within these thermosensitive neuron-enriched areas may be important in thermoregulation of the whole organism.

In conscious mice, a thermal response to TRH is thought to be mediated through catecholamines from the adrenal gland[1]. If the antagonism by TRH of pentobarbital-induced hypothermia in the rat is mediated through catecholamine, we could not use any acute brain lesions to elucidate the site(s) through which TRH exerts its effects. It is well known that lesions or stimulation in various brain areas, including the hypothalamus[13], result in a stimulation of catecholamine secretion from the adrenal glands. From this point of view, the present finding that adrenal demedullectomies have no essential effect on the brain temperature response to TRH is of importance.

The septum has been shown to be the most sensitive site for the antagonism of narcosis by TRH[14], suggesting that TRH-induced analepsis is mediated through this brain structure. This hypothesis has been supported by recent findings[10] that TRH-immunoreactive materials are present in high concentration in neural terminals on the surface of certain cell soma in the septum. TRH binding capacity has also been identified in this area[22]. In the present paper, however, septal deafferentation was found to have no essential effect on the antagonism by TRH of pentobarbital-induced hypothermia. Therefore, the antagonism of hypothermia may involve different neuroanatomical substrate(s) from analepsis by TRH. Different sensitive sites have already been elucidated between the thermal and the analeptic responses, using a microinjection method[14].

It has been reported[2] that TRH administered to the mPO can induce a moderate hypothermia in unanesthetized rats, suggesting a TRH-sensitive thermoregulatory center within this nucleus. In contrast, the present findings show that mPO lesions, as well as posterior hypothalamic lesions, have no effect on the antagonism of hypothermia. This seems to indicate the existence of another site(s) important for the exerting of TRH effects on thermoregulation. Injection of TRH into the 4th ventricle was able to reverse the hypothermia induced by pentobarbital even in rats with midbrain deafferentations. These findings lend support to an important role of the lower parts of the brain as neuroanatomical substrates for the antagonism by TRH. Many TRH neurons have been identified in the lower brain stem and the spinal cord[18]. Several studies are in progress in our laboratory to elucidate a possible role of TRH neurons within the brain areas in thermoregulation.

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Effects of thyroidectomy and thyroxine replacement on the responsiveness of the anterior pituitaries from male rats to thyrotropin-releasing hormone in vitro

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Summary. Thyroidectomy decreased prolactin concentrations in the anterior pituitary (AP) and serum of the male rat. The amount of basal and thyrotropin-releasing hormone (TRH)-stimulated release of prolactin by AP in vitro was lower in thyroidectomized (Tx) rats than in sham Tx rats. These results suggest that the inhibitory effects of thyroidectomy on pituitary and serum prolactin in male rats are mediated in part by the reduction of the production and spontaneous release of prolactin and the responsiveness of prolactin to TRH.

Key words. Thyroidectomy; TRH; rat prolactin; rat TSH.

Synthetic thyrotropin-releasing hormone (TRH) has been shown to increase serum prolactin (PRL) and thyrotropin (TSH) in rats[1-8]. The release of prolactin in response to TRH in vitro was demonstrated using rat pituitary cells or tissue in culture[9-13]. Thyroxine (T4) has been observed to stimulate the secretion of prolactin in male rats[14]. However, TRH-stimulated secretion of rat prolactin by pituitary tumor cells[11,13] and neonatal pituitary cells[12] is inhibited by the administration of thyroid hormones in vitro. Since some models employed in these studies may not reflect the in vivo or physiological situation, the role of the thyroid gland in regulating prolactin secretion is still open to question.

The purpose of this investigation was to examine the effects of thyroidectomy and T4 replacement in vivo on basal and TRH-induced prolactin release and production in vitro by the pituitary of male rats.

Materials and methods. Animals. Male Sprague-Dawley rats (160–240 g) were housed in an air-conditioned room with 14 h of artificial illumination daily (06.00–20.00). All animals were given food and tap water ad libitum. They were subjected to one of three treatments: 1) thyroidectomy, followed by T4 replacement, 2 µg/100 g BW, once daily for 42 days; 2) thyroidectomy, followed by injection of 0.9% NaCl solution; or 3) sham thyroidectomy, followed by injection of 0.9% NaCl solution. T4 solution was prepared by dissolving L-thyroxine (Sigma) in a few drops of 0.1 N NaOH then diluting with 0.9% NaCl solution to a concentration of 20 µg/ml before using for s.c. injection. Twenty hours after the last injection, rats were sacrificed. Blood

