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Brain Serotonin Metabolism in Hibernation

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POPOVA, N. K. AND N. N. VOITENKO. *Brain serotonin metabolism in hibernation.* PHARMAC. BIOCHEM. BEHAV. 14(6) 773–777, 1981.—It has been shown that notwithstanding 2-fold decreased monoamine oxidase (MAO) activity in brain of hibernating ground squirrels *(Citellus erythrogenys major, Brandt)*, serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels in most of the brain areas studied were not significantly different from the ones in active ground squirrels. However, marked changes were revealed in 5-HT and 5-HIAA brain level in entering hibernation (body temperature 11–9°C) and arousing (body temperature 22°C) animals. In entry into hibernation an increase in brain 5-HT, decrease in 5-HIAA level and lowered MAO activity was found. In arousal from hibernation 5-HT was decreased, 5-HIAA was increased and MAO activity was found to be increased to the level of the active ground squirrels.

Brain serotonin Monoamine oxidase Hibernation

IT IS now generally accepted that in spite of being unique, natural hibernation is accomplished by physiological mechanisms which also function in non-hibernators [7].

There is some evidence suggesting an involvement of serotonin (5-HT) in the control of hibernation [5, 6, 9, 10, 14, 16]. However, the literature on brain 5-HT in hibernation is contradictory. Some investigators reported increased [6, 8, 16], decreased [14], or unchanged [17], brain 5-HT during hibernation, although the latter authors found an increase of tryptophan hydroxylase activity, the rate-limiting enzyme for 5-HT synthesis, in brainstem of hibernating woodchuck.

The discrepancy may be due to differences in the brain areas and in the phases of hibernation studied. It has been previously shown that the changes were significant only in some brain areas [6] and they were especially pronounced at critical time points such as entry into hibernation and arousal from hibernation [11].

The present study was undertaken to evaluate brain 5-HT metabolism in hibernation. For this purpose 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) levels and monoamine oxidase (MAO) activity were determined. Special attention was paid to the entry into hibernation and arousal marked by drastic changes in behaviour and the state of physiological systems.

METHODS

Animals

Males of red-cheeked ground squirrels *(Cittelus erythrogenys major, Brandt)* weighing 250–300 g were used. These animals, widespread in West Siberia, are the typical representatives of seasonal hibernators. Their hibernation period lasts up to 7 months, during which ground squirrels neither eat nor drink.

The captured animals were individually caged and, in autumn, they entered into natural hibernation in a special chamber with constant temperature (2–3°C) and housing conditions similar to natural ones. The body temperature during the entry into hibernation gradually lowered and in hibernating animals the "core" temperature was near 4°C. While hibernating, the ground squirrel took a typical hibernating posture with tail and feet curled around the head. The cardiac and breathing rates were very slow.

The experiments were performed in active ground squirrels with body temperature of about 37°C; in animals entering hibernation (body temperature 11–9°C), in hibernating ground squirrels (body temperature 4–5°C); and in animals in a state of partial arousal (body temperature 22°C). The arousal was provoked by transferring the animals to a room with an ambient temperature of 21–22°. The animals were decapitated when their body temperature increased to 22°. At this time restoration of the orthostatic reflex was noted, they turned over, paws down, but their eyes remained closed.

Procedure

After a quick decapitation the brain of ground squirrel was immediately removed. The hippocampus, hypothalamus, hindbrain and midbrain were rapidly excised, weighed and placed into test-tubes on ice. 5-HT and 5-HIAA were determined fluorometrically [12] using a "Hitachi" spectrofluorometer. For the determination of 5-HT catabolism by MAO [1], brain regions studied were homogenized in 9 volumes of Krebs-Ringer phosphate solution with pH 7.4. The preincubation of homogenates was made for 30 min at the temperature adjusted to body temperature of the animal. The experiments were performed with the amount of homogenate corresponding to 100 mg of brain tissue, to which 4 μg of 5-HT were added. The incubation was carried out at 7°, 22°

