Thyrotropin releasing hormone (TRH): its changes in discrete hypothalamic areas after treatment with triiodothyronine, thyroidectomy and acute cold exposure.

Jiro Yamauchi*
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Abstract

In order to elucidate the specific thyrotropic area in the hypothalamus, thyrotropin releasing hormone (TRH) content and concentration were measured in discrete hypothalamic nuclei and areas after triiodothyronine (T3) administration (T3 10 micrograms/rat/day for 6 days), thyroidectomy (TX) and acute cold exposure in male rats. In the TX and T3 groups, serum TSH levels were significantly increased in TX group and markedly decreased in T3 and TX with T3 groups as compared to the sham operated control group (Sham). TX produced a slight but nonsignificant decrease in TRH content in most of the hypothalamic nuclei examined as compared with the Sham group. However, a significant increase in TRH contents was seen in the anterior hypothalamic nucleus (AHN), median eminence (ME) and posterior pituitary (PP) in TX with T3 group as compared to the rats with only TX. In the acute cold stress experiments, serum TSH levels were elevated from 15 to 30 min of 4 degrees C exposure. Together with these peripheral changes, TRH content and concentration in the suprachiasmatic nucleus (SC) were increased significantly at 15 min and had returned to the normal level by 30 min after 4 degrees C cold exposure. However, in the paraventricular nucleus (PV) and dorsal premmamillary nucleus (PMD), marked decrease in TRH concentrations were observed with this stress. Therefore, 1) decreased TSH release in TX rats treated with T3 was induced by the block of TRH release from the AHN and ME as compared with the TX group, and 2) elevated serum TSH levels in 4 degrees C cold stress might be induced by the release of TRH from the PMD and PV. These experiments demonstrate that the specific hypothalamic area for TSH release was located in some of the anterior and posterior hypothalamic nuclei and in the ME.

KEYWORDS: thyrotropin releasing hormone (TRH), hypothalamic distribution, thyroidectomy, triiodothyronine, cold exposure.

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THYROTROPIN RELEASING HORMONE (TRH): ITS CHANGES IN DISCRETE HYPOTHALAMIC AREAS AFTER TREATMENT WITH TRIIODOTHYRONINE, THYROIDECTOMY AND ACUTE COLD EXPOSURE

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Abstract. In order to elucidate the specific thyrotropic area in the hypothalamus, thyrotropin releasing hormone (TRH) content and concentration were measured in discrete hypothalamic nuclei and areas after triiodothyronine (T₃) administration (T₃ 10 µg/rat/day for 6 days), thyroidectomy (TX) and acute cold exposure in male rats. In the TX and T₃ groups, serum TSH levels were significantly increased in TX group and markedly decreased in T₃ and TX with T₃ groups as compared to the sham operated control group (Sham). TX produced a slight but nonsignificant decrease in TRH content in most of the hypothalamic nuclei examined as compared with the Sham group. However, a significant increase in TRH contents was seen in the anterior hypothalamic nucleus (AHN), median eminence (ME) and posterior pituitary (PP) in TX with T₃ group as compared to the rats with only TX. In the acute cold stress experiments, serum TSH levels were elevated from 15 to 30 min of 4°C cold exposure. Together with these peripheral changes, TRH content and concentration in the suprachiasmatic nucleus (SC) were increased significantly at 15 min and had returned to the normal level by 30 min after 4°C cold exposure. However, in the paraventricular nucleus (PV) and dorsal premamillary nucleus (PMD), marked decrease in TRH concentrations were observed with this stress. Therefore, 1) decreased TSH release in TX rats treated with T₃ was induced by the block of TRH release from the AHN and ME as compared with the TX group, and 2) elevated serum TSH levels in 4°C cold stress might be induced by the release of TRH from the PMD and PV. These experiments demonstrate that the specific hypothalamic area for TSH release was located in some of the anterior and posterior hypothalamic nuclei and in the ME.

Key Words: thyrotropin releasing hormone (TRH), hypothalamic distribution, thyroidectomy, triiodothyronine, cold exposure.

