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Alteration of the Turnover of Dopamine and 5-Hydroxytryptamine in Rat Brain Associated With Hypothermia

CHIEKO OKUDA, AKIKO SAITO, MASAO MIYAZAKI AND KINYA KURIYAMA*[1]

*Departments of Anesthesiology and *Pharmacology, Kyoto Prefectural University of Medicine Kamikyo-Ku, Kyoto 602, Japan*

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OKUDA, C., A. SAITO, M. MIYAZAKI AND K. KURIYAMA. *Alteration of the turnover of dopamine and 5-hydroxytryptamine in rat brain associated with hypothermia.* PHARMACOL BIOCHEM BEHAV **24**(1) 79–83, 1986.—The alteration of monoamines and their metabolites in the brain in response to hypothermia was studied using rats subjected to a cold and immobilization stress. The experiments were designed to compare the responses in the "hypothermal" rats with those in the "normothermal" ones which received the same stress except for the change in body temperature. It has been found that the contents of norepinephrine (NE) and 5-hydroxytryptamine (5-HT) in various cerebral regions were significantly decreased during hypothermia. These decreases were readily reversed by the rewarming of animals. Moreover, the increase in the content of 5-hydroxyindole-3-acetic acid (5-HIAA), the metabolite of 5-HT, was also detected in some cerebral regions where the decrease of 5-HT was observed. Although the dopamine (DA) contents in all cerebral regions examined were found to be unaltered, its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and/or homovanillic acid (HVA) contents in most regions in the brain showed a significant elevation during and/or after the occurrence of hypothermia. These results suggest that the metabolic turnovers of 5-HT and DA in various cerebral regions may be accelerated during hypothermia.

Hypothermia Cerebral monoamines Dopamine turnover 5-HT turnover Stress

VARIOUS stressors, including hypothermal stress, are known to induce the alteration in the metabolism and/or turnover of monoamines in the central nervous system (CNS). The decrease in cerebral contents of norepinephrine (NE) and epinephrine (EPI) has been demonstrated during cold swim stress [15,18] and cold and immobilization stress [11]. These changes have been thought to reflect the increased turnover of NE and EPI [18]. The alteration in the turnover rates of dopamine (DA) and 5-hydroxytryptamine (5-HT) has been also observed in these animals [11, 15, 18]. The reports on the latter alteration induced by cold and immobilized stress, however, are more conflicting in the direction of the changes and are less clear about the physiological significance as compared with the cases of NE and EPI. Similarly, controversial results have been reported with regard to the changes in DA and 5-HT in the brain following the application of other type of stress such as by footshock [8, 9, 20] and by immobilization [4,13].

It has been reported that the response of central monoaminergic systems, especially DA and 5-HT neurons, to stress depends on the type, severity and/or duration of stress [10]. This fact strongly suggests that it is necessary to employ animal models to examine the particular factors of stress.

The rapid change of body temperature has been known to be stressful. Although artificial hypothermia has long been employed clinically as a mean of protecting the CNS from the damage due to hypoxia, little attention has been paid on the effect of hypothermia itself on the central neuronal functions. In this study, we have used animals subjected a cold and immobilization stress [11] and have analyzed the effect of body temperature changes on the activities of cerebral monoaminergic neurons. To distinguish the stress caused by the change of body temperature from other stress such as immobilization and being immersed into water, the experiment was designed to compare the responses of the "hypothermal" animals with those in the "normothermal" ones which received the same stress except for the changes in the body temperature, by being immersed in water of 38°C.