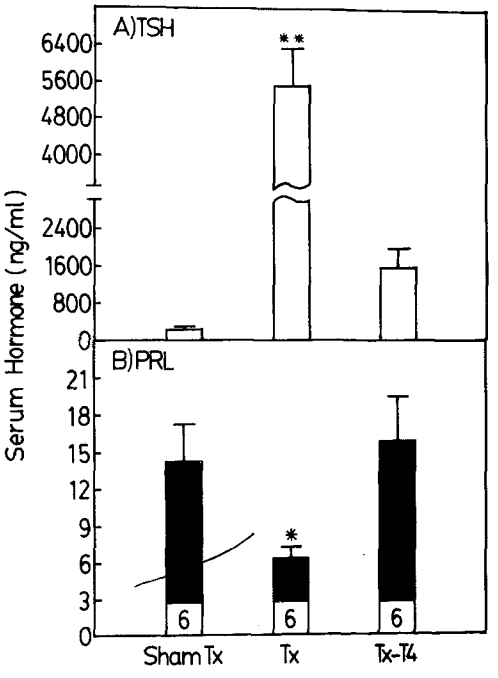

Figure 1. Effects of thyroidectomy and T4 replacement with 2 µg/100 g BW/day for 42 days on serum TSH (A), and prolactin (B) in male rats. Results are expressed as mean ± SEM. * $p < 0.05$ versus sham Tx or Tx-T4 group, respectively; ** $p < 0.01$ versus sham Tx or Tx-T4 group, respectively. Each group consisted of 6 animals.

samples were obtained by decapitation. Serum was separated by centrifugation at 1000 × g for 30 min and stored at −20 °C until assayed for prolaction and TSH by radioimmunoassay (RIA). *Incubation procedure.* After decapitation of the rats, the anterior pituitary glands (APs) were removed and bisected. One hemi-AP was placed in a flask containing 2 ml of Krebs-Ringer phosphate buffer which contained 0.1% ascorbic acid, 0.1% bovine serum albumin, 10 mM glucose and 0.05% bacitracin (KRB), and had been gassed with 95% O_2 and 5% CO_2 at room temperature. Preincubation at 37 °C was performed for 1 h after replacing the turbid medium with 1 ml of fresh KRB. The tissues were then incubated with 1 ml of fresh KRB containing 0 or 2.8 nM (1 ng/ml) TRH at 37 °C in a shaking water bath for 4 h. There were 5–7 flasks for each dose of TRH. At the end of the incubation, the medium was removed and centrifuged at 1000 × g for 30 min. The AP tissues were weighed and homogenized with 0.9% NaCl solution before centrifugation at 1000 × g for 30 min. Both AP extract and supernatant of the medium sample were stored at −20 °C until assayed for prolactin and TSH by RIA. *Hormone assays.* The concentrations of prolactin and TSH in the serum, medium and AP extracts were measured by the double-antibody RIA. Both rat prolactin and rat TSH RIA kits were provided from NIAMDD, USA. Rat PRL-I-5 and rat TSH-I-5 were iodinated with ^{125}I by a modification of the chloramine-T method of Greenwood et al.[15]. Anti-rat PRL-S-8 and anti-rat TSH-S-5 were diluted with ethylenediaminetetraacetate in phosphate buffered saline (0.05 M EDTA-PBS, pH 7.0) containing 0.25% normal rabbit serum and 0.01% merthiolate. Rat PRL-RP-2 and rat TSH-RP-1 served as standards. Two hundred µl of antiserum with optimal dilution and 50 µl of tracer, circa 10000 cpm were mixed with 300–500 µl of 1% ovalbumin in PBS containing standard hormone or sample in a 12 × 75 plastic tube. The mixture was incubated for 48 h at 4 °C before addition of the second antibody (sheep anti-rabbit gamma globulin serum). After an additional 48-h incubation, 2 ml of ice-cold PBS were added into each assay tube and the samples were centrifuged at 1000 × g at 4 °C for 30 min. The radioactivity in the precipitate was then measured by an automatic gamma counter. Each sample was assayed at one or two dilutions in duplicate. The sensitivity was 20 pg per tube for prolactin RIA and 20 ng per tube for TSH RIA.

Statistical analysis. The data were analyzed by one-way analysis of variance. When F was significant, the least significant difference test was employed to determine the difference between means.