It has been generally accepted that thyrotropin releasing hormone (TRH) stimulates the release of thyrotropin (TSH) from the pituitary gland (1-3). It is therefore to be expected that the different environmental stimuli which accompany observed changes in serum TSH level may change the release of TRH from the hypothalamus.

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Acute cold exposure has been shown to stimulate the secretion of TSH in many species (4-6). The activation of TSH secretion is followed by increased thyroid activity and there is general agreement that the primary event leading to this change is an increased release of TRH from the hypothalamus. However, there are many reports of serum TSH rise in response to cold exposure without any change in hypothalamic TRH content (7-11).

In contrast to cold exposure, there are many reports concerned with thyroidectomy and thyroid hormone treatments on hypothalamic TRH content. Shinha and Meites (12) reported that thyroidectomy decreased hypothalamic TRH content unchanged by T₄ treatment measured by bioassay. Motta et al. (13) failed to demonstrate any substantial decrease in bioassayable hypothalamic TRH after thyroidectomy. However, it is well known that somatostatin inhibits the effect of TRH on the release of TSH (14), so bioassay of TRH content in hypothalamus is inappropriate. Recent studies using radioimmunoassay (RIA) of TRH (7, 10, 15) have shown that thyroidectomy resulted in a decrease in hypothalamic TRH content. T₄ replacement restored hypothalamic TRH content in thyroidectomized rat. Nevertheless, in other reports, no change was found in hypothalamic TRH content of either thyroid hormone treated or thyroidectomized rat (8, 16).

Recent advance in RIA technique and micropunch methods for quantifying TRH in discrete brain areas have made it possible to demonstrate the presence of this hormone in most nuclei and areas of the brain of rats (17-19) and humans (20, 21). High concentration of this peptide has been found in several distinct areas: in the median eminence-arcuate nucleus, dorsomedial nucleus, medial preoptic area and anterior part of the ventromedial nucleus (18, 19). However, nothing is known about the role of this hormone in the so-called “thyrotropic” area with respect to the regulation of TSH secretion. Since TRH participates in hormonal regulatory systems and may participate in neural regulatory systems as well, it is reasonable to inquire to what extent hypothalamic and brain TRH content might be altered by various hormonal and environmental manipulations.

The purpose of the present study was to investigate the effects of various treatments that alter endocrine function on hypothalamic and other brain content of immunoreactive TRH.

MATERIALS AND METHODS

Animal experiments. Adult male Wistar strain rats weighing about 250g were used. They were maintained under controlled illumination (light on 7:00-19:00h) and temperature (22 ±2°C) for two weeks. Laboratory chow and tap water were available at all times. In the first experiment, rats were divided into four groups of 5 to 6 rats each. Two groups were thyroidectomized (TX) and another two were sham operated (Sham) under light ether anesthesia. Beginning on the next day after surgery, the Sham and TX groups were intraperitoneally injected daily with 1 ml saline for 6 days; other Sham and TX groups were treated with 10 µg triiodothyronine (T₃) (Sigma Chemical Company)
daily for 6 days. The rats were sacrificed by decapitation 24 h after the last injection. In the second experiment, three groups of 5 rats were used. After being housed as above, two groups of rats were moved into the cold room kept at 4°C, and a control group was transferred to the laboratory room maintained at 22°C. The animals were killed under 22°C as a control group and at 15 and 30 min of exposure to 4°C by decapitation. In the third experiment, one group of rats was subjected to free swimming in the ice cold water (0°C) for 15 min, other control group was maintained at 22°C room. In all experiments, whole brain was removed immediately after decapitation and frozed with powdered dry ice; and blood samples were collected from the trunk. All the treatments and decapitation were performed between 11 and 12 a.m. Trunk blood was centrifuged and the serum stored at −20°C until TSH, prolactin and GH were assayed.