METHOD

Application of Hypothermal and Normothermal Stress

Male Wistar rats (180–220 g) were housed under the condition of controlled lighting (lights on at 7:00 a.m. and off at 6:00 p.m.) and given free access to food and water. All experiments were started at 10:00 a.m. in order to avoid possible

[1]Requests for reprints should be addressed to Dr. Kinya Kuriyama, Department of Pharmacology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamikyo-Ku, Kyoto 602, Japan.

circadian variations in the functional status of central monoamine neurons. Animals were divided into two groups, a "hypothermal" group and a "normothermal" group. Both groups of rats were confined individually in metallic restraint cages and were immersed in water bath, as described previously [10, 11, 23]. The water temperature was maintained at 38°C for the initial 60 min in order to give the same stress without body temperature change. Subsequently, rats of the hypothermal group were transferred into the water bath of 20°C and were kept in it for 120 min, then the water temperature was gradually elevated to 38°C by the use of a thermostat according to the schedule which took 90 min. The normothermal group continued to be immersed in water of 38°C for the same period as that applied to the hypothermal group (total immersion at 38°C was 270 min). Body temperature of the rat was monitored with thermistor probe inserted 6 cm into the rectum and fixed by binding on the tail with thread, and it was recorded by a thermometer (Thermo-Finer, Terumo Co., Tokyo, Japan) during each experiment. Following the application of such stress, one part of animals in both groups were sacrificed by focussed microwave irradiation (5 KW for 0.7 sec) for the determination of cerebral monoamines, and other animals were killed by decapitation to collect trunk blood for the measurement of plasma level of corticosterone, respectively. Sampling points were 70 min, 120 min, and 270 min from the beginning of the experiment. These points represented, in the hypothermal group, 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 reached to the lowest temperature), and the recovery from hypothermia, respectively.

Measurement of Cerebral Monoamines and Their Metabolites

The brain was dissected on a chilled plastic plate, according to the method described by Glowinski and Iversen [7], homogenized by the use of Polytron in 2.5 vol of ice-cold 0.05 M perchloric acid which contained a calibrated amount of 3,4-dihydroxybenzylamine (DHBA) as an internal standard, and centrifuged at 48,000 g for 20 min at 4°C. The resultant supernatant was stored at −80°C and was subjected to the determination of monoamines and their metabolites within one week. The contents of DA and its metabolites (3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)), 5-HT and its metabolite (5-hydroxyindole-3-acetic acid (5-HIAA)), and NE were determined by ion-pairing reverse phase HPLC with electrochemical detection, according to the method of Wagner *et al.* [22] with some simplifications.

Measurement of Plasma Corticosterone

The trunk blood was collected into ice-cold heparinized test tubes. The plasma was separated after centrifugation and stored at −80°C until measurements. Corticosterone levels were determined fluorometrically by the method of Mattingly [14] with a slight modification.

Statistics

Results were expressed as mean±S.E.M. and the statistical significance was determined by Student's *t*-test.

RESULTS

Changes in Rectal Temperature and Behavior in Hypothermal and Normothermal Rats

Rectal temperatures of rat were satisfactorily controlled by changing water temperatures. At the points of sacrificing rats for the determination of cerebral monoamines, i.e., 70 min, 120 min and 270 min from the beginning of the experiment (see the Method section), the rectal temperatures of the hypothermal rats were 34.4±0.5, 24.0±0.2 and 37.9±0.1°C, whereas those of the normothermal group were 38.1±0.1, 38.3±0.1 and 38.0±0.3°C, respectively. The body temperature of control rats (without any stressful manipulations) was 38.1±0.1°C. In preliminary experiments, it was found that a progressive drop of body temperature caused gradual muscle relaxation, and finally the loss of righting reflex became evident when the rectal temperature reached to approximately 21°C. These changes in behavior and in vital signs induced by the hypothermia were completely restored as soon as

