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Antagonism by Thyrotropin-Releasing Hormone of Brain Temperature in Response to Pentobarbital in the Rat: Possible Involvement of Cholinergic Mechanism (42433)

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Abstract. The effects of thyrotropin-releasing hormone (TRH) on brain temperature in response to pentobarbital were examined in male rats. After intraperitoneal injection of pentobarbital sodium (55 mg/kg body wt), the rats were fixed stereotaxically and received intraventricular (ivt) injection of varying doses (0.03–30 nmol) of TRH and 17 nmol atropine. Following the injection of 3 nmol TRH, 100 nmol of carbocholine was administered in the same manner. A thermocouple microprobe was unilaterally placed in the midbrain reticular formation so that brain temperature was continuously monitored at room temperature. Brain temperature after pentobarbital injection progressively decreased. While ivt injection of saline did not affect this change in temperature, ivt administration of TRH produced a dose-dependent antagonism of the brain hypothermia induced by pentobarbital. Atropine injection also reversed the pentobarbital-induced decrease in brain temperature. Carbocholine injection led to a significant decrease in brain temperature in response to TRH administration. The present study indicates that brain TRH may play a pivotal role in brain thermoregulation and its mechanism may involve at least in part the central cholinergic pathway in the rat.

There is a general consensus that the brain substantially participates in thermoregulation, and several neuropeptides existing in the brain have been observed to have thermoregulatory functions (1). Apart from its original endocrinological actions, a number of reports have indicated the possibility of thyrotropin-releasing hormone (TRH) as a potent thermoregulatory substance in the brain. Prange *et al.* (2) found that intraperitoneal administration of TRH prevented a decrease in body temperature induced by pentobarbital anesthesia. The same phenomenon was also observed following injection of the tripeptide via the intraventricular (ivt) route (3, 4). The data suggest that the mechanism of the pentobarbital antagonism by TRH is central in origin. The possibility has been recently raised that the mechanism mediating some central actions of TRH may involve cholinergic neurons in the brain (5). However, little is known concerning the effects of TRH on temperature in the brain. Recent studies have shown that measurement of brain temperature is important in determining the extent to which TRH influences the arousal status (6). A critical thermoregulatory center in the brain resides in the midbrain reticular formation (mrf) which has pivotal interregulatory connections with many brain regions including the hypothalamus (7, 8).

In view of these observations, we have attempted to clarify the effects of TRH on temperature changes in the mrf in response to pentobarbital and to determine the degree of involvement of the central cholinergic mechanism in this action in the rat.

Materials and Methods. Adult male rats of Wistar strain which weighed 300–350 g were fed a Purina laboratory chow diet and given tap water to drink. The animals were caged for at least 1 week before the experiments in a temperature-controlled room (23 ± 1°C) with the light going on at 6:30 AM and off at 6:30 PM. TRH was supplied by Tanabe Pharmaceutical Company (Osaka, Japan), and atropine and carbocholine were purchased from Sigma Chemical Company (St. Louis, Mo.). All neuroactive substances were dissolved in saline, and 10 μl of each substance was administered intraventricularly as mentioned previously (9).

The rats were intraperitoneally injected with 55 mg/kg body weight of pentobarbital sodium

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(Sigma Chemical Co.). Thirty minutes later, the animals stereotaxically received lateral ventricular injection of TRH in doses of 0.03 to 30 nmol, or atropine in a dose of 17 nmol. Further, to evaluate the interaction between TRH and cholinergic activities in regulating brain temperature, 100 nmol carbocholine was intraventricularly administered 30 min after injection of 3 nmol TRH. Because carbocholine injection did not apparently affect the decrease in brain temperature under pentobarbital anesthesia in the preliminary study, sole injection of carbocholine was not done.

Brain temperature was continuously determined at room temperature by using a thermocouple microprobe (Type IT-21, Sensortek Inc.) which was unilaterally placed in the mrf, according to the stereotaxic coordinate of Paxinos and Watson (10) (posterior = 5.8 mm behind bregma; lateral = 1.5 mm from the midline; vertical = 6.0 mm from the skull surface).

Statistical significance was done by Duncan's multiple comparison (11).

