Neuronal Activity in the Dorsal Hippocampus after Lateral Hypothalamus Stimulation: Effects of Delta-Sleep-Inducing Peptide

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We studied central effects of delta-sleep-inducing peptide in the mechanisms of positive emotional state formation in rats. In Wistar rats preliminary tested in an open field, the reactions of 57 neurons of the dorsal hippocampus were analyzed during lateral hypothalamus stimulation and microionophoretic application of delta-sleep-inducing peptide. It was found that the number of neurons not responding to stimulation in the lateral hypothalamus surpassed the number of sensitive neurons (63 and 37%, respectively). Hippocampal neurons in active animals were less sensitive to stimulation of the lateral hypothalamus than in passive rats (33 *vs*. 42%) After application of delta-sleep-inducing peptide, only 28% neurons responded to stimulation. Thus, delta-sleep-inducing peptide reduced the sensitivity of hippocampal neurons to stimulation of the lateral hypothalamus.

Key Words: *delta-sleep-inducing peptide; lateral hypothalamus; ventromedial hypothalamus*

Emotions play a key role in the organization of goal-directed behavior [9]. They tune the organism to satisfying the basic biological demands by inducing a complex of behavioral and somatoautonomic reactions. Positive emotional states of laboratory animals can be modeled by stimulation of the positive emotiogenic center of the lateral hypothalamus (LH); stimulation of this center induces searching and food-procuring behavior [2]. Despite the hippocampus is a key emotiogenic structure related, among other things, to detection of signals of hardly probable events with low probability of demand satisfaction [8], the effects of LH stimulation on parameters of neuronal pulse activity in the dorsal hippocampus in rats with different resistance to stress were never studied. Hypothetically, peculiarities of generalization of emotional excitation provoked by LH to hippocampal neurons directly determine individual stress resistance.

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Thus, LH stimulation produces a specific effect on dorsal hippocampal neurons, but the effect of regulatory oligopeptides, substances maintaining stable body functions under normal and pathological conditions, remains poorly understood [4]. Delta-sleep-inducing peptide (DSIP) exhibiting stress-protective activity and participating in transmitter interactions also belongs to this family [5,6,12].

Here we studied the influence of stimulation of hypothalamic structures on neuronal activity in the hippocampus and the effects of DSIP on activity of hippocampal neurons under these conditions.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 230-280 g (n=10). The animals were maintained at room temperature with had free access to food and water. The experiments were performed with strict adherence to Order No. 267 of the Ministry of Health of the Russian Federation (19.06.2003) and Regulations for Conducting Animal Experiments

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On the basis of open-field behavior parameters, the rats were divided into groups of active, passive, and intermediate animals. To this end, a special coefficient (K) was used:

$$K = \frac{\text{Peripheral ambulation+central ambulation}}{\text{Latency of first movement+ Latency of visiting the center}},$$

Active rats (K=2-6) are prognostically more resistant to emotional strain, while passive animals (K=0.2-0.6) are characterized by low resistance to stress [3].

The animals were anesthetized with chloral hydrate (450 mg/kg, intraperitoneally) and a metal electrode was implanted into the lateral hypothalamus (coordinates: AP=1.2 mm; LP=1.0 mm; H=8.6 mm, slope 0°). The position of the electrode was verified on the next day after implantation by stimulation of the structure with rectangular electrical pulses (50-200 mA, 100 Hz, 1 msec pulse duration) over 1 sec; stimulation threshold was adjusted individually. In case of correct position of the electrode, stimulation induced feeding behavior and self-stimulation reaction. The animals were then narcotized with urethane (2 g/kg) and scalped. Urethane produces dissociation anesthesia preserving the properties of neurons and autonomic reflexes [10]. Body temperature was maintained at 37-38°C. Glass three-channes microelectrodes were introduced through the trephine hole into the brain according to the stereotaxic coordinates (Stoelting stereotaxis apparatus). Hole coordinates were AP=4 mm; LP=2 mm; H=2-4 mm; slope 10° medially.

One channel of the microelectrode was filled with 3 M NaCl and other with DSIP (100 μg/ml; Serva). The third channel for microionophoresis was filled with NaCl solution for injections (control). The current applied during microionophoresis of DSIP or water was 20 nA and holding current 5 nA. The indifferent electrode was implanted into the nasal sinuses. The position of microelectrode tips was controlled on brain sections after decapitation of rats [11].