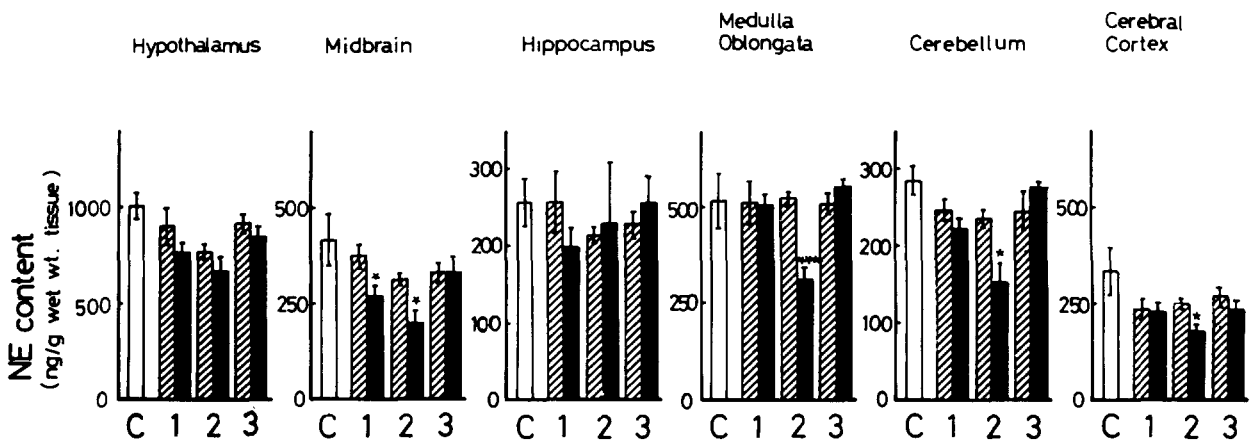

FIG. 1. Changes in the content of NE in various brain regions in rats of control (white columns), normothermia (hatched columns) and hypothermia (black columns) groups. Normothermal rats were restrained and immersed in a water bath of 38°C throughout the experiments. The water temperature for hypothermal rats was changed from 38°C (for 60 min) to 20°C (for 120 min) and again gradually returned to 38°C (for 90 min). The rats were sacrificed by focussed microwave irradiation at the points of 1, 70 min; 2, 120 min; and 3, 270 min from the beginning of the experiment, respectively. Each value represents the mean ±SEM obtained from 4 to 5 separate experiments. *$p<0.05$; ***$p<0.005$, compared with each value in the normothermal group.