_Tissue preparation and extraction._ All techniques used in this work were described in detail in the previous report (19), and therefore, only the essential techniques are given here. The extirpated brains were sectioned in the frontal plane at −20°C using a freezing-microtome. A single 100 μm section was taken before each 400 μm section in order to verify section location for histological determination of lesion extent and placement. The hypothalamic nuclei and various brain areas were removed from the 400 μm sections under a stereomicroscope using specially designed needles. The dissected nuclei and areas were homogenized with ice cold methanol acetic acid solution. The supernatants were dried and then reconstituted in 0.1% gelatin with 0.1M EDTA in 0.01M phosphate 0.15M saline buffer, pH 7.6, and assayed by specific RIA of TRH.

Not all of the nuclei were punched out completely because of anatomical reasons. Therefore, the TRH content in each nuclei might be slightly greater than the levels in this paper. However, identical nuclei in individual rats were punched out with the same needles.

TRH measurement was carried out using triplicate samples of all extracts for RIA. Statistical significance was performed by student’s t test.

_Radioimmunoassay of TRH and pituitary hormones._ TRH measurement was performed according to the method described elsewhere (19). Antiserum to TRH was kindly supplied by Dr. Utsumi. Synthetic TRH (Takeda Chemical Industries, Osaka, Japan) served as material for reference and iodination.

Serum TSH, prolactin and GH were assayed using the rat TSH, prolactin and GH RIA kits provided by NIAMDD of NIH, U.S.A.

**RESULTS**

_Effect of TX or treatment with T₃ or their combination on serum TSH, prolactin and GH concentration._ Table 1 shows the various pituitary hormone levels after TX and treatment with T₃. There was a significant (p<0.001) increase in serum TSH level after TX compared to the Sham group and also a marked (p<0.01) decrease in TSH after treatment with T₃. TX-induced TSH increase was completely blocked by the administration of T₃. There was also a marked (p<0.05) decrease in the prolactin level of the TX group compared to the Sham group. No change was observed in GH levels after these treatments.

_Effect of TX or treatment with T₃ or their combination on TRH content in discrete hypothalamic nuclei._ The content of TRH in various hypothalamic nuclei and other
J. Yamauchi

**Table 1. Effects of Thyroidectomy and Triiodothyronine (T₃) Treatment on Serum TSH, Prolactin, and GH Concentrations.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>TSH ng/ml</th>
<th>Prolactin ng/ml</th>
<th>GH ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>6</td>
<td>628±104⁺*</td>
<td>13.2±3.3</td>
<td>59.6±21.2</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>2187±682ᵇ</td>
<td>5.0±1.0ᵈ</td>
<td>55.2±12.9</td>
</tr>
<tr>
<td>T₃ treatment</td>
<td>5</td>
<td>139±12ᶜ</td>
<td>15.5±4.9</td>
<td>62.5±45.7</td>
</tr>
<tr>
<td>Thyroidectomy + T₃</td>
<td>5</td>
<td>194±25ᵈ</td>
<td>11.5±8.4</td>
<td>46.2±20.4</td>
</tr>
</tbody>
</table>

*a: Mean±SE, b: p<0.001, c: p<0.01, d: p<0.05 vs Sham group.
Sham: sham-operated control group.
T₃: triiodothyronine was given at a dose of 10μg/day for 6 days.
N: number of rats in each group.

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**Fig. 1.** Effect of thyroidectomy and/or triiodothyronine (T₃) (10 μg/day for 6 days) on TRH content in individual preoptic area, anterior hypothalamic nuclei and posterior pituitary. Five to six rats in each group. Abbreviations: see Table 2.
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brain areas was measured by direct RIA, as shown in Figs. 1 and 2 after TX and T3 treatments. Thyroidectomy produced a slight decrease in TRH content in the lamina terminalis, anterior hypothalamic nucleus, suprachiasmatic nucleus, supraoptic nucleus and posterior pituitary compared with the Sham group. Treatment with T3 to the TX rats produced a statistically significant increase in TRH levels in the anterior hypothalamic nucleus and posterior pituitary compared with TX group: values were from 200.1±31.7pg to 335.5±32.9pg (p<0.02) in the anterior hypothalamic nucleus and from 164.0±31.9pg to 309.9±57.7pg (p<0.05) in the posterior pituitary (Fig. 1). Fig. 2 shows the TRH changes in the medial and posterior hypothalamus after the same treatment with the same fashion as in Fig. 1. There was a nonsignificant decrease in TRH content due to TX in almost all nuclei examined in these areas. In particular, administration of T3 to TX rats caused a significant increase in TRH levels in the median eminence as compared with the TX group (738.9±88.6 vs 1224.0±139.0pg).

