or 120 min and rats treated with T4 (10 μg/100 g body weight) 3 hr previously were exposed for 60 min in the same manner.

All the rats were sacrificed by decapitation under light ether anesthesia. Blood samples were collected from the cervical trunk. After excision of the upper cranial bones, the whole brain was removed as rapidly as possible and frozen on dry-ice. The dissection of hypothalamus was performed as follows: The hypothalamus was extended from the optic chiasma anteriorly to the mamillary body posteriorly, and was bordered by the pyriform lobe laterally and at a depth of 2 mm dorsally. The wet weight of hypothalamus was measured quickly by torsion balance. The hypothalamus was homogenized immediately in one ml of ice-cold 0.01 M phosphate buffer saline (PBS), pH 7.6 and extracted with an addition of 5 volumes of ice-cold methanol. The resulting mixture was centrifuged at 3,000 rpm for 15 min and the supernatant was lyophilized under vacuum. The lyophilized extract was dissolved in two ml of ice-cold PBS and centrifuged at 3,000 rpm for 15 min. The supernatant was stored at -20°C until TSH assay.

TRH radioimmunoassay

Synthetic TRH (Takeda Chemical Industries, Ltd., Osaka, Japan) was used for the production of antibody to TRH, radioiodination and assay standard. The radioimmunoassay for TRH has been described elsewhere (Utsumi et al., 1975). The minimum detectable level of TRH in this assay system was 10 pg/tube. The intraassay coefficient of variation in three different concentrations of the hypothalamic extract was 2.0%, 3.5% and 7.2%, respectively. The interassay coefficient of variation of the same specimens in 6 assays was 7.5%, 4.4% and 5.6%, respectively. The recovery rate of synthetic TRH added to rat hypothalamic homogenates was 81.8% to 102.4%.

Rat-TSH radioimmunoassay

Plasma and pituitary TSH levels in rats were determined by homologous radioimmunoassays, using the rat TSH kit kindly supplied by NIAMDD (Utsumi, 1976). The assay sensitivity was 10 ng/tube of NIAMD-Rat TSH-RP-1 and the results were expressed in μg/ml of plasma and μg/mg of anterior pituitary gland.

Statistical analysis

Results were expressed as mean±SE and analyzed using paired Student’s t test.

Results

Hypothalamic TRH level in normal male rats

The hypothalamic TRH content and concentration in the four groups of normal male rats at different experimental date are shown in Fig. 1. The weight of hypothalamus (mean±SE) ranged from 20.5±0.9 mg to 24.4±0.6 mg and the TRH content ranged from 5.16±0.39 ng to 6.04±0.10 ng per hypothalamus. The TRH concentration, expressed as pg TRH per mg of hypothalamus, was 261±37 pg to 237±15 pg/mg hypothalamus.

Effect of T4 on hypothalamic TRH level in normal rats

The single T4 administration 24 hr before decapitation in normal rats failed to alter
Fig. 1. Hypothalamic TRH content (ng/hypothalamus) and concentration (pg/mg hypothalamus) in normal male rats of different body weight. Numbers in the bracket represent animal numbers.

the hypothalamic TRH levels. In contrast, the plasma TSH level was reduced significantly, while the pituitary TSH level did not change (Table 1). As shown in Table 2, the repetitive T4 treatment for 7 days in normal rats resulted in a significant and dose-dependent increase of the hypothalamic TRH level. The hypothalamic TRH content increased from 5.16 ± 0.30 ng in the control group to 5.59 ± 0.25 ng, 6.76 ± 0.28 ng and 8.21 ± 0.70 ng in the T4-treated (1, 5 and 20 μg/100 g B.W.) groups, respectively.