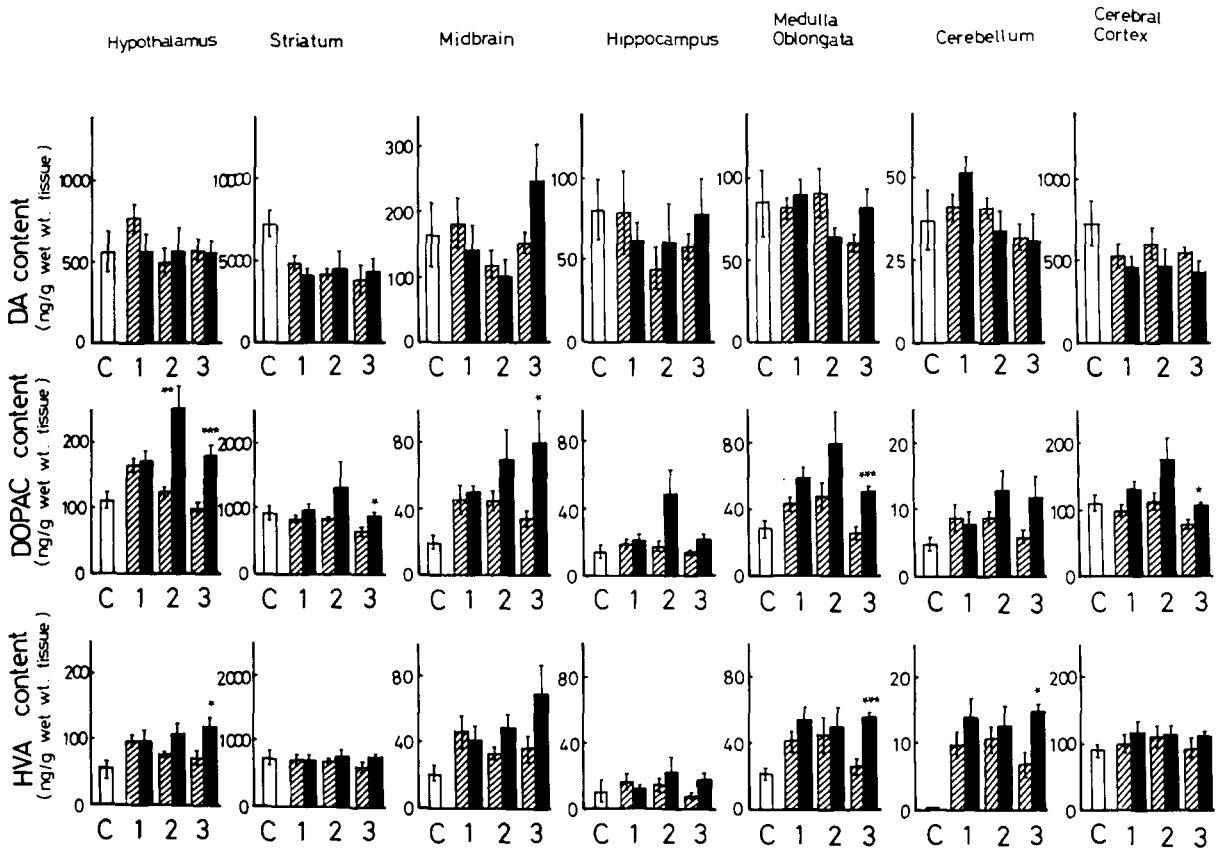

FIG. 2. Changes in the contents of DA, DOPAC and HVA in various brain regions in rats of control (white columns), normothermia (hatched columns) and hypothermia (black columns) groups. For details, see the legend of Fig. 1. Each value represents the mean±S.E.M. obtained from 4 to 5 separate experiments. *$p<0.05$; **$p<0.01$; ***$p<0.005$, compared with each value in the normothermal group.

their body temperatures became normal. In the present experimental conditions, it was found, however, that no complete recovery was obtained if body temperature of these animals exhibited less than 19°C.

Effect of Changes in Rectal Temperature on the Content of Monoamines and Their Metabolites in Various Cerebral Regions

As shown in Fig. 1, NE contents in the midbrain, medulla oblongata, cerebellum and cerebral cortex showed a significant decrease during hypothermia as compared with those in normothermal rats. These decreases were, however, completely reversed by the rewarming of animals. The most rapid change in NE content was seen in the midbrain, where the decrease of NE appeared at the initial stage of hypothermia.

Hypothermia did not induce a significant change in DA contents in all brain regions examined, while DOPAC and HVA contents showed significant increases in most brain regions following the recovery from hypothermia. In the hypothalamus, there was a significant increase in DOPAC during hypothermia, and it tended to restore to the control value after the rewarming. Such a tendency was also seen in other brain regions, such as the striatum, hippocampus, medulla oblongata and cortex (Fig. 2).

The 5-HT content showed a decrease during hypothermia in the midbrain, medulla oblongata and cerebellum, and it was also returned to the control level after rewarming. In the midbrain and cerebellum, the decrease in 5-HT occurred at the initial stage of hypothermia. On the other hand, the content of 5-HIAA showed an increase during and after hypothermia in all brain regions examined except the cerebellum (Fig. 3).

Effect of Changes in Rectal Temperature on the Plasma Level of Corticosterone

The plasma corticosterone in the "hypothermal" rats showed higher levels than those in the "normothermal" ones in all stages examined such as the initial stage, the steady-state of hypothermia and also after the rewarming (Fig. 4), respectively.

