Antagonism by thyrotropin-releasing hormone (TRH) of pentobarbital-induced hypothermia in rats with brain lesions

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Summary. Anesthesia with a large dose of pentobarbital (55 mg/kg, i.p.) caused a sustained decrease in brain temperature (Tb), which was monitored with a probe placed in the midbrain reticular formation. The administration of TRH to the lateral ventricle antagonized this hypothermia. None of the acute surgeries examined in this paper (adrenal-demedullectomy, septal knife cuts, electrolytic lesions of the hypothalamus and midbrain knife cuts) had any essential effect on this antagonism by TRH. These results suggest that centrally-administered TRH exerts its effect on thermoregulation, at least in part, through brain structure(s) caudal to the midbrain.
Key words. Thermoregulation; electrolytic lesion; TRH; septum; hypothalamus; reticular formation.

Thyrotropin-releasing hormone (TRH) was first isolated[6] from the hypothalamus on the basis of its function in stimulating pituitary thyrotropin release. This tripeptide has since been shown to be widely distributed in the central nervous system (CNS)[5], and to have multiple effects on the CNS[3, 14, 23]. The effects of TRH on thermoregulation have also been studied extensively in various species[7], and it has been suggested that endogenous TRH participates in the thermoregulation of the whole body[20]. However, little information is available concerning the site(s) through which TRH exerts these effects. In rats, it is well-known that injection of TRH into the brain can antagonize the hypothermia induced by certain drugs, such as pentobarbital[15], ethanol[16] and bombesin[4]. The hypothermia induced by pentobarbital is significantly reversed by microinjection of TRH in the medial preoptic nucleus (mPO) and/or adjacent areas[14]. Since this region contains both TRH neurons and thermosensitive neurons, the mPO might play an important role in the antagonism by TRH. The present study was designed to investigate the effect of electrolytic lesions in the mPO on the antagonism by TRH of pentobarbital induced hypothermia. In addition, we carried out similar investigations using rats which had undergone knife cuts in the septum or the midbrain, or electrolytic lesions in the posterior hypothalamus. Each of these brain structures has been shown to be densely populated with TRH positive neurons[8, 10, 17].

In the present study, temperature was monitored with a small probe placed in the midbrain reticular formation (mRF), because this brain structure contains many thermosensitive neurons. These neurons are thought to be important in thermoregulation of the whole organism[9]. We report herein for the first time that the temperature within this thermosensitive-neuron enriched area is strongly modified by both TRH and pentobarbital treatments.

Methods. Experiments were performed in male Wistar rats (280–350 g). On the experimental day, animals were anesthetized with sodium pentobarbital (55 mg/kg, i.p.) and positioned in a stereotaxic apparatus, as previously described[12]. One thermocouple microprobe (OD: 0.41 mm, IT-21, Bailey Inc, USA) was placed unilaterally in the mRF using the following stereotaxic coordinates; 5.8 mm posterior to the bregma, 1.5 mm lateral to the midline, 6.0 mm below the surface of the skull. Ambient temperature was also monitored and maintained at 22.0–24.0 °C. Forty minutes after pentobarbital treatment, they were given an intralateral (ilv) or 4th ventricular (AC) injection of TRH (1 µg in 20 µl of saline), or saline alone by means of a microcannula (OD: 0.3 mm) connected to a 50 µl Hamilton microsyringe.

In order to determine whether TRH exerts its effect through circulating catecholamine(s), the same procedure was performed in bilaterally adrenal-demedullectomized rats.

The following studies were made in an attempt to elucidate the site(s) through which TRH acts on the CNS. The experimental protocol was the same as that described above, except that the animals underwent septal deafferentation, midbrain knife cuts, mPO lesions, posterior hypothalamic lesions or sham operation immediately after pentobarbital treatment. In order to achieve complete deafferentation of the septum, an L-shaped knife (width; 0.35 mm, height; 2.5 mm, length of the horizontal part; 2.0 mm) was inserted vertically into the brain so that the angle of the knife was located 6.0 mm below the bregma. It was moved bilaterally ± 1.5 mm across the sagittal suture, then move back to the original position. In sham-operated animals, the knife was simply inserted 6.0 mm below the surface of the skull. mPO or posterior hypothalamic lesions was made bilaterally by applying a 2.0 mA direct anodal current for 10 s through an insulated insect pin (0.3 mm in diameter), as described previously[12]. Midbrain knife cuts were made with a 2 mm wide stainless-steel knife, according to the methods described previously[11]. To identify the locations and extents of the knife cuts and lesions, the brains were excised at the end of each experiment and fixed in 10% formalin. Following fixation, the brain was cut in 40-µm sections, which were then stained with cresyl violet.