Fig. 2. Effect of thyroidectomy and/or T3 (10 μg/day for 6 days) on TRH content in individual medial and posterior hypothalamic nuclei. Five to six rats in each group. Abbreviations; see Table 2.
(p<0.02). There was a marked increase of TRH content in the ventromedial nucleus from 204.2±24.3pg in the TX group to 418.5±73.3pg in the T3 dosed group (p<0.02). Also, 165%, 153% and 236% increments were observed in the arcuate nucleus, median eminence and dorsal premammillary nucleus respectively in the T3 dosed group compared to the TX group.

Effect of acute 4°C cold exposure on serum TSH concentration. The data presented in Fig. 3 shows that acute cold exposure of 4°C produced a significant increase in serum TSH levels from 15 to 30 min after beginning hypothermia.

![Graph showing serum TSH levels](image)

Fig. 3. Effect of 4°C cold exposure and 0°C ice cold water stress on serum TSH levels in normal male rats. Vertical bars represent one standard error of the mean. *p<0.01, **p<0.02.

Effect of acute 4°C cold exposure on TRH content and concentration in discrete hypothalamic nuclei and other brain areas. The TRH content in discrete hypothalamic areas under 4°C cold stress are depicted in Figs. 4 and 5. As shown in Fig. 4, the TRH content in the suprachiasmatic nucleus increased from 62.5±4.0pg to 162.4±21.3pg at 15 min (p<0.01) and returned to the control level 30 min after the cold exposure. However, TRH content in the preoptic region and other anterior hypothalamic nuclei did not change with this stimulation. There was a significant (p<0.02) decrease in content of this hormone in the dorsal premammillary nucleus after 15 min of cold stress compared to the control group.
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Fig. 4. Effect of acute 4°C cold exposure on TRH content in individual preoptic region and anterior hypothalamic nuclei. Five rats in each group. Abbreviations: see Table 2.

Fig. 5. Effect of acute 4°C cold exposure on TRH content in individual medial and posterior hypothalamic nuclei. Five rats in each group. Abbreviations; see Table 2.
(98.4±15.2 vs 40.3±11.0pg). In contrast, the TRH content in the medial hypothalamus (containing the ventromedial nucleus, dorsomedial nucleus, arcuate nucleus and median eminence) showed no change after 4°C.

Cold stress. Table 2 shows the TRH concentration, expressed as ng per mg protein, in various hypothalamic nuclei and other brain regions after exposure to 4°C. The TRH concentration in the suprachiasmatic nucleus was markedly increased (p<0.05) at 15 min after the stimulation, but in the paraventricular nucleus, significant (p<0.05) decrease of this peptide was seen after 30 min of stress. There was also a sustained decrease in the TRH concentration from 15 to 30 min in the dorsal premamillary nucleus (p<0.05). No significant change was observed in the TRH concentration of the discrete medial hypothalamic nuclei, preoptic region, septum or accumbent nucleus.

![Graph showing TRH content in various hypothalamic nuclei](image)

*Fig. 6. Effect of acute 0°C cold water stress on TRH content in individual hypothalamic nuclei, the preoptic region and the posterior pituitary. Four rats in each group. Abbreviations; see Table 2.*

*Effect of 0°C cold water stress on serum TSH concentration.* As shown in Fig. 3, there was no particular change in serum TSH concentration in the rats treated with 0°C cold water stress for 15 min.