Results. Figure 1 shows the typical changes in brain temperature induced by pentobarbital injection, followed by injection of TRH and carbocholine. Brain temperature in rats anaesthetized with pentobarbital decreased progressively, and reached 33.6°C 30 min after injection. Injection (ivt) of saline did not prevent the progressive decrease in brain temperature (Fig. 1a). In contrast, as shown in Fig. 1b, ivt injection of TRH significantly attenuated the decrease in brain temperature, and this antagonistic effect was dose-dependent (Fig. 2). The smallest dose of TRH (0.03 nmol) injection was observed to significantly antagonize the brain hypothermic action of pentobarbital 50 min after injection (a saline-injected group, -2.8 ± 0.1 vs a TRH-injected group, -1.4 ± 0.3°C, $P < 0.05$).

As shown in Fig. 3, ivt injection of atropine also antagonized the decrease in brain temperature in response to pentobarbital.

Figure 1c depicts the effect of carbocholine on the increase in brain temperature induced by TRH. The TRH-induced increase in brain temperature was significantly antagonized by ivt administration of carbocholine, as shown in Fig. 4.

Discussion. A number of studies have shown that administration of TRH induces

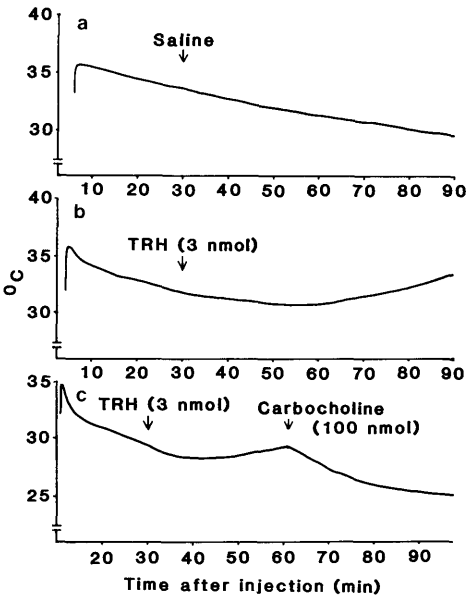

FIG. 1. A typical change in brain temperature after pentobarbital injection. The rats were intraperitoneally injected with pentobarbital sodium (55 mg/kg body wt). Thirty minutes later, saline (a) or 3 nmol TRH (b) was administered intraventricularly. Thirty minutes after 3 nmol TRH injection, 100 nmol carbocholine (c) was administered intraventricularly. Brain temperature in the midbrain reticular formation was continuously monitored with a thermocouple microprobe.

an increase in body temperature (2–4), but whether the tripeptide affects brain temperature has remained unknown. It is clear from the present study that ivt administration of TRH had a significant inhibitory effect on pentobarbital-induced changes in brain temperature monitored in the midbrain reticular formation, an important thermosensitive center in the brain (7, 8). In the present study, the significant inhibition of pentobarbital-induced hypothermia in the brain was produced by ivt injection of TRH in a dose of 0.03 nmol, which is within the physiological range of TRH concentrations observed in the rat brain (12). The data strongly suggest that TRH is a thermoregulatory factor in the brain. Moreover, the present study showed that ivt injection of atropine acted, as TRH did, in preventing the brain hypothermia induced by pentobarbital. In contrast, ivt injection of carbocholine antagonized the increase in brain temperature produced by TRH, leading to the brain hy-

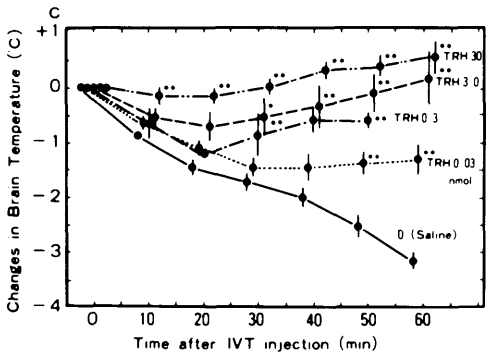

FIG. 2. Effects of ivt injection of varying doses of TRH on brain temperature. Rats received ivt injection of TRH in doses of 0–30 nmol after pentobarbital injection as described in Fig. 1. Changes in brain temperature were expressed as decrement from the level before TRH injection. Vertical bars indicate means ± SE. The number of animals used was five in each group. Significant analysis was done between each of TRH-injected groups and a saline-injected group at each time (*$P < 0.05$, **$P < 0.01$).