Results. Serum TSH and prolactin. Thyroidectomy produced a significant increase in serum TSH (fig. 1, upper panel, $p < 0.01$). Administration of 2 µg T4/100 g BW reduced TSH levels. Thyroidectomy significantly decreased serum prolactin in male rats (fig. 1, bottom panel, $p < 0.05$). Replacement of physiological dose of T4 returned prolaction to that of sham Tx animals.
Prolactin release in response to TRH in vitro. TRH significantly increased the medium prolactin in all three groups by 0.8 to 1.8-fold (fig. 2, panel A). With respect to prolactin release by rat

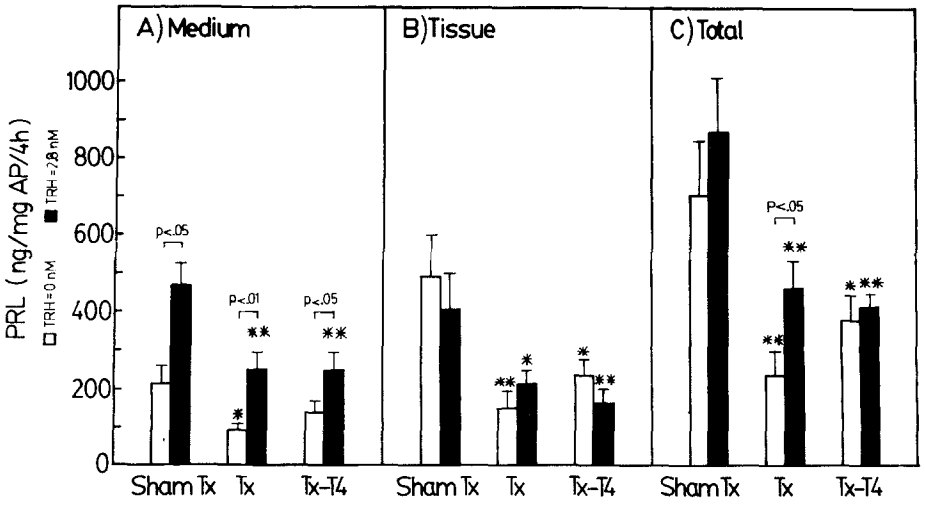

Figure 2. Effects of thyroidectomy in male rats on the concentration of prolactin in the medium, tissue, and total system after AP incubation. Immediately after thyroidectomy, the rats were injected s.c. with either T4 (2 µg/100 g BW/day) or 0.9% NaCl solution for 42 days. Sham thyroidectomized (TX) rats served as controls. On day 43, they were sacrificed by decapitation. The APs were quickly removed, bisected, and preincubated for 30–60 min in Krebs-Ringer phosphate buffer at 37 °C before 4-h incubation with or without TRH. Each flask contained one piece of hemi-AP. Results are expressed as mean ± SEM based on 5–7 replicates. * $p < 0.05$ versus sham Tx group; ** $p < 0.01$ versus sham Tx group.

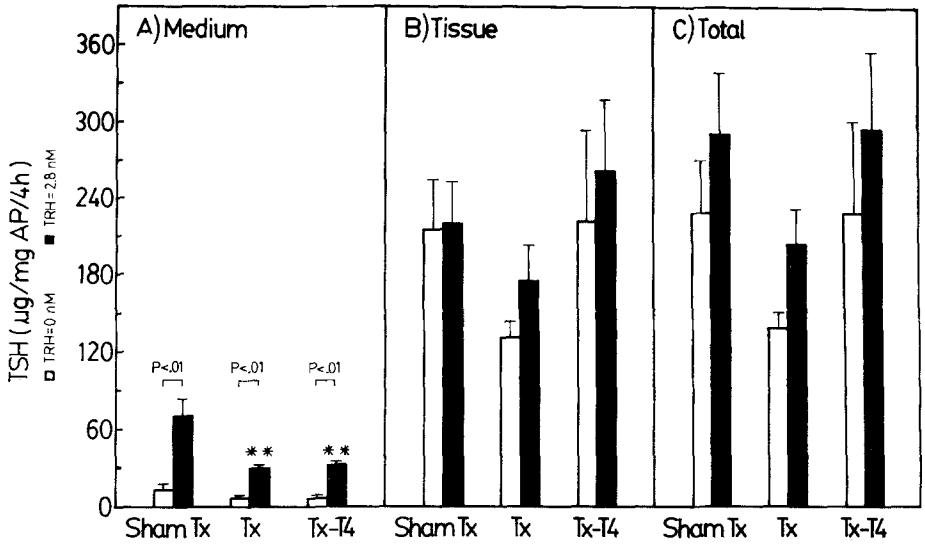

Figure 3. Effects of thyroidectomy in male rats on the concentration of TSH in the medium, tissue, and total system after AP incubation. See legend to figure 2 for further details.