*Effect of 0°C cold water stress on TRH content and concentration in discrete hypothalamic nuclei and other brain areas.* Results presented in Fig. 6 show the TRH
Hypothalamic TRH and Various Treatments

Table 2. Effect of acute 4°C cold exposure on TRH concentrations in individual hypothalamic nuclei and other brain areas.

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Abbreviation</th>
<th>TRH concentration (ng/mg protein)</th>
<th>Before</th>
<th>15 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Preoptic region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoptic area</td>
<td>POA</td>
<td>0.77±0.19</td>
<td>0.95±0.26</td>
<td>1.17±0.33</td>
<td></td>
</tr>
<tr>
<td>Medial preoptic area</td>
<td>MPO</td>
<td>3.77±0.52</td>
<td>3.42±0.17</td>
<td>3.59±0.53</td>
<td></td>
</tr>
<tr>
<td>Lamina terminalis</td>
<td>LT</td>
<td>1.71±0.21</td>
<td>2.34±0.73</td>
<td>2.16±0.76</td>
<td></td>
</tr>
<tr>
<td>2. Hypothalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus hypothalamicus anterior</td>
<td>AHN</td>
<td>2.77±0.33</td>
<td>2.04±0.21</td>
<td>2.01±0.15</td>
<td></td>
</tr>
<tr>
<td>Nucleus suprachiasmaticus</td>
<td>SC</td>
<td>1.22±0.12</td>
<td>2.60±0.49</td>
<td>0.91±0.11</td>
<td></td>
</tr>
<tr>
<td>Nucleus supraopticus</td>
<td>SO</td>
<td>0.55±0.15</td>
<td>0.42±0.11</td>
<td>0.30±0.06</td>
<td></td>
</tr>
<tr>
<td>Nucleus paraventricularis</td>
<td>PV</td>
<td>2.79±0.32</td>
<td>2.03±0.25</td>
<td>1.84±0.10a</td>
<td></td>
</tr>
<tr>
<td>Lateral hypothalamic area</td>
<td>LAH</td>
<td>1.44±0.10</td>
<td>1.45±0.15</td>
<td>1.14±0.15</td>
<td></td>
</tr>
<tr>
<td>Nucleus ventromedialis</td>
<td>VM</td>
<td>2.39±0.27</td>
<td>2.59±0.22</td>
<td>2.42±0.19</td>
<td></td>
</tr>
<tr>
<td>Nucleus dorsomedialis</td>
<td>DM</td>
<td>3.65±0.46</td>
<td>2.44±0.55</td>
<td>2.42±0.38</td>
<td></td>
</tr>
<tr>
<td>Nucleus arcuatus</td>
<td>ARC</td>
<td>5.12±1.16</td>
<td>3.44±0.29</td>
<td>5.00±0.63</td>
<td></td>
</tr>
<tr>
<td>Median eminence</td>
<td>ME</td>
<td>22.57±2.56</td>
<td>22.24±1.97</td>
<td>18.94±2.77</td>
<td></td>
</tr>
<tr>
<td>Nucleus hypothalamicus posterior</td>
<td>PHN</td>
<td>0.64±0.15</td>
<td>0.61±0.08</td>
<td>0.62±0.08</td>
<td></td>
</tr>
<tr>
<td>Nucleus premammillaris dorsalis</td>
<td>PMD</td>
<td>2.06±0.56</td>
<td>0.43±0.08a</td>
<td>0.70±0.11a</td>
<td></td>
</tr>
<tr>
<td>Nucleus premammillaris ventralis</td>
<td>PMV</td>
<td>1.70±0.31</td>
<td>1.03±0.30</td>
<td>1.33±0.22</td>
<td></td>
</tr>
<tr>
<td>3. Septum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus lateralis</td>
<td>SL</td>
<td>1.39±0.32</td>
<td>1.19±0.26</td>
<td>1.73±0.36</td>
<td></td>
</tr>
<tr>
<td>Nucleus medialis</td>
<td>SM</td>
<td>0.64±0.09</td>
<td>0.39±0.09</td>
<td>0.62±0.11</td>
<td></td>
</tr>
<tr>
<td>4. Nucleus accumbens</td>
<td>ACB</td>
<td>0.27±0.06</td>
<td>0.24±0.01</td>
<td>0.32±0.07</td>
<td></td>
</tr>
</tbody>
</table>

\( a: \) Mean±SEM, \( b: \) p<0.05 vs before group.
Five to six rats were used in each group.