Neuronal activity was performed extracellularly. The amplified signal was digitized and feed into a computer. For obtaining frequency histograms and patterns of neuronal activity, special software for spike isolation and analysis of spike number per time unit was used. After recording baseline neuronal activity in the hippocampus, pulse-spike activity was recorded over 1.5 min after LH stimulation and then neuronal activity recovered over 2 min. After that, DSIP was applied and neuronal activity was recorded over 30 sec before and over 1.5 min after repeated LH stimulation.

Changes in neuronal activity were evaluated using Student *t* test. The differences of the mean spike activity over 30 sec with a significance level of $p<0.05$ were considered as significant increase or decrease of spike frequency. All calculations for Student *t* test and χ^2 test were performed using Statistica 8.0 software.

RESULTS

A total of 57 neurons of the dorsal hippocampus were recorded. Of them, 21 neurons (37%) were sensitive to LH stimulation. Thus, the most of the dorsal hippocampal neurons were insensitive to LH stimulation. It was can assumed that generalization of positive emotional excitation has a more limited pattern than that of negative. However, additional experiments with stimulation of the ventromedial hypothalamus (the negative emotional center) are required for verification of this assumption. Among the neurons sensitive to LH stimulation, the greater part, 17 neurons (81%), responded by enhancement of pulse activity, while in 19% neurons pulse activity was suppressed. Thirty-six neurons (63%) were insensitive to LH stimulation.

In active animals, 33 hippocampal neurons were recorded; of them, 11 neurons (33%) changed the pattern of pulse activity after LH stimulation (sensitive neurons): activation and inhibition were observed in 9 (27%) and 2 (6%) neurons, respectively. Twenty-two hippocampal neurons (67%) were insensitive to LH stimulation.

In passive animals, 24 hippocampal neurons were recorded. Of them, 10 neurons (42%) responded to LH stimulation: spike frequency increased in 8 (34%) and decreased in 2 (8%) neurons. Fourteen neurons (58%) were insensitive to LH stimulation. Thus, hippocampal neurons in active animals were less sensitive to stimulation of the lateral hypothalamus than in passive rats (33 *vs*. 42%; Table 1).

Since hippocampal neurons in prognostically stress-resistant rats were less sensitive to LH stimulation than neurons of prognostically stress-prone animals, this regularity probably reflects lower emotionality of stress-resistant animals.

After preliminary application of DSIP, 16 neurons (28%) were sensitive to LH stimulation; spike frequency increased in 12 (21%) and decreased in 4 (7%) neurons. Forty-one hippocampal neurons (72%) were insensitive to LH stimulation after DSIP application, which considerably surpassed the corresponding value before DSIP treatment (63%).

In active animals, 7 of 33 recorded neurons (21%) were sensitive to LH stimulation after DSIP application: spike frequency increased in 5 (15%) and decreased in 2 (6%) neurons. Insensitive neurons constituted 79%, which considerably surpassed the corresponding parameter in active rats before DSIP treatment (67%).

TABLE 1. Activity of Hippocamplal Neurons in Rats during LH Stimulation before and after DSIP Application

Number of neurons	Activation		Inhibition		No effect	
	abs.	%	abs.	%	abs.	%
Primary LH stimulation						
in all animals	17	30	4	7	36	63
active animals	9	27	2	6	22	67
passive animals	8	34	2	8	14	58
LH stimulation after preliminary microionophoretic application of DSIP						
in all animals	12*	21	4	7	41*	72
active animals	5*	15	2	6	26*	79
passive animals	7	29	2	8	15	63

Note. *$p<0.05$ in comparison with primary LH stimualtion.

In passive rats, 9 of 24 neurons (38%) responded to LH stimulation: spike frequency increased in 7 (29%) and decreased in 2 (8%) neurons (Table 1).

DSIP did not change the number of inhibitory reactions, but considerably reduced the number of cases of activation of spike activity, especially in animals prognostically resistant to stress. Thus, the sensitivity of hippocampal neurons to LH stimulation was reduced after DSIP treatment. Since hippocampal neurons in stress-resistant rats are initially less sensitive to LH stimulation, the decrease in their reactivity to LH stimulation under the effect of DSIP probably reflects an important mechanism of antistress action of this peptide. It has been demonstrated [7] that administration of antistress peptides, in particular, DSIP and ACTH (4-10) inhibited stress-induced expression of the early *c-fos* gene in emotiogenic brain structures, including the hippocampus. Since DSIP has been shown to reduce the sensitivity of neurons to glutamate [1], the involvement of glutamatergic mechanisms into activation of hippocampal neurons during emotional excitation of the hypothalamic center could be hypothesized. However, this hypothesis requires further verification.

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