DISCUSSION

It was demonstrated that the NE and 5-HT contents in various cerebral regions decreased significantly during hypothermia. These decreases were readily reversed by the rewarming of animals. In the case of 5-HT, the decrease during hypothermia was accompanied by an increase in 5-HIAA contents in the midbrain and medulla oblongata following the recovery from hypothermia. In other cerebral regions except the cerebellum, an increase of 5-HIAA was also

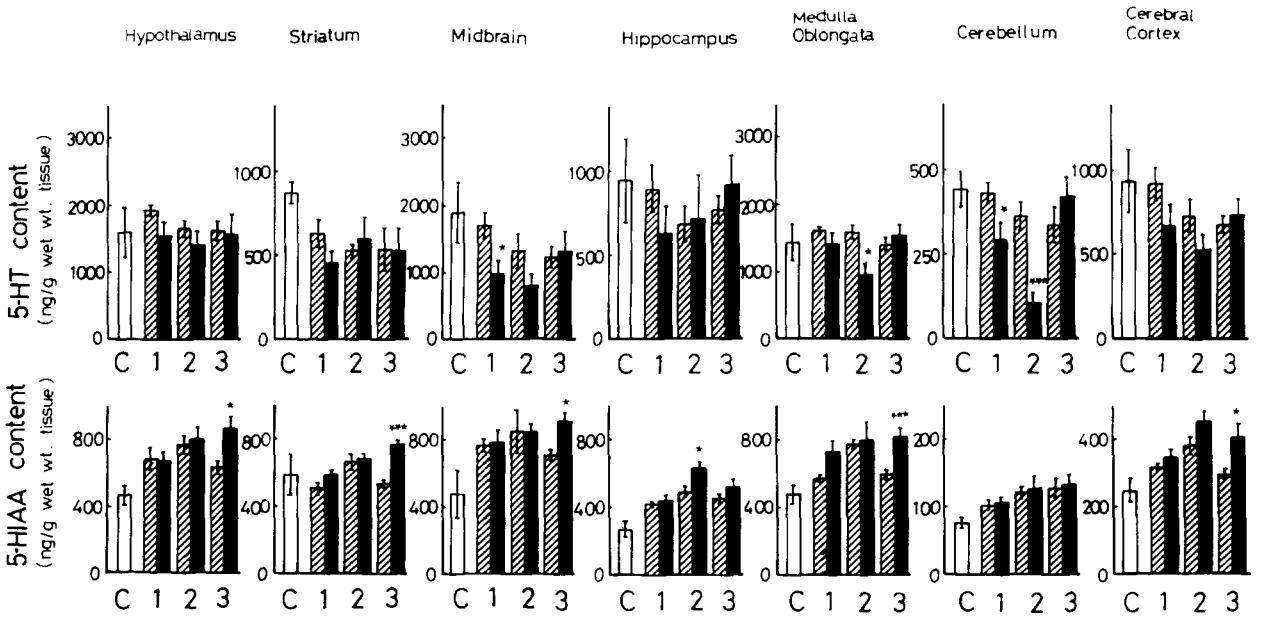

FIG. 3. Changes in the contents of 5-HT and 5-HIAA in various brain regions in rat of control (white columns), normothermia (hatched columns) and hypothermia (black columns) groups. For details, see the legend of Fig. 1. Each value represents the mean±SEM obtained from 4 to 5 separate experiments. $*p<0.05$; $***p<0.005$, compared with each value in the normothermal group.

observed, although the contents of 5-HT did not change significantly. On the other hand, DA contents in all cerebral regions were unaffected, but DOPAC and HVA contents showed an elevation during and after hypothermia. These results suggest that the metabolic turnovers of 5-HT, DA and probably NE in various cerebral regions may be accelerated in hypothermal rats. It is also likely that the increase in the turnover rates of these monoamines may be induced not only during the cooling process but also during the rewarming process.