Content in various brain areas after 0°C stress. A significant decrease in TRH content was observed in the posterior hypothalamic nucleus after 15 min of stimulation compared to the 22°C control group; values were from 145.0±29.7 pg to 54.9±9.3 pg (p<0.05). However, when expressed as ng per mg protein, there was no statistically significant change in all areas examined under these conditions (Table 3).

Discussion
I have previously discussed the great variation in TRH content and concentration in various regions of the brain in rats (19). TRH was found in almost all areas of the brain examined and especially high concentrations of this hormone were found in relatively restricted areas: in the median eminence, arcuate nucleus, dorsomedial nucleus and the medial preoptic area. The present studies were undertaken to determine whether there is a specific region which reacts during various thyroidal conditions in these hypothalamic nuclei and other brain.
Table 3. Effect of acute 0°C cold water stress on TRH concentrations in individual hypothalamic nuclei and other brain areas.

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>TRH concentration (ng/ng protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>1. Preoptic region</td>
<td></td>
</tr>
<tr>
<td>Preoptic area</td>
<td>0.97±0.11*</td>
</tr>
<tr>
<td>Medial preoptic area</td>
<td>4.16±0.59</td>
</tr>
<tr>
<td>2. Hypothalamus</td>
<td></td>
</tr>
<tr>
<td>Nucleus hypothalamicus anterior</td>
<td>1.57±0.18</td>
</tr>
<tr>
<td>Nucleus suprachiasmaticus</td>
<td>1.55±0.22</td>
</tr>
<tr>
<td>Nucleus supraopticus</td>
<td>1.73±0.18</td>
</tr>
<tr>
<td>Nucleus paraventricularis</td>
<td>3.45±0.61</td>
</tr>
<tr>
<td>Lateral hypothalamic area</td>
<td>1.74±0.25</td>
</tr>
<tr>
<td>Nucleus ventromedialis</td>
<td>2.01±0.19</td>
</tr>
<tr>
<td>Nucleus dorsomedialis</td>
<td>6.18±0.75</td>
</tr>
<tr>
<td>Nucleus arcuatus</td>
<td>4.72±0.47</td>
</tr>
<tr>
<td>Median eminence</td>
<td>33.71±3.81</td>
</tr>
<tr>
<td>Nucleus hypothalamicus posterior</td>
<td>3.82±1.13</td>
</tr>
<tr>
<td>Nucleus premammillaris dorsal</td>
<td>1.64±0.50</td>
</tr>
<tr>
<td>Nucleus premammillaris ventral</td>
<td>2.80±0.16</td>
</tr>
<tr>
<td>3. Septum</td>
<td></td>
</tr>
<tr>
<td>Nucleus lateralis</td>
<td>2.21±0.34</td>
</tr>
<tr>
<td>Nucleus medialis</td>
<td>0.75±0.32</td>
</tr>
</tbody>
</table>

*: Mean±SEM, Four rats were used in each group.

areas.

