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Serotonin in the Control of the Sleep-Like States in Frogs

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POPOVA, N. K., I. I. LOBACHEVA, I. G. KARMANOVA AND N. V. SHILLING. *Serotonin in the control of the sleep-like states in frogs.* PHARMACOL BIOCHEM BEHAV 20(5) 653–657, 1984.—It has been established that brain serotonin (5-HT) is involved in the control of behavioral phenomena in amphibians. The transition of frogs from active wakefulness to sleep-like states was accompanied by alteration of brain 5-HT metabolism, i.e., in sleep-like state with plastic muscle tone 5-hydroxyindoleacetic acid (5-HIAA) level was reduced and in sleep-like state with rigid muscle tone both brain 5-HIAA and 5-HT were decreased. An experimental decrease of brain 5-HT level by inhibition of its biosynthesis by p-chlorophenylalanine (200 mg/kg) induced the sleep-like state of catatonic type, i.e., the state which was characterized by lowered brain 5-HT.

Serotonin Sleep-like states Amphibians

SEROTONIN (5-HT) has been demonstrated in the nervous tissues of animals at all steps of the evolutionary scale [18], however most of the studies have been concerned with its functions in mammals. There are now many reports of the 5-HT involvement in mechanisms of sleep [4] and hibernation [12] in mammals. A decrease of 5-HT levels in cold has been found in representatives of cold-blooded vertebrates, i.e., in brain of frogs [3], lizards [19], tortoises [13]. However it has hitherto not been clear if it reflected only the fall of body temperature in poikilotherms and therefore lowered general metabolism or connected with the concomitant changes in behavior of animals.

In this connection it was interesting to clear up whether there are any alterations in brain 5-HT level in frogs kept at a constant ambient temperature but changing periodically their behavioral activity.

It has been previously shown [5,11] that besides wakefulness, there are three forms of rest in the frog, which differ from wakefulness and from each other in the tone of somatic muscles and in some behavioral, somato-vegetative and neurophysiological characteristics. The first form of sleep-like state is characterized by a plastic muscle tone of cataleptic type (SLS-1) and observed predominantly in day-time. The second form with a rigid muscle tone of catatonic type (SLS-2) is found mainly at night. The third resting state (SLS-3) is characterized, like the higher vertebrates sleep, by the relaxation of the skeletal musculature: the animals lay flat on the bottom of the terrarium, extremities sprawled, eyes closed [6,7]. A comparative physiological study of stages of sleep evolution permitted to regard these forms of the sleep-like rest as the "primary sleep" in the phylogenesis of vertebrates inherent in fishes and amphibians [5].

In this study we tried to elucidate the role of brain 5-HT in the emergence of the sleep-like states in frogs.

METHOD

Animals

Frogs, Rana temporaria, weighing 22–25 g were used. Frogs were collected from the wild in the fall and maintained in the laboratory at 7°C in a special chamber. The experiments were performed in winter on frogs transferred from 7°C to 20°C and acclimated at this ambient temperature.

Procedure

To identify different forms of sleep-like states in frogs at the ambient temperature 20°C, their behavior was observed and electrocardiogram (ECG) and actogram were recorded by the non-contact method [14]. Graphite electrodes placed in the aquarium served as the receiver of the cardiac signals, and the signals induced by the frog's movements. Such a method of recording does not disturb the natural behavior of the animals in water.

In a special series of experiments, chronic polygraphic recording of ECG and several parameters of bioelectric activity via electrodes implanted in the forebrain and crainial part of the longitudinal back muscle was made using a 8-canal electroencephalograph "Orion." In each experiment frequency components of EEG were determined with the help of widezonal analyzer-integrator. The frequency ranges of the zonal filters of the analyzer (1, 5–3; 7–14; 14–20 Hz) corresponded to the biorhythms S, θ, α, β. The integration period was 30 sec during which EEG might be regarded as a stationary process.