TABLE 1

EFFECT OF TEMPERATURE ON MAO ACTIVITY IN DIFFERENT PHYSIOLOGICAL STATES OF GROUND SQUIRRELS

State of animals and body temperature, C°	Incubation temperature C°	Number of experiments	MAO activity in μg of deaminated 5-HT per 100 mg of brain tissue wet weight for 15 min (Mean ± SE) Midbrain	Hypothalamus	Hippocampus
Active	37°*	5	3.12 ± 0.10	3.23 ± 0.09	2.87 ± 0.06
state,	22°	5	2.39 ± 0.24	2.62 ± 0.12	2.41 ± 0.17
36.5–37°	7°	5	1.96 ± 0.15	2.07 ± 0.06	2.01 ± 0.09
Entrance into	37°	5	3.11 ± 0.10	3.46 ± 0.02†	3.05 ± 0.02†
hibernation,	22°	5	2.41 ± 0.06	2.80 ± 0.05	2.18 ± 0.01
11–9°	7°*	5	2.48 ± 0.07†	2.77 ± 0.07†	2.28 ± 0.01†
Hibernation,	37°	8	3.47 ± 0.10†	3.69 ± 0.08†	3.65 ± 0.07†
3–4°	22°	8	2.81 ± 0.08†	3.22 ± 0.07†	2.77 ± 0.09
	7°*	8	1.70 ± 0.16	1.54 ± 0.12†	1.58 ± 0.09†
Arousal,	37°	4	3.71 ± 0.03†	3.79 ± 0.03†	3.68 ± 0.02†
22°	22°*	4	2.57 ± 0.21	3.07 ± 0.10†	2.74 ± 0.31
	7°	4	1.56 ± 0.07†	1.59 ± 0.25†	1.38 ± 0.30

*"Real" MAO activity corresponding to the body temperature in this phase of hibernation.
†$p<0.05$ vs correspondent incubation temperature in active state.

and 37°C with shaking in a Warburg apparatus for 15 min. The reaction was stopped by addition of 1 N perchloric acid. MAO activity was evaluated by the quantity of 5-HT metabolized and expressed in μg of metabolized 5-HT per 100 mg of wet tissue weight for 15 min. The Student t-test was applied to test the significance of the difference between mean values of different groups.

RESULTS

It was found that 5-HT and 5-HIAA levels in examined regions of the brain in awake active ground squirrels were similar to those in the nonhibernators' brain. 5-HT to 5-HIAA ratio was close to 1, which corresponds to values found in rats [3].

Studies on the entry to hibernation revealed marked changes in 5-HT level and catabolism. The beginning of the hibernation was characterized by an increase in brain 5-HT level, and by significant decrease of 5-HIAA content in all regions of the brain studied (Fig. 1). The most pronounced changes were found in the hippocampus. 5-HT/5-HIAA ratio in hippocampus was elevated by 6 times (0.7 in active ground squirrels and 4.3 ratio in animals entering hibernation). In the rest of the areas of the brain studied there were similar changes, although less pronounced as compared to hippocampus.

MAO activity in ground squirrels entering hibernation was lowered in comparison with that in awake animals. The MAO activity at 7°C, i.e., close to the body temperature of ground squirrels entering hibernation, decreased by 21% in the midbrain and hippocampus and by 15% in the hypothalamus (Table 1). Potential activity of this enzyme, i.e., the activity measured at 37°C, in hippocampus and hypothalamus of ground squirrels entering hibernation was higher than that in active ones. At the same time at the temperature of brain homogenates incubation of 22°C MAO activity was not different from the "real" one (at 7°C) in all brain regions under study. Therefore a marked decrease in temperature dependence of 5-HT oxidative deamination in the ground squirrel's brain was found to be within the limits of low temperatures (22–7°C) during entry into hibernation.

In a deep hibernation, 5-HT and 5-HIAA brain concentrations were not significantly different from the values obtained in active euthermic ground squirrels, although in the hypothalamus of hibernating animals 5-HT level was slightly increased (0.71 ± 0.04 μg/g in active state and 0.88 ± 0.05 μg/g, $p<0.05$, in hibernating ground squirrels). However, in the study of 5-HT metabolism in hibernating animal brain homogenates it was found that the "real" MAO activity (temperature of incubation 7°C) was lowered. These values of MAO activity were twice as low as the "real" enzyme activity in active ground squirrels. Potential MAO activity in hibernating ground squirrels (temperature of incubation of brain homogenates 22° or 37°C) exceeded the "real" one and also was found to be significantly higher than that in active animals at corresponding temperatures (Table 1).