In the first experiment, the effects of TX and T₃ treatment on serum TSH and hypothalamic TRH were examined. The results show that TX produced a significant increase in serum TSH levels whereas the elevated TSH level induced by TX was completely abolished by the administration of T₃. The variations of the TRH levels in discrete hypothalamic nuclei under these circumstances were as follows. Nonsignificant decrease in TRH content was observed in most of the anterior, middle and posterior hypothalamic nuclei in contrast to the increase in serum TSH levels in TX rats as compared with the Sham group. In the group treated with T₃ for 6 days, no definite change of this hormone was also observed in these areas. Treatment with T₃ of the TX rats also produced no definite tendency in TRH levels compared with the Sham group. However, a significant increase in TRH content was seen in the anterior hypothalamic nucleus, median eminence and posterior pituitary in TX plus T₃ rats as compared with the TX group. Therefore, it is apparent that T₃ produced a significant increase in this hormone in these three areas of TX rats. Also, TRH levels in the ventromedial nucleus, arcuate nucleus, median eminence and dorsal premammillary nucleus in the T₃ group were significantly higher than those in the TX group.
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These data indicate that under relatively chronic stimulation of TX and T₃, not only the medial hypothalamus where comparatively large amounts of TRH were detected, but also the anterior and posterior hypothalamus were participated in homeostasis of the hypothalamo-pituitary-thyroid axis. It was also obvious that blockade of TRH release from the anterior hypothalamic nucleus and median eminence in TX rats treated with T₃ produced a similar prevention of peripheral TSH release from the pituitary. In view of the presence of large quantities of TRH in the posterior pituitary in the present experiment and others (22, 23), and a dose-related pulsatile arginine vasopressin (AVP) release by TRH from perfused rat pituitaries (24), it is certainly possible that the posterior pituitary TRH content reflects hypothalamic TRH secretion; moreover, that the large quantities of TRH in the posterior lobe modulates the physiologic release of AVP.

It is generally accepted that the area from the anterior hypothalamus to the rostral portion of medial hypothalamus plays an essential role as a "thyrotropic area" for regulation of pituitary TSH secretion (25-29). The areas where TRH changes were observed in the present experiments were partially in agreement with this classic "thyrotropic area" of hypothalamus.

After isolation and synthesis of TRH (1, 2), direct measurement of this hormone reflected the dynamics of this peptide in the hypothalamus and peripheral blood under various thyroid states. Bassiri and Utiger (7) first reported, by utilizing antibody to this hormone, that a single dose of T₄ resulted in no change in the hypothalamic TRH content of rats. However, in some chronically T₄-treated rats, reduction in hypothalamic TRH content were slight, unsustained and apparently of little biological significance. Therefore it was assumed that T₄ and TSH have little effect on hypothalamic TRH content. Also, according to these authors in another experiment (16), no consistent changes in TRH content in the hypothalamus or brain of the rats followed thyroidectomy or administration of T₄. Montoya et al. reported similar results (8). On the other hand, other laboratories reported different results in which chronic administration of T₄ resulted in a dose-dependent increase in hypothalamic TRH content, but in thyroidectomized rats, the hypothalamic TRH levels were slightly reduced. This reduction was completely restored by chronic administration of T₄ (10, 15). Thus, no consistent conclusion was obtained from these reports from a number of laboratories concerning the effects of different thyroidal states on hypothalamic TRH content and peripheral TRH concentration. These discrepancies might result from measuring the TRH content in the whole hypothalamus. Because so much TRH was contained in the hypothalamo-median eminence complex, it was inadequate to measure TRH levels as a whole under these conditions.

The aim of the present experiments was to clarify the effect of various thyroid conditions on TRH content in discrete hypothalamic areas and nuclei and to elucidate the specific thyrotropic area. The results are basically in agree-
ment with Utsumi et al. (10) and Roti et al. (15). TRH content in the anterior hypothalamic nucleus and median eminence was significantly higher in the TX treated with T3 than in the TX group. It is interesting that the TRH content in the preoptic region was not affected at all in the present experiment. This area is known as one of the important "thyrotropic area" of the brain (29). The reason for this result is unclear.

