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Effects of Hypothermia on Thyrotropin-Releasing Hormone Content in the Rat Brain

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OKUDA, C., T. MIZOBE AND M. MIYAZAKI. *Effects of hypothermia on thyrotropin-releasing hormone content in the rat brain.* PHARMACOL BIOCHEM BEHAV 30(4) 941–944, 1988.—The thyrotropin-releasing hormone (TRH) content in the brain was determined in normothermic and hypothermic rats subjected to immobilization stress. TRH contents in the hypothalamus, midbrain and cerebral cortex significantly decreased during mild hypothermia (body temperature about 34°C), but not during profound hypothermia (about 24°C). The decreases in the TRH content during mild hypothermia were readily reversed by rewarming the animal. These results indicate that cerebral TRH is involved in the response to a mild body temperature drop when the animal is exposed to a cold environment.

Cerebral TRH Mild hypothermia Profound hypothermia Thermoregulation

SEVERAL studies have indicated that thyrotropin-releasing hormone (TRH) influences the central nervous system (CNS) independent of its action on the hypothalamo-pituitary axis. In addition to its ability to stimulate pituitary TSH secretion, centrally administered TRH produces hyperthermia in rodents [3] by elevating skeletal muscle tonus [6], increasing sympathetic nervous system activity [4], and affecting behavioral activity [15]. The physiological role of TRH in thermoregulation is further supported by the observation that passive immunization against endogenous TRH by anti-TRH sera causes a decrease in body temperature [13]. Brown has suggested that cerebral TRH may initiate a series of coordinated effects when animals are exposed to a cold environment [5].

However, the mechanism whereby TRH influences thermoregulation is not well understood. There are conflicting reports about the changes in the cerebral TRH content in the animals exposed to cold [2,10]. In addition, whether or not exogenous TRH increases the body temperature has been reported to depend on the ambient temperature [12].

We previously reported that the body temperature of the rat can be readily controlled by changing the water temperature in which the animal is immersed [11]. In the present study, we measured the cerebral TRH content in rats subjected to immobilization under different environmental temperatures.

METHOD

Hypothermic and Normothermic Stress

Male Wistar rats (180–220 g) were subjected to hypothermic and normothermic stress as described previously [11]. In brief, animals were kept individually in wire cages (inner size, 18.0×5.0×4.5 cm), each of which was equipped with a springy metallic plate to keep the rat immobilized. Then the cages were immersed vertically in a plastic tank that was filled with water up to the rat's neck. The water temperature was maintained at 38°C for the initial 60 min, after which the animals were divided into 3 groups: the normothermia group (A), in which the animals remained immersed in the water bath of 38°C for another 210 min, the mild hypothermia group (B) and the profound hypothermia group (C). For inducing hypothermia, the rats were placed in a water bath of 30°C (group B) or 20°C (group C) for 120 min, then the water temperature was gradually elevated to 38°C by a thermostat according to the time course shown in Fig. 1B and 1C. The rectal temperature of the rat was monitored continuously. At 70, 120 and 270 min from the beginning of the experiment, the rats in each group were decapitated for the measurement of brain TRH. These points represent, in the hypothermic groups, that the initial stage of hypothermia (e.g., 10 min after the beginning of body temperature drop), the steady-state in hypothermia (e.g., the body temperature reaches to the lowest temperature) and the recovery from hypothermia, respectively. Rats not subjected to cold or immobilization were used as controls. All experiments were started at 10:00 a.m. to avoid possible circadian variations in cerebral TRH contents [10].