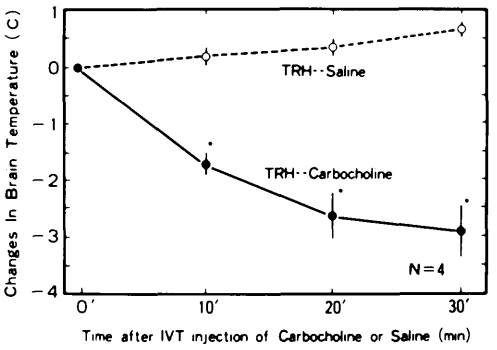

FIG. 4. Effects of carbocholine on TRH-induced changes in brain temperature. As described in Fig. 1, rats were injected with 100 nmol carbocholine or saline 30 min after TRH injection. Response of the brain temperature was expressed as the changes from the level before carbocholine or saline injection. Vertical bars indicate means ± SE. The number of animals used was four in each group. (*$P < 0.01$ differs from a saline (TRH—saline)-injected group.)

pothermia. Because much attention has been focused on the possibility of involvement of the cholinergic mechanism in TRH action in the central nervous system (5), the present data imply that the thermoregulatory action of TRH may be attributed, at least in part, to a cholinergic mechanism in the brain. Although it remains to be seen whether TRH could affect cholinergic receptor mechanisms, the present study supports the possibility that TRH may inhibit a pentobarbital-induced cholinergic activity in the brain.

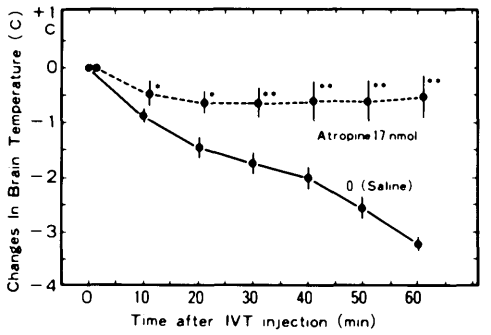

FIG. 3. Effects of atropine on brain temperature in the rats. Rats received ivt injection of atropine or saline 30 min after pentobarbital injection. Changes in brain temperature were expressed as decrement from the level before atropine or saline injection. Vertical bars indicate means ± SE. The number of animals used was five in each group. (*$P < 0.05$ and **$P < 0.01$ differ from a saline (atropine 0)-injected group.)

TRH not only attenuates the decrease in body temperature, but shortens sleeping time in response to pentobarbital. Whereas the former function was not affected by atropine (3, 4), the latter was antagonized by the same cholinergic antagonist (3, 13, 14). The discrete nature of TRH effects on barbiturate hypothermia and anesthesia, both of which are affected differently by cholinomimetics, has not yet been elucidated. Indeed, no consensus of opinion has been reached with respect to the interactions of TRH with the cholinergic excitation of the brain neurons. A facilitatory effect of microiontophoretically applied TRH on acetylcholine-induced excitation of neurons was observed by one group of authors (15, 16), but not by others (17, 18). Miyamoto *et al.* (14) reported that ivt injection of atropine antagonized the shortening action of TRH on sleeping time induced by pentobarbital when the tripeptide was injected into the hypothalamus, but not into the other brain regions or intraventricularly. They suggested that certain neuroanatomical sites may be responsible for mediating the antagonistic action of TRH on pentobarbital-induced sleep. The brain regions critical to this arousal action of TRH include the posterior hypothalamic regions (14) and/or the medial septal nucleus (19). In contrast,

many investigators have proposed that the sites most sensitive to the TRH action in inducing hyperthermia are the preoptic/anterior hypothalamic nuclei in the rat. Cohn *et al.* (20) reported that a sustained increase in body temperature was observed when TRH was injected into the preoptic and anterior hypothalamic nuclei. An extensive search for the brainstem at which TRH-elicited hyperthermia in the rats was done by Boshi and Rips (21) and Kalivas and Horita (22), and the same brain nuclei were found to be important sites in the hyperthermic action of TRH. The present data show that TRH may play a physiological role in brain thermoregulation. Further, they indicate that the central thermoregulatory and arousal mechanisms of TRH differ from each other regarding interaction with cholinergic systems in the brain.

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