There is no doubt that hypothermia, especially at the initial stage of hypothermia, is stressful to these animals, because the behavior of the "hypothermal" rats is more distressful than that of the "normothermal" ones. More struggling, vocalization, increased defecation and urination, in addition to a higher plasma corticosterone level, were observed in the "hypothermal" animals. Such a stress-induced response has been also reported in rats given corticotropin-releasing factor (CRF) intracerebroventricularly [2]. Moreover, the activation of noradrenergic neurons in the locus coeruleus was reported to occur when CRF was administered [21]. Considering these findings along with the present observations, it seems likely that the activation of cerebral NE neurons in hypothermal rats relates, at least in part, to the general response to stress, as found in animals received various other types of stress [4,6].

At the steady-state of hypothermia, animals became relatively inactive. It seems unlikely that NE neurons are still activated at this stage. It is difficult, however, to determine whether or not the activities of NE neurons are actually enhanced at this stage, because it has been reported that there is a time lag between the duration of stress and the initiation of stress-induced alteration in NE content in various regions in the brain [19]. Although similar delay may exist in the cases of DA and 5-HT, it is noteworthy that the

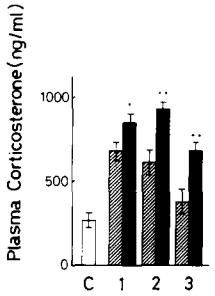

FIG. 4. Changes in plasma level of corticosterone in control (white columns), normothermia (hatched columns) and hypothermia (black columns) groups. The rats were sacrificed by decapitation and the trunk blood was collected. For other details, see the legend of Fig. 1. Each value represents the mean±SEM obtained from 5 to 6 separate experiments. $*p<0.05$; $**p<0.01$, compared with each value in the normothermal group.

increase in DOPAC, an intermediate metabolite of DA, has been observed only after the steady-state of hypothermia, and this increase in most cerebral regions examined has been eliminated after rewarming of animals. These results strongly suggest that the activity of DA neurons may be enhanced during hypothermia.

The physiological role of monoamines in the thermoregulation has been well documented in the hypothalamus, particularly in the preoptic area. Although there are a number of controversial reports, it has been suggested from pharmacological studies that the stimulation of cold receptors may induce the release of 5-HT, whereas the stimulation of warm receptors is thought to cause NE release [3]. DA agonists are also known to cause hypothermia, probably due

to a downward shift in the thermoregulatory set point [1]. It has been also pointed out that not only the hypothalamus but also various brain regions are involved in the central mechanism of thermoregulation [16]. The thermoregulatory mechanism is expected, however, to be operable only for a short period, since it has been reported this mechanism is eliminated easily when the rectal temperature drops below 28°C [5]. Since hypothermal animals used in this study achieved such a hypothermal state within 20 min after the initiaton of cooling, it is unlikely that the observed changes in the function and metabolism of monoamines are involved in the operation of thermoregulatory mechanism during the steady-state in hypothermia.

On the other hand, it has been reported that stress-induced hyperglycemia promotes insulin release, which is in turn to stimulate monoamine release, mediated by insulin receptors in the CNS [17]. Therefore, it seems of interest to further investigate the correlation between glucose and/or insulin levels and these changes of monoamines in the brain during hypothermia. Likewise, the metabolism of cerebral γ-aminobutyric acid (GABA) has been also reported to be altered by the cold and immobilization stress [23]. Since GABA and muscimol, a GABA agonist, have been known to stimulate [^{3}H]DA release in caudate nuclei [12], a possible presence of the alteration of DA turnover secondary to the change of GABAergic neurons may not be ruled out.

In summary, the effect of hypothermia was explored in stressed animals and it was compared to that found in normothermal but identically stressed ones. In this model, the increased turnovers of DA and 5-HT in various cerebral regions were observed during and/or after the occurrence of hypothermia. If hypothermia is applied to non-stressed or mildly stressed animals, it may have different effects on monoaminergic systems in the brain. This fact seems one of the reasons for the controversial results obtained in various models for hypothermal stress. The present experimental design seems adequate to examine neurochemical changes associated with hypothermia in animals bearing various stresses.

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