Measurement of Cerebral TRH

The brain (excluding the cerebellum) was rapidly removed and dissected on a chilled plastic plate according to the method described by Glowinski and Iversen [8]. It was then homogenized by the use of Polytron in 10 vol. of ice-cold acidified ethanol consisting of equal parts of ethanol and 0.1 N HCl. The homogenate was centrifuged at 15,000×g for

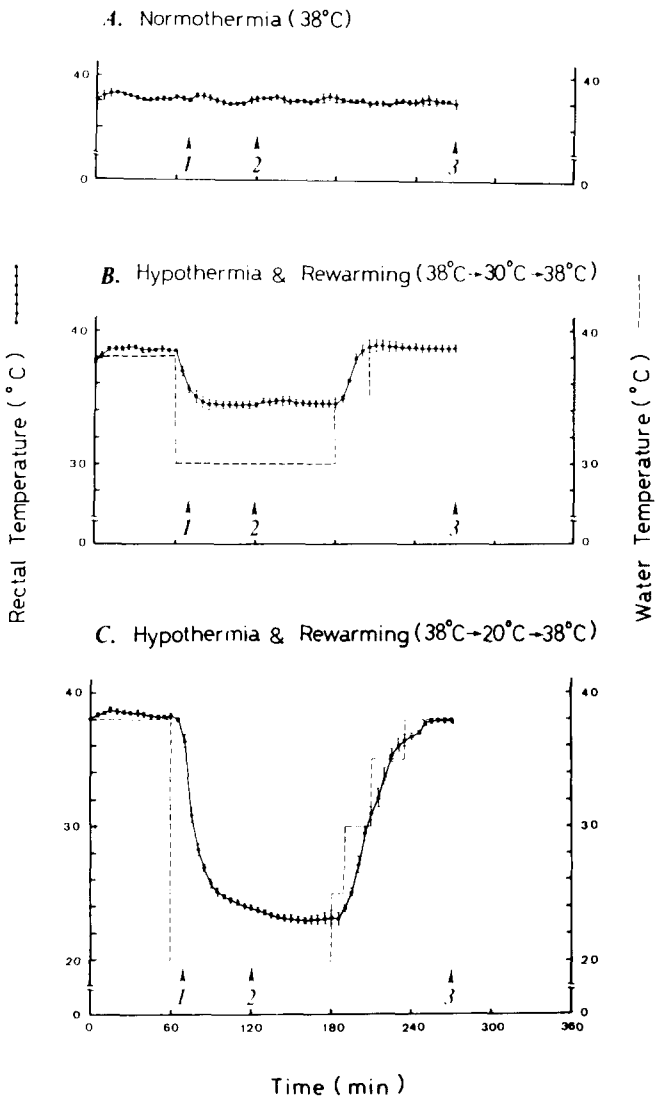

FIG. 1. Changes in rectal temperature in rats restrained and immersed in a water bath at the temperature shown by dashed lines. The rectal temperature was measured every 5 min and each value represents the mean±SEM (n=3). At (1) 70 min (2) 120 min and (3) 270 min from the beginning of the experiment, animals were sacrificed for the determination of brain TRH.

30 min at 4°C. The supernatant was evaporated to dryness by a centrifugal concentrator (cc-180, Tomy Seiko Co., Ltd., Tokyo). The dried supernatant was dissolved in 0.01 M phosphate buffer (pH 7.4)–0.15 M NaCl containing 2% BSA (2% BSA-PBS) immediately before radioimmunoassay (RIA) of TRH. RIA was performed as follows: 100 μl of a serial dilution of standard or sample, 100 μl of a specific TRH-antibody diluted 1:10,000 (final), 100 μl of ^{125}I-TRH (about 30,000 cpm) and 200 μl of 2% BSA-PBS were mixed. The mixture was then incubated for 36 hr at 4°C. Bound and free ligands were separated by means of the double antibody method. The lowest limit for the detection of TRH in the assay was 20 pg/tube. Little cross-reaction was found with Glu-TRH (2.5%), TRH-OH (0.02%) and His-Pro-diketopiperazine (0.001%).

Statistics

Results were expressed as mean±S.E.M. Statistical analysis was performed using one-way analysis of variance followed by Student's *t*-test to determine differences during the experimental period in each group and those between groups.