In arousal from hibernation changes in 5-HT and 5-HIAA content quite opposite to those which took place in entry to hibernation were revealed. It was found that 5-HT was decreased and 5-HIAA concentration in brain increased (Fig. 2). Especially significant changes of 5-HT metabolite were found in the hypothalamus. The "real" level of MAO activity (at 22°C) in arousal was increased and was found to be similar to the active ground squirrel's "real" (at 37°C) level of enzyme activity (Table 1).

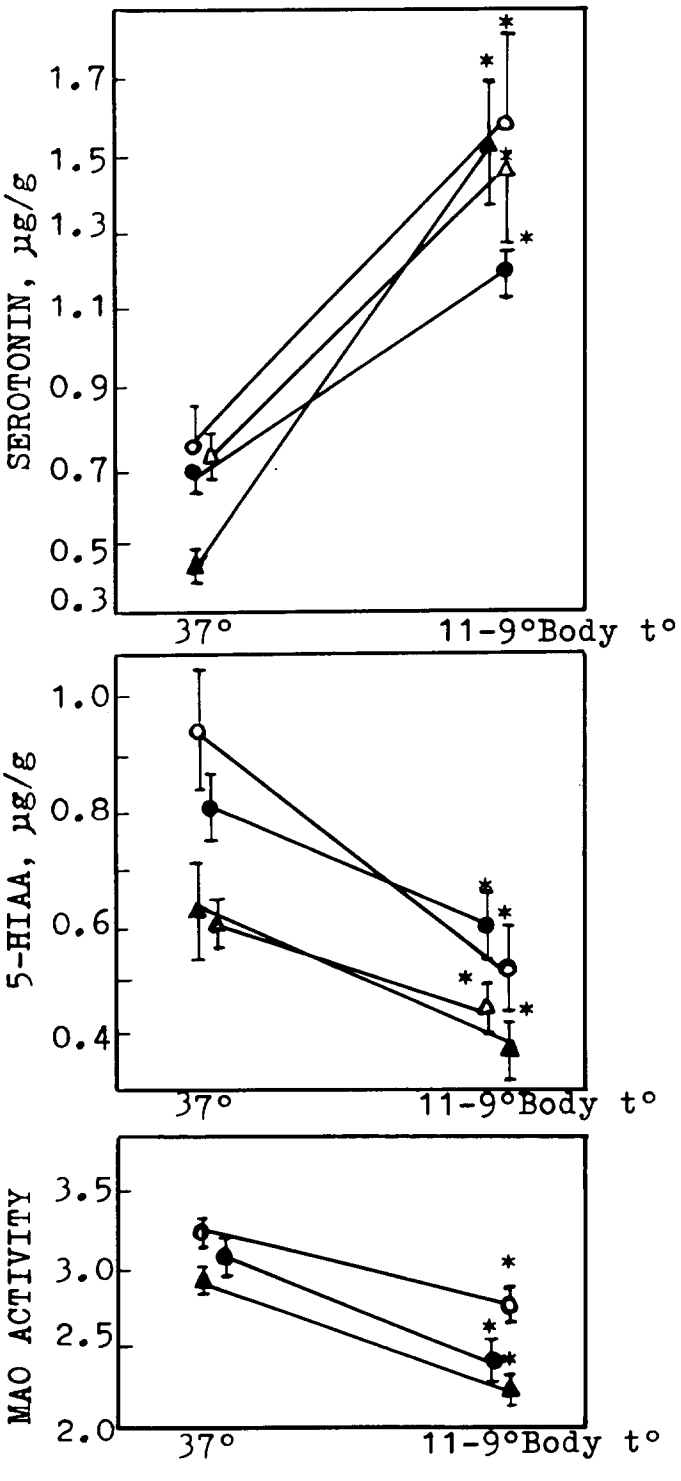

FIG. 1. Changes in brain serotonin, 5-hydroxyindoleacetic acid (5-HIAA) levels and monoamine oxidase (MAO) activity in entering hibernation ground squirrels. Groups of 7 ground squirrels were decapitated in active state in summer (Mean body t° 37°C) and in October when during entry to hibernation their body t° lowered to 11–9°. MAO activity was evaluated in μg of deaminated serotonin per 100 g of brain tissue weight after incubation for 15 min (at 37° for active animals and at 7° for entering hibernation). ○—hypothalamus, ●—midbrain, ▲—hippocampus, △—hindbrain. *$p<0.05$ vs active state (body t° 37°).