In the second experiment, the effects of acute cold exposure of 4°C and 0°C on serum TSH and hypothalamic TRH were examined. Serum TSH concentrations were significantly increased from 15 to 30 min after starting the 4°C stress compared to the control group. In contrast to these increments in peripheral TSH, the TRH content and concentration of discrete nuclei in the medial hypothalamus were not affected. However, concomitant increase of this hormone in the suprachiasmatic nucleus of anterior hypothalamus was observed. In contrast to the elevated peripheral TSH, TRH concentrations in the paraventricular nucleus of anterior hypothalamus and dorsal premammillary nucleus of the posterior hypothalamus were significantly decreased. It is interesting that the increment of TRH content and concentration was observed in the suprachiasmatic nucleus after cold exposure of 15 min. This nucleus is also known as one of the central generators of diurnal rhythm (30). Unlike 4°C cold stress, the TRH content in the posterior hypothalamic nucleus was significantly decreased in the rats treated with 0°C cold water. However, the concentration in this nucleus was not influenced.

The data presented here indicate that under the circumstances of relatively acute stress of the present experiments, TRH in the medial hypothalamus was not involved in the release of TSH from the pituitary. TRH in the anterior and posterior hypothalamus participated in the regulation of TSH. The reason the serum TSH concentration was not influenced by 0°C cold water stress is unclear. It is also unclear why TRH in the preoptic region, known as one of the most important part of thermoregulation (31-35) was not significantly influenced. Cooling of the preoptic anterior hypothalamic area induces, in many species, a conspicuous rise of body temperature and rapid release of thyroid hormone, through specific and rapid mechanisms for decreasing heat loss and increasing heat production (36-38).

It is well known that acute cold stress stimulates the thyroid gland and the release of TSH from the pituitary in rats (5, 39, 40). Acute TSH release by cold may be mediated via peripheral cold receptors, because the hypothalamic core temperature was unaffected or slightly elevated during acute cold stress (39). In general, skin temperature was constantly regulated through information from both skin and deep thermoreceptors. These deep thermoreceptors are situated in the anterior hypothalamus of the CNS and visceral region. Information on temperature from input signals arise in many body structures among which the following have been identified experimentally: the preoptic-anterior
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hypothalamus, posterior hypothalamus, midbrain, medulla, motor cortex, thalamus, spinal cord, skin, respiratory tract and viscera. Convergence of input signals from these regions may occur in many areas but has only been identified experimentally in the preoptic-anterior hypothalamus, posterior hypothalamus and midbrain (34, 41, 42). Therefore, both surface and deep body receptors are presumed to connect functionally with the preoptic anterior and posterior hypothalamic thermoregulating centers. This was proved by the facts that bilateral destruction of these areas produced an impediment in autonomic thermoregulation (33). It has also been shown that there is a population of thermosensitive neurons in the anterior hypothalamus which serve as a reference of core temperature (41), and that the autonomic responses to cold exposure involve an interaction between core temperature and peripheral cold receptors. On the other hand, it has been reported that thyroid hormone was released due to decrease of hypothalamic core temperature (36). Therefore, it seems that TSH was released by central and peripheral thermoreceptor input to these temperature regulating centers.

To investigate the release of TSH from the pituitary under acute cold stress, hypothalamic and peripheral TRH contents were measured in many reports (7-11, 43, 44). Montoya and his co-workers reported that serum TSH and TRH were significantly increased after acute cold exposure (8). However, no reduction in hypothalamic TRH content was observed. This elevation of serum TSH was blocked by prior administration of thyroid hormone, but no reduction was observed in peripheral TRH. Meanwhile, plasma TSH was reported to be increased by acute cold exposure, but plasma TRH was slightly increased (43) or not affected (44). Effects of acute cold exposure on plasma TRH levels were not definite. Hypothalamic TRH levels were not affected either (7, 9-11, 44).

Numerous papers concerning the effect of acute cold exposure on plasma TSH and TRH secretion reported that plasma TSH levels were elevated under these conditions. Therefore, it was suggested that hypothalamic TRH plays a major role in the activation of pituitary thyrotrophs under these circumstances. The reason for the invariability of the TRH content in the hypothalamus after acute cold exposure in these reports might be the fact that only a part of such change was observed in the hypothalamus. Consequently, it is not appropriate to express the TRH content in whole hypothalamus.

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