AP in vitro, TRH at 2.8 nM elicits a half-maximal response (data not shown). Thyroidectomy resulted in a significant decrease in basal ($p < 0.05$) and TRH-stimulated ($p < 0.01$) release of prolactin. After incubation, prolactin concentrations in AP tissue from Tx animals were lower than in that from sham Tx animals (fig. 2, panel B).

Total prolactin (medium plus tissue) was significantly reduced by thyroidectomy ($p < 0.01$, fig. 2, panel C). Administration of T4 failed to return the prolactin in medium and total system to sham Tx levels.

TSH release in response to TRH in vitro. The pituitary TSH response to TRH in vitro was shown in figure 3. TRH produced a 2.8 to 4.4-fold increase in medium TSH released by APs ($p < 0.01$, fig. 3, panel A).

Basal release of TSH was not influenced by thyroidectomy, whereas thyroidectomy significantly ($p < 0.01$) reduced release of TSH in response to TRH by 40%. TSH in both tissue and the total system was unaffected by thyroidectomy (fig. 3, panels B and C).

Discussion. These results suggest that 1) TRH stimulated the release of prolactin and TSH in vitro by APs of male rats; 2) thyroidectomy decreased the concentration of prolactin in the serum and AP of male rats; 3) thyroidectomy reduced both basal and TRH-induced release of prolactin; 4) replacement of Tx rats with physiological doses of T4 restored serum prolactin concentrations in vivo, but neither AP prolactin content nor basal and TRH-induced release of prolactin in vitro to the euthyroid levels.

In agreement with previous studies of pituitary cells[9-13], we have demonstrated that TRH directly stimulates both prolactin and TSH release from AP of adult male rat in vitro. Both the spontaneous and TRH-induced release of prolactin by AP were lower in Tx rats than in sham Tx rats (fig. 2). However, the increase of prolactin induced by TRH was 180% in Tx group and 110% in sham Tx group. It is evident that in male rats the relative increase over basal levels of prolactin release in response to TRH is elevated by thyroidectomy. This finding is in agreement with the observations from propylthiouracil-induced hypothyroidism in the male rats[10].

The medium plus tissue prolactin, i.e. the total content of prolactin in rat AP, was reduced by thyroidectomy. This result suggests that thyroidectomy in male rats decreased the production or synthesis of prolactin. Our finding is similar to that reported by Chen and Meites[16] and would imply a reduction of the number of lactotrophs in male rat AP following thyroidectomy. We also found that TRH resulted in a significant increase of prolactin production in Tx rats, but this production was still lower than that in sham Tx group. We therefore believe that the inhibitory effect of thyroidectomy on serum prolactin in male rat is mediated at least in part by the decrease of the prolactin production from the AP.

Compared to untreated Tx rats, T4-treatment of Tx rats increased appreciably the release of prolactin, as indicated by the circulating concentration of prolactin. Indeed, the release of prolactin in T4-treated Tx rats was comparable to that seen in untreated, sham Tx rats. However, under in vitro conditions the release of prolactin from APs of untreated Tx rats and T4-treated Tx rats were quite different from that seen in vivo. We found the basal in vitro release of prolactin from APs of untreated Tx rats and T4-treated Tx rats to be essentially the same, and in both cases to be less than that seen from APs of intact rats. We also found the TRH-stimulated in vitro release of prolactin from APs of untreated Tx rats and T4-treated Tx rats to be the same, and in both cases to be significantly less than that seen from APs of intact rats. The reasons for this lack of effect are unclear but may be due to the loss of TRH receptors in AP of Tx rat following a long-term administration of thyroid hormones[11-13]. Since no hypothalamic tissue was applied in our in vitro incubation system and it is well known that the secretion of prolactin can be controlled by both prolactin release-inhibiting factor (PIF) and prolactin releasing factor (PRF) from hypothalamus, the possible effect of T4 on the production and release of prolactin through an action on the release of PIF or PRF from the hypothalamus is uncertain. It is apparent that other mechanisms excluding TRH are involved in the regulation of the prolactin levels in rat serum by the thyroid hormones. It has been shown that a surgical interruption of anterolateral neural connections of the mediobasal hypothalamus in male rats reduces the prolactin content in AP[17], the prolactin response to stress and the basal release of prolactin from perifused APs[18]. Brown et al.[19] have demonstrated that T4 treatment in male rats decreases the concentration of dopamine, a physiological prolactin release-inhibiting factor, in anterior and middle hypothalamus and suggested that a dopaminergic mechanism was involved in the action of T4 on hypothalamus. These observations reflect that T4 may regulate the secretion of prolactin by acting at the hypothalamus and/or higher levels in rat brain.