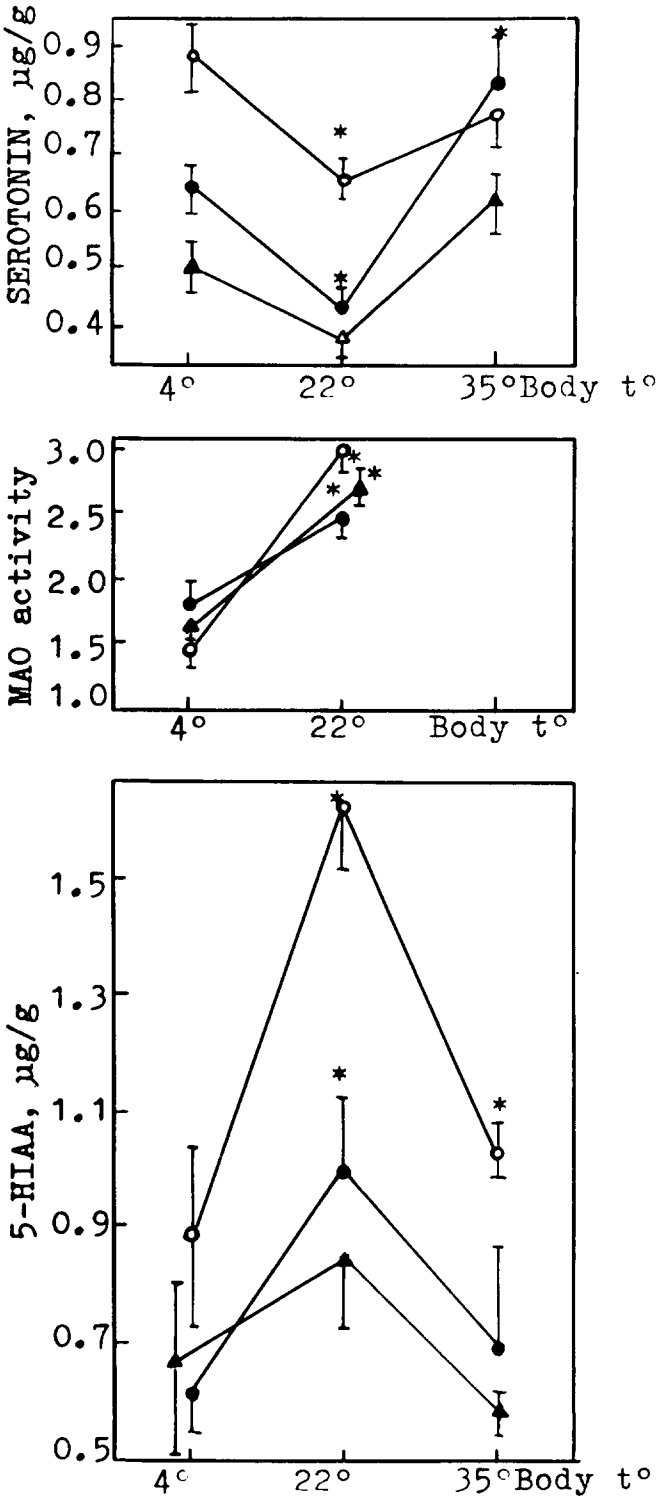

FIG. 2. Changes in brain serotonin, 5-hydroxyindoleacetic acid (5-HIAA) levels and monoamine oxidase (MAO) activity during arousal from hibernation. Groups of 5 ground squirrels were decapitated in April in hibernation (body t° 4°C) and after transferring them into the warm room (t° 21–22°C) when their body temperature had increased to 22° and 35°C. MAO activity was determined after incubation of brain of hibernating animals at 7°C and arousing (body t° 22°) at 22°C. ○—hypothalamus, ●—midbrain, ▲—hippocampus. *$p<0.05$ vs previous body temperature.