Effect of T4 in thyroidectomized rats and hypophysectomized rats

Table 3 shows that the hypothalamic TRH level in thyroidectomized rats was...
Table 3. Hypothalamic TRH level, plasma and pituitary TSH concentration in thyroidectomized and repetitive T4-treated thyroidectomized rats

<table>
<thead>
<tr>
<th></th>
<th>control (5)</th>
<th>thyrex (5)</th>
<th>thyrex T4 (10 µg) (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamic weight (mg)</td>
<td>27.9±2.0</td>
<td>25.6±1.0</td>
<td>24.7±1.3</td>
</tr>
<tr>
<td>Hypothalamic TRH ng/hypothalamus</td>
<td>5.30±0.35</td>
<td>4.33±0.21</td>
<td>7.03±0.33** ***</td>
</tr>
<tr>
<td>pg/mg hypothalamus</td>
<td>192±18</td>
<td>171±14</td>
<td>287±21***</td>
</tr>
<tr>
<td>Plasma TSH (µg/ml)</td>
<td>0.56±0.06</td>
<td>2.10±0.31**</td>
<td>&lt;0.10***</td>
</tr>
<tr>
<td>Pituitary TSH (µg/mg pituitary gland)</td>
<td>175±29</td>
<td>70±23**</td>
<td>36±5** ***</td>
</tr>
</tbody>
</table>

Animals were sacrificed 7 days after thyroidectomy (thyrex). T4 was given in doses of 10 µg/100 g B.W. for 7 days beginning the day after the operation.

*p <0.01  **p <0.001 in comparison to the control group.
***p <0.01 ****p <0.001 in comparison to the thyroidectomized group.

Table 4. Hypothalamic TRH level and plasma TSH concentration in hypophysectomized and repetitive T4-treated hypophysectomized rats

<table>
<thead>
<tr>
<th></th>
<th>control (5)</th>
<th>hypox (7)</th>
<th>hypox T4 (1 µg) (5)</th>
<th>hypox T4 (5 µg) (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamic weight (mg)</td>
<td>22.5±0.2</td>
<td>19.0±0.5</td>
<td>21.5±1.0</td>
<td>24.3±1.9</td>
</tr>
<tr>
<td>Hypothalamic TRH ng/hypothalamus</td>
<td>5.31±0.25</td>
<td>3.41±0.20**</td>
<td>4.61±0.31***</td>
<td>6.17±0.12****</td>
</tr>
<tr>
<td>pg/mg hypothalamus</td>
<td>237±15</td>
<td>187±13**</td>
<td>214±14***</td>
<td>258±14****</td>
</tr>
<tr>
<td>Plasma TSH (µg/ml)</td>
<td>0.56±0.06</td>
<td>&lt;0.10***</td>
<td>&lt;0.10***</td>
<td>&lt;0.10***</td>
</tr>
</tbody>
</table>

Hypophysectomy (hypox) was performed 7 days before decapitation. T4 was given in doses of 1 µg and 5 µg/100 g B.W. for 7 days beginning the day after the operation.

*p <0.05  **p <0.001 in comparison to the control group.
***p <0.05 ****p <0.001 in comparison to the hypophysectomized group.

slightly but not significantly reduced, while the TRH level in thyroidectomized rats given 10 µg/100 g B.W. of T4 was greater than that in the thyroidectomized or normal group.

Hypophysectomy resulted in a significant reduction of the hypothalamic TRH level (Table 4). The TRH content was 5.31±0.27 ng in the control group and 3.41±0.20 ng in the hypophysectomized group 7 days after the operation. The hypothalamic TRH level in hypophysectomized rats treated with T4 (1 and 5 µg/100 g B.W.) for 7 days was restored dose-dependently to that of the normal group.

Effect of acute cold exposure

The results of studies of acute cold exposure are shown in Fig. 2. There was no significant change in the hypothalamic TRH levels during cold exposure. Plasma TSH levels rose significantly from 0.39±0.08 µg/ml in the control group to 0.71±0.05, 0.76±0.06 and 0.86±0.07 µg/ml in rats exposed to cold for 30, 60 and 120 min and pituitary TSH levels decreased from 188±5 µg/mg pituitary to 137±13, 144±16 and 143±25 µg/mg pituitary, respectively. The treatment of T4 prior to cold exposure inhibited the increase of plasma TSH levels and the decrease of pituitary TSH levels. The hypothalamic TRH levels in the T4-pretreated group did not change by acute cold exposure.
Discussion

In our laboratory, the mean hypothalamic TRH content in normal male adult rats of different body weight ranged from 5.16 ng to 6.04 ng, which was altered subsequently by the weight of the hypothalamus. It has recently been proved that the radioimmunoassayable TRH is present in most parts of the brain of various animals (Winokur and Utiger, 1974; Oliver et al., 1974b; White et al., 1974; Winters et al., 1974; Jackson and Reichlin, 1974b). However, there is individual difference of the hypothalamic TRH contents in rats ranging from 3.4 ng to 15.7 ng in previous reports (Bassiri and Utiger, 1974; Oliver et al., 1974b). These differences might be, in part, responsible for the dissection of the hypothalamus. The present results demonstrate that the correct and reproducible dissection of hypothalamus is important for the serial measurement of the hypothalamic TRH level.