RESULTS

When the animals were sacrificed for the determination of cerebral TRH at 70, 120 and 270 min from the beginning of the experiment, the rectal temperature in group A (normothermia) was around 38°C, that in group B (mild hypothermia) 34.6±0.4, 34.4±0.2 and 38.7±0.3°C, respectively, and that in group C (profound hypothermia) 34.4±0.5, 24.0±0.2 and 38.0±0.3°C, respectively (Fig. 1).

The analysis of variance revealed no significant changes in the TRH contents over time in any brain region examined in groups A and C (including the control group). However, in group B, the variances were statistically significant in the hypothalamus, cerebral cortex, midbrain and medulla oblongata. In these regions, except for medulla oblongata, the TRH content was significantly decreased at 120 min from the beginning of the experiment, i.e., when their body temperature mildly dropped to about 34°C, as compared to control values and/or those at 70 min. The differences between group B and either A or C group were also significant at 120 min. No significant decrease in the TRH content was observed after the animals were rewarmed. In the medulla oblongata in group B, the TRH content was significantly increased at 70 min, however, it was similar to the control level at 120 min and increased again after rewarming. Similar but not significant changes were observed in groups A (only at 70 min) and C. Thus, there were no significant differences among the three groups at any sampling point in this region (Table 1).

DISCUSSION

The present study demonstrated that the TRH contents in the hypothalamus, midbrain and cerebral cortex decreased during the mild hypothermia and readily increased when the animal was rewarmed but such changes were not observed during the profound hypothermia.

The use of immobilization and immersion in water during induction of the hypothermia may have some effects on the TRH content. To exclude them, the experiment was designed to compare the responses of the hypothermic rats with those of the normothermic ones that were subjected to the same stress except for the changes in the body temperature. In addition, animals in the hypothermic groups were subjected to 60 min of prestress in the water of 38°C before induction of hypothermia (Fig. 1) to separate the induction of the hypothermia from the beginning of the application of immobilization and immersion in water. No significant changes were seen in the TRH contents over time in any brain region examined in the normothermic group (Table 1) or during the prestress period in the hypothermic groups (data not shown). Thus, in this model, although animals are subjected to stress, we can evaluate the specific effect of hypothermia on the TRH content in the brain.

Several studies found no changes in the cerebral TRH content in rats exposed to cold [2,10]. In these studies, freely moving rats were placed in a cold room at about 4°C for 5 to 60 min. We found that animals under similar conditions maintained a normal body temperature for as long as 180 min

TABLE 1

CHANGES IN THE CONTENTS OF TRH IN VARIOUS BRAIN REGIONS IN RATS RESTRAINED AND IMMERSED IN A WATER BATH AT VARIOUS TEMPERATURES

	TRH contents (ng/g wet wt. tissue)			
	Control	1 (70 min)	2 (120 min)	3 (270 min)
Hypothalamus				
A.		100.5 ± 5.8	108.4 ± 5.0 ‡	108.2 ± 11.6
B.	117.3 ± 9.9	100.0 ± 9.9 †	79.0 ± 5.7 ‡ †	87.5 ± 8.6
C.		103.2 ± 12.0	107.9 ± 6.4 †	102.3 ± 8.3
Cerebral Cortex				
A.		4.5 ± 0.8	4.6 ± 0.3 *	4.1 ± 0.7
B.	4.2 ± 0.8	6.5 ± 0.7 ‡	2.8 ± 0.5 *	4.8 ± 0.3 †
C.		4.3 ± 0.4	4.7 ± 0.5 *	4.5 ± 0.5
Midbrain				
A.		8.4 ± 0.4	9.3 ± 1.0 *	9.1 ± 1.7
B.	9.7 ± 0.9	9.5 ± 1.2 *	5.9 ± 0.7 * *	7.8 ± 0.2
C.		8.9 ± 0.9	8.3 ± 0.7 *	9.6 ± 1.0
Medulla Oblongata				
A.		18.1 ± 2.3	14.7 ± 1.7	14.9 ± 2.7
B.	13.6 ± 0.4	19.3 ± 2.0 *	14.4 ± 0.4 *	21.2 ± 1.5 *
C.		15.8 ± 1.4	15.5 ± 2.6	18.9 ± 1.0
Hippocampus				
A.		5.1 ± 1.2	6.5 ± 1.3	7.0 ± 1.4
B.	3.8 ± 1.5	5.2 ± 0.8	4.9 ± 2.1	8.3 ± 1.3
C.		6.2 ± 1.9	7.8 ± 1.4	6.7 ± 1.9
Striatum				
A.		5.4 ± 0.1	6.7 ± 0.9	7.0 ± 0.7
B.	5.7 ± 0.9	6.5 ± 1.0	3.6 ± 1.4	6.9 ± 1.2
C.		6.2 ± 1.9	7.8 ± 1.4	6.7 ± 1.9