Septal knife cuts severed the septum horizontally at the level just above the anterior commisure, and the line of the cuts extended laterally to the bed nucleus of the stria terminales just below the lateral ventricle. The structure was also separated from the hippocampus at the level of the anterior commisure in antero-posterior direction. Midbrain knife-cuts severed the central aquaduct at the midbrain level, as described previously[11]. mPO lesions ablated the mPO and the anterior part of the anterior hypothalamus, and frequently deformed the third ventricle.

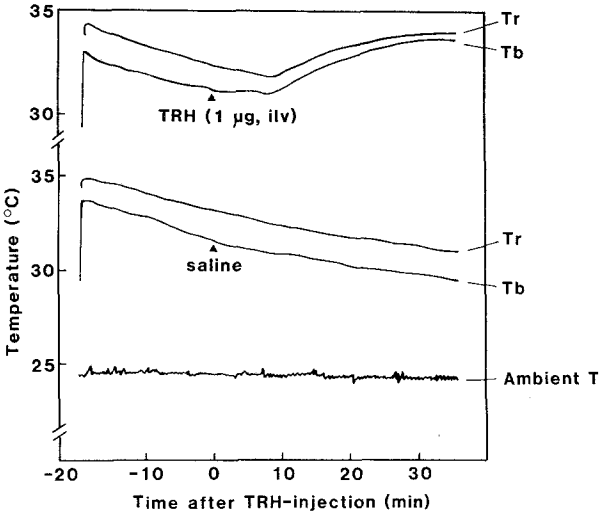

Figure 1. Representative tracing showing responses of brain (Tb) and rectal (Tr) temperatures to TRH injection in pentobarbital-anesthetized rats. Tb could be monitored after the animals were positioned on a stereotaxic apparatus (circa 5–10 min after i.p. injection of pentobarbital). TRH (1 µg in 20 µl of saline) or saline was injected into the lateral ventricle (ilv) exactly 40 min after pentobarbital (55 mg/kg i.p.) treatment.

Student's t-test was used to estimate the significance of differences within and between groups, and differences were considered significant at the 0.05 level.

Results. Tb in normal rats anesthetized with pentobarbital decreased gradually as shown in figure 1. Tb levels were almost always 1.0–1.6 °C lower than those of rectal temperature (Tr). Administration of TRH diminished this hypothermic effect of pentobarbital, and Tb levels began to rise within 10 min of TRH injection. The net increase in Tb within 40 min after TRH injection was 3.4 ± 0.3 °C, which was significantly higher than that in Tr (ΔT, 1.5 ± 0.5 °C). Adrenal demedullectomies had basically no effect on the antagonism by TRH of pentobarbital hypothermia. In demedullectomized rats, Tb values at 8 min were lower than those in sham-operated controls, as shown in figure 2A. At 40 min, however, no significant difference was observed between the two groups. There was no difference between the two groups in basal Tb levels, either (control; 31.4 ± 0.6 versus demedullectomized; 30.6 ± 0.4).

Septal deafferentation, mPO lesions or posterior hypothalamic lesions failed to inhibit the antagonistic effect of TRH. The table and figure 2B show these results. Tb levels from each group increased after TRH injection in a similar manner to those from the corresponding control groups. Data obtained from the rats with midbrain knife cuts are summarized in figure 3. When TRH was injected into the lateral ventricle, the antagonistic effect of the drug was almost abolished. The Tb continued to decrease even after TRH was injected, and the time course was very similar to that observed in saline-injected animals. However, when TRH was administered into the 4th ventricle of these deafferentiated rats the Tb levels increased shortly after TRH injection as observed in sham-operated rats. During the whole observation period, there were no significant differences in Tb levels between the deafferentiated and the sham-operated groups.

Discussion. The results of the present study show clearly that brain temperature can be modified strongly by both TRH and pentobarbital treatments in a manner similar to core temperature[15,16]. The magnitude of Tb response to TRH was more than twice that of core (rectal) temperature. It is interesting that the changes in Tb after TRH injection were very similar to changes in brain temperature observed in hibernating squirrels following TRH treatment[21]. These findings support an assumption[19] that changes in brain temperature are mediated through different components of the thermoregulatory system from those acting on core temperature.