To compare the different resting forms with the state evoked by p-chlorophenylalanine (pCPA) pretreatment method of computer analysis (power spectral analysis) was used as well as the conventional visual analysis of EEG.

5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels were determined in brains of frogs acclimated at the ambient temperature of 20°C but being in different states: 1—wakefullness; 2—sleep-like state with plastic muscle tone (SLS-1); 3—sleep-like state with rigid muscle tone (SLS-2); 4—sleep-like state with muscle relaxation (SLS-3). Brain 5-HT and 5-HIAA were also determined in frogs being at 7°C in a state of hypobiosis, and animals 2 or 24 hr after pCPA administration. After a quick decapitation the brain of frog was immediately removed, weighed and placed into test-tubes on ice. 5-HT and 5-HIAA were determined fluorometrically [1] using a "Spekol" spectrofluorometer.

pCPA methylester (Serva), an inhibitor of brain tryptophan hydroxylase [8], the rate-limiting step in 5-HT biosynthesis, was administered in the dose 200 mg/kg b.wt. into the dorsal lymph sack of frogs kept at the ambient temperature of 20°C. The control frogs received saline.

The Student *t*-test was applied to test the significance of the difference between mean values of different groups.

RESULTS

Brain 5-HT and 5-HIAA Levels in Different Resting Forms and in Hypobiosis of Frogs

It was found that in active awake frogs acclimated to the ambient temperature of 20°C, brain 5-HT level was considerably higher than in mammals, which corresponded to the previous reports [3,18]. Brain 5-HIAA level was significantly lower than that of 5-HT and the ratio of 5-HT to 5-HIAA' concentrations which is close to 1 unit in mammals [17] exceeded 4 units.

Significant changes of 5-HT metabolism were found in two states of primary sleep: SLS-1 and SLS-2 (Fig. 1). Frogs in the sleep-like state with plastic muscle tone (SLS-1) were characterized by a decrease of brain 5-HIAA level by 25%, without significant changes in brain 5-HT. Far more pronounced changes took place in the sleep-like state with rigid muscle tone (SLS-2). It was found that both 5-HIAA and 5-HT levels were lowered in brains of frogs in this resting form. A decrease of brain 5-HT was also found in hypobiotic animals acclimated to low temperature (7°C). The degree of decrease of 5-HT level in the state of hypobiosis was similar to that in frogs acclimated to 20°C but being in the sleep-like state with rigid muscle tone.

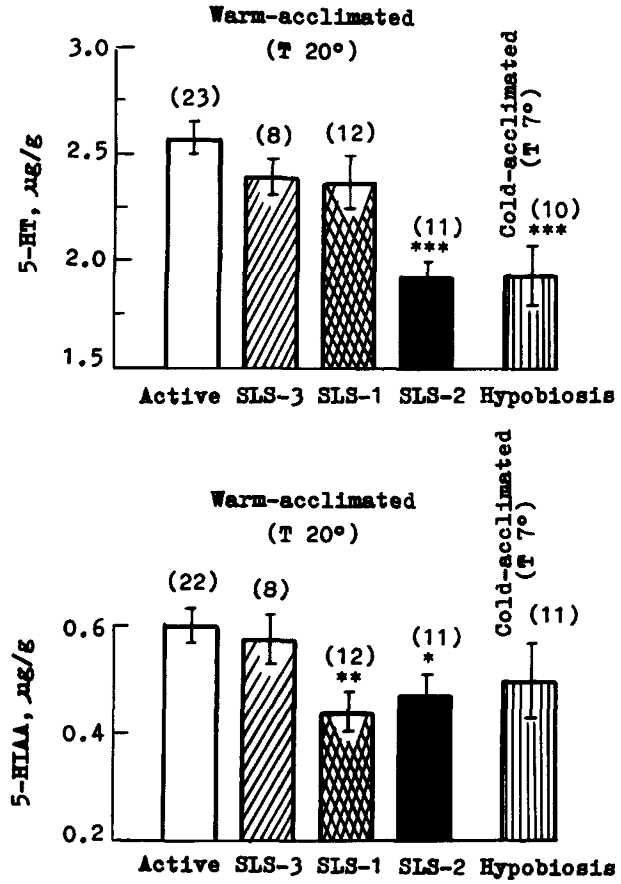

FIG. 1. Brain 5-HT and 5-HIAA in active frogs, in frogs in different forms of rest and in hypobiosis. Values given in micrograms per gram wet weight are means±SE.