Thyroidectomy in male rats resulted in a significant increase in serum TSH. Administration of T4 to Tx rats reduced serum TSH levels to a level with no difference to those of intact rats. These results are conceivable and in consistent with earlier reports[20-22]. However, thyroidectomy had no effect on the basal release of TSH, and reduced the release of TSH in response to TRH by rat AP in vitro. These results favor a view that the negative feedback control of TSH secretion by thyroid hormones might be mainly at the level of the hypothalamus. This concept is supported by Berelowitz et al.[20], who suggested that the elevated TSH levels seen in primary hypothyroidism may result in part from a decrease in the tonic inhibitory effect of hypothalamic somatostatin. However, our data do not rule out the possibility that T4 may also act at the anterior pituitary level as well.

A deficient effect of T4 replacement in vivo on the TSH release in response to TRH in vitro is similar to those on the prolactin release in the present study. These results could be explained by the loss of TRH receptors in both thyrotrophs[12] and lactotrophs[11,13] of the rat AP after chronic treatment of thyroid hormones. The deficiency of the dose of T4 therapy may be another possibility even the same dose of T4 is sufficient to restore the prolactin concentration in serum of Tx rats. Whereas the different results of the total hormone production between prolactin and TSH in the AP of Tx rats replaced with T4 reflect a possibility of different mechanisms in regulating the hormone synthesis between prolactin and TSH in vivo.

In summary, the present results suggest that the inhibitory effects of thyroidectomy on AP and serum prolactin in male rats are mediated at least in part by the reduction of the total production and basal release of prolactin and the responsiveness of prolactin to TRH.

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7-Dehydrosterols in prothoracic glands of the silkworm, *Bombyx mori*

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Summary. Sterol analysis of prothoracic glands of the silkworm, *Bombyx mori* revealed the presence of 7-dehydrocampesterol and 7-dehydrositosterol together with 7-dehydrocholesterol. It was also found that the amounts of these 7-dehydrosterols were increased in proportion to the ecdysone titer.

Key words. 7-Dehydrocholesterol; 7-dehydrocampesterol; 7-dehydrositosterol; *Bombyx mori;* ecdysone; prothoracic gland.

Usually, 7-dehydrocholesterol represents only a few percent of the total sterol content in most insects[2], but high titers of this sterol have been found in the prothoracic glands and in eggs[3,4]. The importance of 7-dehydrocholesterol has been described in relation to the biosynthesis of ecdysteroids[5]. The sterols of the silkworm, *Bombyx mori* were reported to contain cholesterol (**1a**), campesterol (**1b**), and sitosterol (**1c**) along with very small amounts of fucosterol[6], isofucosterol[6], desmosterol[7] and fucosterol epoxide[8]. We describe here the identification of 7-dehydrocampesterol (**2b**) and 7-dehydrositosterol (**2c**) in addition to 7-dehydrocholesterol (**2a**), in prothoracic glands. The peak titers of these 7-dehydrosterols occur on days 5 and 9 of the 5th larval instar.

Prothoracic glands (3200) of 5th instar larvae of *B.mori* were extracted with chloroform-methanol (2:1). The extracts were applied to precoated TLC plates (Merck, Kiesel-gel 60 F254) and developed with benzene-acetone (10:1). The UV absorbing band corresponding to 7-dehydrocholesterol was scraped off and extracted with ethyl acetate. Gas-liquid chromatography (GLC) analysis of the extract after TMS (trimethylsilyl imidazol) derivatization detected cholesterol, campesterol and sitosterol as well as three additional sterols, which have slightly longer retention times than the respective Δ^5 (fig. 1). These sterols were identified as 7-dehydrocholesterol (**2a**), 7-dehydrocampesterol (**2b**), and 7-dehydrositosterol (**2c**) by the use of GC-MS analysis[9].

We then analyzed the sterol fractions (eluted with hexane-ethyl acetate (4:1) on a column of silica gel) isolated from hemolymph (2 ml), prothoracic glands (10 pairs) or fat body (2 g wet wt) of the 5th instar larvae (day 2 of wandering). GLC analysis of these fractions after TMS derivatization revealed cholesterol, campesterol and sitosterol in all three tissues, whereas no 7-dehydrosterols were detected in the samples of hemolymph and fat body.

Encouraged by these observations, we then went on to measure the changes in the amounts of 7-dehydrosterols in the protho-