EFFECT OF GAMMA-AMINOBUTYRIC AND GAMMA-HYDROXYBUTYRIC ACIDS ON AWAKENING OF SUSLIKS FROM HIBERNATION

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Hibernation is a unique physiological state characterized by inhibition of all metabolic processes, while a certain level of their integration is preserved. Although the mechanism of this state has attracted the attention of investigators for many years, the role of mediator systems in it was studied originally only with a view of elucidating the role of adrenergic processes [1]. Later, however, it was shown that one factor determining the maintenance of natural hypobiosis is the state of the serotoninergic system and its balance with the catecholaminergic system [2]. The system of the GABA shunt has been studied less than the other mediator systems in this respect. Facts such as the hypothermic effect of some GABA-ergic substances [7], participation of gamma-hydroxybutyric acid (GHBA) in the regulation of sleep processes [6], and, finally, the high levels of endogenous GABA observed during hibernation [3, 8] point to the value of a study of the role of GABA and its metabolites in processes of hibernation. One possible approach to the investigation of this problem is a study of the effects of GABA-ergic analyzers on the phases of hibernation.

The aim of this investigation was to study the effect of GABA-ergic substances on awakening of animals from hibernation.

Considering the authors' views [10] on the significant differences between the pharmacological activity of GABA and GHBA, the two principal members of the GABA shunt, analyzers which affect each of these metabolites to some degree selectively also were used.

EXPERIMENTAL METHOD

Experiments were carried out on male red-cheeked susliks (Citellus erythrogenys major), caught in Novosibirsk Region. These animals hibernated in the fall. They were kept in a special room for this purpose, in which a constant temperature of 3–3.5°C was maintained and the conditions were close to natural. Awakening from hibernation was studied at the end of March and beginning of April, when the animals were already prepared for awakening from hibernation. The susliks were transferred to a room at a temperature of 20–21°C, which served as the stimulus for awakening. The initial body temperature of the susliks was measured with a thermometer or electrothermometer introduced per rectum to a depth of 8 cm, and it was measured subsequently every 15 min until normothermia was reached. The times of formation of the orthostatic reflex and the body temperature at which it appeared were recorded. The body temperature and the time when the susliks opened their eyes also were noted. The time of opening of the eyes and appearance of active movements was taken to be the time of awakening.

To change the GABA level in the brain tissue, thiosemicarbazide (TSC), an inhibitor of glutamate decarboxylase, which lowered the brain GABA level (in a dose of 5 mg/kg), and aminohydroxyacetic acid (AHAA), an inhibitor of α-ketoglutarate-GABA transaminase (GABA-T), which causes accumulation of GABA in brain tissue (in doses of 20 and 50 mg/kg), were used. GHBA was given in the form of its sodium salt, sodium hydroxybutyrate (100–300 mg/kg). Depakine (sodium n-dipropylacetate) also was used as a substance which, in small doses, inhibits succinic semialdehyde dehydrogenase [13], and, in large doses, reduces GABA-T activity [12].

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TABLE 1. Effect of GABA-ergic Drugs on Awakening of Susliks from Hibernation (M ± m)

Substance injected	Dose, mg/kg	Number of experiments	Time when body temperature rose to 35°C, min	Recovery of orthostatic reflex		Awakening	
				time, min	body temperature, °C	time, min	body temperature, °C
Control	—	23	119±4	81±4,3	20,2±0,5	106±4	31,3±0,7
TSC	5	7	116±4	82±2,2	20,5±0,7	108±1	31,3±0,6
AHAA	20	6	115±8	82±6,7	22,2±1,6	105±8	33,1±0,3
	50	4		86±3,2	25,0±2,0	107±6	33,0±0,5
Depakine	150	5	135±5*	103±4,8**	18,3±1,4	126±4**	33,1±1,1
	300	4	127±12	73±6,4	19,1±3,3	127±24	34,8±0,3***
	400	7	135±6*	91±3,5	26,1±0,4***	161±9***	36,2±0,3***
Sodium hydroxybutyrate	100	6	130±5,6	78±3,9	19,2±1,7	118±8	32,5±0,9
	200	7	137±9,4	123±16,3*	30,5±2,4***	156±7***	35,9±0,3***
	300	4	270±12,3***	260±19,5***	34,3±0,7***	271±19,5***	34,8±0,3***

Legend. *P < 0.05, **P < 0.01, ***P < 0.001. When AHAA was given in a dose of 50 mg/kg, all the animals died before the end of the experiment.

The compound was used in doses of 150–400 mg/kg. With the exception of TSC, which was injected subcutaneously, all the test substances were injected intraperitoneally. The effect of each dose of the above-mentioned substances was studied on a group consisting of 4–7 susliks, and altogether 73 animals were used. The significance of differences compared with the control (animals receiving 0.9% NaCl solution) was determined by Student's t test.