Effect of pCPA Administration

First of all it was shown that pCPA administration resulted in a depletion of brain 5-HT though not so pronounced as in endothermic animals. Two hr after pCPA pretreatment in a dose of 200 mg/kg, brain 5-HT level decreased from 2.97±0.27 µg/g (n=10) in control frogs to 2.23±0.09 µg/g (n=10, $p<0.05$). Brain 5-HIAA did not change.

However, pCPA effect was less prolonged in frogs than in rats since the decrease of 5-HT level in rat brain was marked in 48–72 hr after pCPA injection and remained low at least for a week [8]. In frogs the decrease of 5-HT level was found two hours after pCPA administration, but in 24 hr brain 5-HT returned to the control level.

It was found that in approximately 1.5 hr after pCPA administration in all of the animals a state behaviorally similar to the resting form of catatonic type (SLS-2) developed. In this state the frogs were usually motionless, the threshold of motoric reactions was elevated: animals did not react to a touch or when a foreign body was brought into their field of vision. The muscle tone was rigid: a frog could be moved from one place to another on the bottom of the aquarium and could be shaped into unnatural posture by the experimenter. The heart rate was rhythmic, but somewhat more frequent (at 20%) than in the SLS-2 of the control cluster of animals. This state lasted for 1.5–3 hr.

To identify more precisely the state that developed after pCPA administration, EEGs of pCPA pretreated frogs were compared with those of frogs in different sleep-like states.

Earlier it has been shown that SLS-2 is characterized by the predominance of 4–6 Hz frequency range; 2–3, 4–5 and more than 7 Hz frequency ranges dominate in SLS-1; in SLS-3 the slow component of EEG prevails—0.5–3.0 Hz [7]. It was shown that EEG patterns in pCPA pretreated frogs were very alike to those in SLS-2 (Fig. 2). In both cases EEG was characterized by small amplitude and by polymorphism. No significant differences were revealed by analysis of frequency of these EEGs. Heart rate in pCPA pretreated frogs was somewhat higher, than in animals in SLS-2. It has been established that EEG frequency components in frogs with pCPA administration coincides with that of SLS-2 of intact