DISCUSSION

Therefore, it has been found, that the entry into hibernation, the hibernation itself, and the arousal are considerably different with respect to 5-HT brain metabolism. The entry to hibernation is associated with 5-HT storage in some areas of brain. The increase of 5-HT content in hippocampus and hindbrain has been previously shown [6] in ground squirrels with the body temperature lowered to 16–15°C. In a deeper hypothermia with body temperature of 11–9°C, as it was in the present study, the elevation of brain 5-HT was more pronounced and some significant changes also appeared in the midbrain and hypothalamus.

The increase in 5-HT level was combined with the lowered brain MAO activity and decreased 5-HIAA concentration. Taking into consideration that MAO-induced oxidative deamination of 5-HT, with 5-HIAA as the end breakdown product, is the main pathway of 5-HT catabolism [15], it is reasonable to conclude that the elevation of brain 5-HT is due, at least partially, to the decline of 5-HT degradation.

Thus, the beginning of the hibernation is characterized by 5-HT storage in some areas of the brain depending, to a certain extent, on decreased MAO-induced oxidative deamination of 5-HT. The increase of 5-HT may be due, along with the fall of MAO activity, to some other factors, such as intensified 5-HT uptake and binding or synthesis of this amine.

The relatively temperature-independent MAO activity in ground squirrels entering hibernation was found within the low temperatures (22°–7°C). This phenomenon of temperature compensation of metabolism has been described in poikilothermic vertebrates [4] and possibly reflects the adaptation of metabolism to dramatically changed temperatures.

The changes in 5-HT metabolism evidently are not a simple result of the lowered body temperature. First, as it was previously shown [6], the increase in 5-HT level in animals entering hibernation is consederable only in some regions of the brain. Moreover the 5-HT and 5-HIAA changes found were of different intensity in different regions. The most marked changes were seen in hippocampus where 5-HT/5-HIAA ratio increased 6-fold. In other areas of the brain stem the changes were less pronounced, but nevertheless distinct enough. In the hypothalamus 5-HT/5-HIAA ratio increased 3.6-fold, and in the midbrain and hindbrain 2–2.5-fold. It is probable that dissimilar 5-HT metabolism changes in various regions reflect dissimilar functional activity of these brain areas and their different role in the control of natural hibernation and arousal. Attention is drawn to the data [13] demonstrating that the hippocampus belongs to regions with neuronal activity maintained even in deep hibernation.

While hibernation deepened and body temperature fell to 4–3°C, the changes in 5-HT and 5-HIAA content were not intensified but stabilized. Although MAO activity decreased, 5-HT and its metabolite levels in the brain were not so different from the respective values in awake ground squirrels. Thus, hibernation evidently has been characterized by well balanced 5-HT metabolism notwithstanding 2-fold decreased MAO activity.

Our findings of lowered MAO activity are not in agreement with the data presented previously [8]. These authors reported that in golden hamsters the MAO activity during hibernation was similar to that in awake animals. This statement needs a certain correction, since in these experiments MAO activity was determined in brains incubated at 37°C, which was at the temperature non-conformable to the body temperature of hibernating animals. Therefore, these data are evidently indicative of a potential enzyme activity rather than of real activity in hibernation. As it was shown in Table 1 potential brain MAO activity in hibernating ground squirrels is even higher than that in awake ones. At the same time the "real" activity of the enzyme under these conditions is lowered.

The arousal is associated with an increase in MAO activity, a drop in 5-HT level and rise of its metabolite concentration. However, it seems that this activation of 5-HT metabolism is not a result of functional activation of the serotoninergic system. It has been shown that exogenously administered 5-HT or its precursor 5-hydroxytryptophan inhibited the process of arousal [9,10]. On the contrary, the decrease of forebrain 5-HT level as a result of midbrain raphe nuclei lesions or pretreatment with p-chlorophenylalanine evoked the arousal from hibernation [14]. Therefore, it may be concluded from these and present results that the increase of brain serotonin level is the condition necessary for the entry into hibernation while its decrease is necessary for arousal.

In this connection, attention is attracted to the supposition [2] that it is not only functionally active, but also functionally inactive 5-HT that could be metabolized to 5-HIAA. It may be supposed that the decrease of 5-HT level and increase of 5-HIAA level during arousal is due to intraneuronal metabolism of non-functional brain 5-HT, thus leading to the deficiency of 5-HT and diminished functional activity of 5-HT dependent neurons.

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