It has been well known that TRH plays an important role in the regulation of pituitary-thyroid function and that thyroid hormone inhibits both the synthesis and release of TSH at the pituitary level. (Reichlin et al., 1972) However, the relationship between TRH and thyroid hormone has remained obscure because of the methodological difficulties. This subject has been studied in the past using various experimental models, for instance, the hypothalamic
lesion or the microinjection of thyroid hormone into the hypothalamus or the TRH bioassay of hypophyseal portal blood. (Greer, 1951; Yamada and Greer, 1959; Wilber and Porter, 1970) However, there is a discrepancy in the results as to the effect of microinjection of thyroid hormone into the hypothalamus. (Yamada and Greer, 1959; Bogdanove and Crabill, 1961) Moreover, the systemic administration of thyroid hormone could not reduce the thyrotropin-releasing activity of hypophyseal portal blood. (Wilber and Porter, 1970) Reichlin and his co-workers (1972), on the other hand, have suggested that thyroid hormone would accelerate the synthesis of hypothalamic TRH in vitro.

The recent advance in the radioimmunoassay technique for TRH has made it possible to investigate directly the relationship between hypothalamic TRH and thyroid hormone. It was demonstrated by Bassiri and Utiger (1974) that the hypothalamic TRH content was not significantly influenced by the systemic administration of T4 in rats. In contrast, Jackson et al. (1974) reported that the hypothalamic TRH content was increased after T4 treatment in rats. Our data are essentially similar to those of Jackson et al. The reason for the discrepancy between our results and those of Bassiri and Utiger (1974) or Montoya et al. (1975) is not clear.

In hypophysectomized rats, the hypothalamic TRH contents were markedly reduced at the level of 64% of the control. These findings are in agreement with those of Bassiri and Utiger (1974) though this mechanism remains unclarified. Of much more interest is the effect of T4 on the hypothalamic TRH level in hypophysectomized rats. The repeated administration of T4 induced a restoration of the hypothalamic TRH level to the normal level in these rats. These findings may suggest that T4 acts directly on the level of hypothalamus to increase the TRH content. However, it is difficult to conclude from these results whether the increased TRH synthesis, as hypothesized by Reichlin et al. (1972), or the decreased TRH release is attributable to the mechanism. The hypothalamic TRH level did not change significantly in thyroidectomized rats, while a significant increase was found after T4 treatment. Supplementary observation would be necessary to disclose the hypothalamic TRH fluctuation in the conditions of the rapidly reducing thyroid hormone level.

It has been known that acute cold exposure leads to a rapid increase in plasma TSH concentrations and a decrease in pituitary TSH concentrations and that the pretreatment of thyroid hormone inhibits these effects in various animals. (Yamada et al., 1965; Sakoda and Washio, 1970; Hershman et al., 1970) As usual, it has been suggested that the mechanism is neural and originates from the increased TRH secretion (Knigge and Bierman, 1958; Montoya et al., 1975). The present data showed that the hypothalamic TRH level in rats with or without pretreatment of T4 remained unchanged by acute cold exposure, while the appropriate changes of TRH were induced in the plasma and pituitary gland. This may suggest that the quantity of TRH release into the hypophyseal portal vein is very small, probably picogram order, as compared to that of the TRH content in hypothalamus.

The recent observation by Montoya et al. (1975) indicated that plasma TRH levels were increased by acute cold exposure in the rat. On the other hand, Emerson and Utiger (1975) noted no significant increment of plasma TRH levels by acute cold exposure. Approximately 70% of the total brain TRH content is contained in extrahypothalamic area, and thus plasma TRH may not necessarily reflect the secretion from the hypothalamus (Winokur and Utiger, 1974; Montoya et al., 1975). For the elucidation of these problems it is likely to be necessary
to measure the TRH level in the hypophysial portal vessel.

References