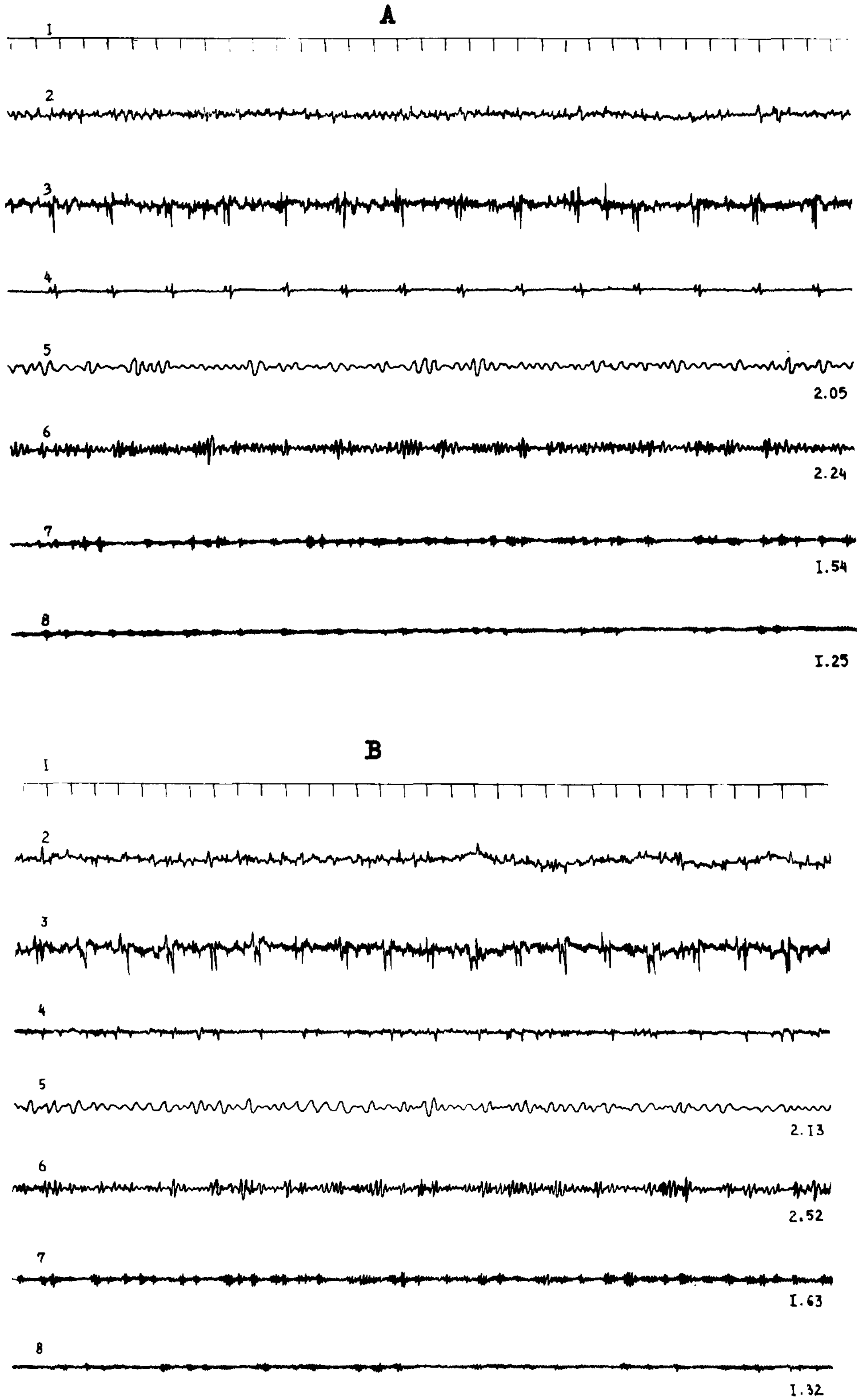

FIG. 2. Polygram of warm-acclimated frogs: A—sleep-like state of rest with a rigid muscle tone of a catatonic type (SLS-2) B—state evoked 2 hr after pCPA administration (200 mg/kg into dorsal lymph sack). Frogs were kept at the ambient temperature of 20°C. 1—time mark 1 sec, 2—EEG of forebrain (primordial hippocampus), 3—EMG, 4—ECG, 5–8—frequency components of EEG for corresponding frequency ranges (1, 5–3; 3–7; 7–14; 14–20 Hz). Calibration 50 mkV. Figures on the right represent results of integration of squared frequency components of EEG in Vs.

frogs in all frequency ranges (Fig. 2). Therefore, the experimental decrease of 5-HT level by inhibition of its biosynthesis induced the sleep-like state with rigid muscle tone, i.e., just that very state in which a significant decrease of brain 5-HT had been revealed.

DISCUSSION

It has been established that brain 5-HT is involved in the control of behavioral phenomena in amphibians. It has been found that the change of functional state of frogs, i.e., the transition from active wakefulness to sleep-like states was accompanied by alteration of brain 5-HT metabolism. Distinct changes were found in two sleep-like states. In SLS-1 with plastic muscle tone brain 5-HT was not altered significantly, but 5-HIAA was reduced. More pronounced changes were found in SLS-2 with rigid muscle tone: not only brain 5-HIAA but also 5-HT levels were decreased. The magnitude of 5-HT decrease was similar to that induced by hypobiosis (7°). It has to be mentioned that the method used [1] does not discriminate between 5-HT and 5-HTP. Therefore a determination of 5-HTP concomitant with 5-HT is possible if 5-HTP brain level is higher in frogs than in mammals where it is negligible.