A: Normothermia (38°C); B: Hypothermia and Rewarming (38°C→30°C→38°C) and C: Hypothermia and Rewarming (38°C→20°C→38°C). 1, 2 and 3 represent the points at which animals were sacrificed as shown in Fig. 1. Each value represents the mean ± SEM (n=4–6). *$p<0.05$, †$p<0.01$ and ‡$p<0.005$.

(unpublished data). However, in the present study, the body temperature of the animals in the mild hypothermic group was maintained at about 34°C for nearly 120 min (Fig. 1B). The cerebral TRH content seems to decrease only when the body temperature has been lowered by the cold exposure. However, there is direct evidence of TRH release in the median eminence during exposure to cold (4°C in a cold room for 40 min) [1]. In the cold-exposed normothermic animals, the decrease of the TRH store in nerve terminals may be quickly compensated for by accelerated biosynthesis by a homeostatic mechanism [9]. If so, there may be no apparent changes in the cerebral TRH content. On the other hand, such a compensatory mechanism for TRH synthesis may be suppressed during the mild hypothermia, leading to a decrease in the TRH content.

Although there was no significant change in the cerebral TRH contents in the profound hypothermia group, it was found, in a preliminary immunohistochemical study, that TRH-positive nerve terminals in some periventricular regions of hypothalamus, such as in the paraventricular nucleus and the dorsomedial nucleus, were greatly reduced in the profound as well as in the mild hypothermic groups as compared with that in the normothermic group (unpublished observation). This suggests that TRH is involved also in the profound hypothermia, at least in the hypothalamus.

During the profound hypothermia, the thermoregulatory

mechanisms seem to be suppressed further. The ability to spontaneously return to normal temperature is lost at around 28°C [7]. This temperature was attained at about 20 min after the initiation of the profound hypothermia as shown in Fig. 1C. Below this temperature, TRH release and, probably biosynthesis and degradation as well, may be greatly suppressed and the cerebral TRH contents may remain relatively unchanged as a whole. In addition, hypothermia does not appear to change the turnover of neuropeptides in a nonspecific manner since neither methionine enkephalin- nor substance P-positive neuronal structures were affected by the mild nor the profound hypothermia (unpublished observation).

Multiple thermoregulatory systems are thought to exist in the brain, and the hypothalamus is at the top of the hierarchically arranged system [14]. Considering the various thermoregulatory effects of exogenously administered TRH [3–6, 15], extrahypothalamic (such as in midbrain and cerebral cortex) as well as hypothalamic TRH may both play a role during the hypothermia.

On the other hand, in medulla oblongata, a change in TRH contents was seen at the initial stage of hypothermia (at 70 min) and after rewarming (at 270 min), but not during steady-state hypothermia (at 120 min). A similar but not significant change was also observed in the normothermic and the profound hypothermic groups. The animal shows distressful behavior and a rise in blood pressure (unpublished observation) especially soon after being immersed in water, and in the hypothermic groups, during the rewarming process. Considering the role of the medulla oblongata as the center for the autonomic reflex control of the circulation, heart and lung [7], the change in TRH contents in this region seems to be related to the general response to stress. To further investigate the functional relevance of this peptide, a comparative morphological and biochemical analysis of TRH in some brain nuclei of the hypothermic rat is now in progress.

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