Considering that many thermosensitive neurons are present in certain brain areas, including the mRF[9], changes in temperature

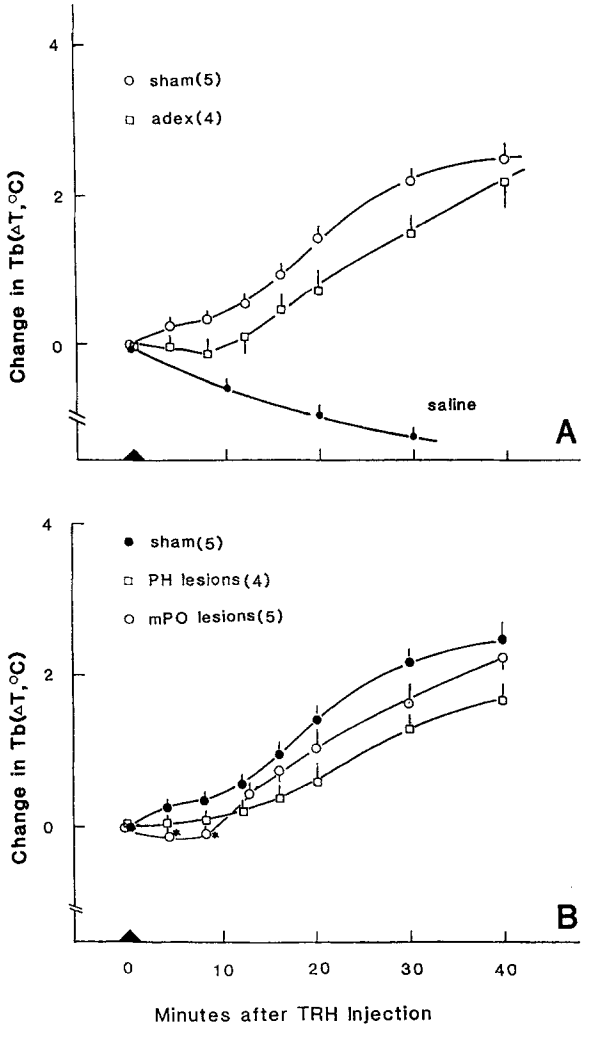

Figure 2. Tb responses to TRH in rats with adrenal-demedullectomies (adex) (A), or electrolytic lesions of the mPO or posterior hypothalamus (PH) (B). Numbers in parentheses indicate the size of each experimental group. *Significant difference at $p < 0.05$ as compared with the sham group. Note that there is no difference between the adrenal-demedullectomized and the sham-operated groups.

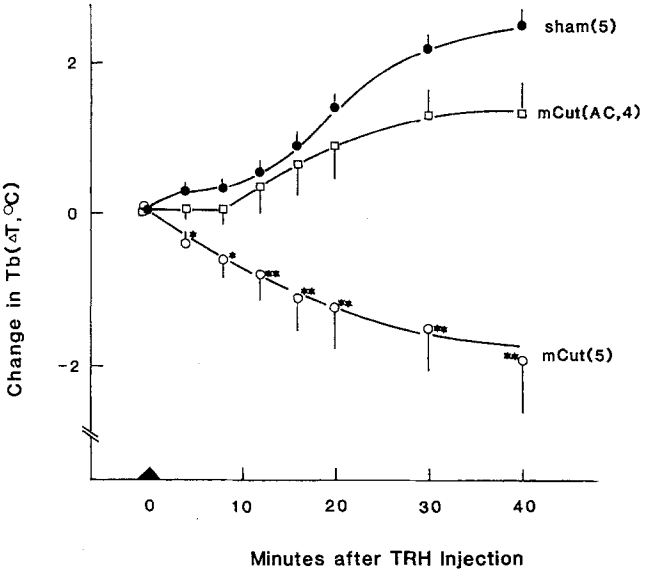

Figure 3. Effects of TRH on brain temperature in rats with midbrain knife cuts or sham-operated rats. Rats with the knife cut were treated with 1 µg TRH into the lateral ventricle (mCut) or the 4th ventricle (mCut; AC). The sham-operated rats received TRH via the lateral ventricle. *Significant difference from the controls at $p < 0.05$. Note that the response to 4th ventricularly-injected TRH in rats with knife cuts is almost the same as that in the control animals.

Changes in Tb after TRH injection in pentobarbital anesthetized rats

Group	0 min	10 min	20 min	30 min	40 min
Saline (5)	0	− 1.0 ± 0.1	− 1.6 ± 0.2	− 2.3 ± 0.3	− 2.7 ± 0.3
TRH/sham (5)	0	0.7 ± 0.1*	1.4 ± 0.2*	2.5 ± 0.1*	3.4 ± 0.3*
TRH/septal cuts (4)	0	0.5 ± 0.1*	0.8 ± 0.1*	1.5 ± 0.3*	2.2 ± 0.4*

TRH (1 µg/20 µl of saline) or the same volume of saline was injected into the lateral ventricle 40 min after pentobarbital (55 mg/kg body wt, i.p.) treatment. Values indicate means (Δ T, C) ± SE. * significant difference from the saline group, at $p < 0.05$.

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