The decrease of brain 5-HT similar to that observed in our experiments was found earlier by Harry [3] in cold-acclimated (5°) winter frogs. However, our principle finding is that significant changes of brain 5-HT and 5-HIAA levels can be found not only in hypobiosis but without any decrease of environmental and body temperature—when frogs fell into the sleep-like state of catatonic type. The data obtained give a reason to conclude that 5-HT plays a role in the control of the evolutionary ancient resting forms.

An earlier report indicated that brain 5-HT in summer frogs is higher than in winter frogs and it was suggested [3] that the decrease of brain 5-HT in cold-acclimated frogs reflected activation of the central 5-HT processes. In our experiments the lowered brain 5-HT and 5-HIAA levels observed in SLS-2 could be related either to the increased activities of 5-HT neurons, when tissue amine resourses were exhausted, or to the decreased activity and, correspondingly, low synthesis and oxidative deamination of 5-HT. Our experiments with pCPA support the latter supposition rather than the former. An experimental decrease of brain 5-HT level by inhibition of its biosynthesis induced the sleep-like state of catatonic type (SLS-2), that was just that state which was characterized by lowered brain 5-HT.

It is worth to be noted that in our experiments pCPA induced a decrease of brain 5-HT similar to that found in sleep-like state with rigid muscle tone and in hypobiosis, i.e., approximately by 25% in all these cases.

It appears that the way of 5-HT control of resting forms in frogs differs from that in regulation of sleep of endothermic animals and of hibernation in hibernators. It was hypothesized that in mammals 5-HT played a role in the set on of sleep [4] and hibernation [12]. The inhibition of 5-HT synthesis by pCPA induces in endothermic animals pronounced decrease of slow-wave and paradoxal phases of sleep [2, 9, 16]. In hibernators, pCPA administration results in arousal from hibernation [12,15]. As for the frogs, the resting form with rigid muscle tone as well as the state of hypobiosis is followed by a decrease of brain 5-HT level, and pCPA-induced inhibition of 5-HT synthesis results in entry into the sleep-like state with rigid muscle tone. It is obvious that this resting form is connected with reduced 5-HT control.

It should be noted that the changes of 5-HT metabolism are not uniform in the resting forms differing in the tone of somatic muscles. In the sleep-like state with relaxation of the skeletal musculature there were not changes in brain 5-HT or 5-HIAA levels. In the sleep-like state with plastic muscle tone, brain 5-HIAA was reduced but there were no changes of 5-HT level. And, finally, the sleep-like state of catatonic type is likely to be connected with decreased 5-HT synthesis, since in this resting form a decrease both of brain 5-HT and 5-HIAA levels was found and a similar state could be induced by the inhibition of 5-HT biosynthesis with pCPA.

So, it would be logical to suppose that in amphibians 5-HT might also contribute to the control of muscle tone. Earlier, in the laboratory of I. P. Lapin [10], it was found that administration of fenfluramin to frogs produced a decrease of 5-HT in the brain, a sedative action and supressed the orthostatic reflec (turning from supine to prone posture). The evidence for the involvement of brain serotoninergic system in mechanisms of catatonia and catalepsy in mammals is supported by a great number of reports, which we reviewed earlier [12].

It is also worth noting that all the forms of sleep-like state studied represent the natural resting behavioral states and that each of them turns up mostly in definite diurnal period. As it was demonstrated, at least two of these resting forms are 5-HT-dependent. Therefore, it seems likely that brain 5-HT may have an important role in diurnal rest-activity cycle in frogs.

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