EXPERIMENTAL RESULTS

Typical hibernating susliks lay curled into a ball during hibernation, did not respond to touch, and their body temperature was 4.4 ± 0.2°C. After the animals had been moved into the room with a temperature of 20–21°C they quickly began to warm up. The times after which the orthostatic reflex recovered and the animals of the control group began to awaken are given in Table 1. They show that the pharmacologic analyzers chosen for study affected the parameters of awakening differently. Substances changing the brain GABA level, whether reducing (TSC) or raising it (AHAA), did not change the time of rewarming of the body to 35°C or the times of recovery of the orthostatic reflex and of complete awakening in these experiments. Meanwhile depakine caused a definite and dose-dependent increase in the times of recovery of the orthostatic reflex. It delayed awakening of the animals and their transition into a state of activity. The interval from the beginning of awakening until the time when the animals opened their eyes also was increased. Delay of awakening also was manifested by the fact that the susliks "overslept" the awakening time and opened their eyes when their body temperature had reached a higher level than in the control animals. The inhibitory effect of the drug on awakening was more marked than its effect on recovery of the orthostatic reflex. Sodium hydroxybutyrate, which also caused dose-dependent delay of awakening of the susliks, had a similar action to depakine; the time of recovery of the orthostatic reflex also was appreciably lengthened. Both these substances, incidentally, exhibit their action in small doses, in which they do not change the body temperature or significantly affect rewarming of animals emerging from deep hypothermia. In this way the action of depakine and sodium hydroxybutyrate differs from the effects of serotonin and, in particular, of its precursor 5-hydroxytryptophan, which block awakening largely because of marked inhibition of thermogenesis [2].

Considering general views of the role of GABA as the mediator of inhibition in certain neuronal systems of the brain, it might be expected that accumulation of GABA would be accompanied by delayed awakening of the animals. However, the results actually showed that elevation of the brain GABA level, even considerable, (AHAA in a dose of 50 mg/kg raises the brain GABA level up to 300% compared with initially [4]) did not change the speed of awakening in hibernating animals. It also remained unchanged when GABA formation was delayed. Meanwhile in these experiments sodium hydroxybutyrate caused definite delay of awakening of the hibernating animals. Since sodium hydroxybutyrate raises the endogenous GHBA level [11], it can be postulated that these results indicate a role for GHBA in the regulation of awakening of hibernating animals. The hypothesis that GHBA has a more important role than GABA in mechanisms of maintenance of hibernation does not contradict the results showing that depakine also has an action similar to that of sodium hydroxybutyrate. Depakine, even in high doses (300–400 mg/kg), unlike AHAA, induces mild inhibition of GABA-T and raises the GABA level by not more than 30% compared with initially. An enzyme which exhibits much greater sensitivity to depakine than GABA-T is succinic semialdehyde dehydrogenase [13]. The high sensitivity of this enzyme to depakine and

the delayed inactivation of GABA produced by it may be the reason why depakine delays the awakening of hibernating animals when administered in doses much smaller than those in which it causes GABA accumulation. It has been shown that the highest concentration of endogenous GHBA, 13–15 times higher than in brain tissue, has been shown to be a feature of the brown fat [9]. Considering the important role ascribed to brown fat in the maintenance of temperature regulation [5], it can be tentatively suggested that this fact is in agreement with the writers' hypothesis of the possible role of GHBA as one of the biologically active substances responsible for the unique pattern of metabolic regulation in hibernating animals. However, it has already been stated that the inhibitory effect of GHBA and depakine on behavioral awakening was more marked than their action on temperature regulation. This suggests that GHBA not only induces a metabolic effect, but also participates in the central mechanisms maintaining this unique adaptive state, namely natural hibernation.

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ELECTROPHYSIOLOGICAL AND CATECHOLAMINE MECHANISMS OF NEGATIVE FEEDBACK IN HYPOTHALAMIC REGULATION OF THE MALE GONADS

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A very important aspect of the problem of control over male gonad function is the relationship between the hypothalamo–hypophyseo–gonadal system (HHGS) and the sympathicoadrenal system (SAS). It has been shown that catecholamines play a determining role in the mechanism of secretion of gonadal releasing hormones [4, 7, 8, 11, 12], and in particular, that excitation of dopaminergic structures of the mediobasal hypothalamus is accompanied by activation of testicular function [8, 9]. These facts indicate that dopamine can be regarded as the leading mediator in the mechanism of direct positive communication at the level of the stage of HHGS control. However, the question of its participation in the realization of negative feedback, and also the role of other hormones and mediators of the catecholamine group requires further research. Hyperandrogenization,

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