Neurotropic Effects of L-Lysine in Formation of Pain-Induced Behavior

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In experiments on Wistar rats L-lysine (0.15, 0.5, 1.5, 5.0, 15.0, and 50.0 μg/kg intraperitoneally) exhibited a dose-dependent algic effect during electrocutaneous stimulation of the tail and dose-dependent effects in aggressive defense behavior caused by electrical painful stimulation of paws. It was found that the effect of L-lysine depended on situation determining the predominance of defense or aggression, rather than on the intensity of painful stimulation.

Key Words: *L-lysine; pain; electrocutaneous stimulation; aggressive defense behavior*

Lysine-rich proteins predominate among functionally important neuronal proteins; their content directly correlates with structural and functional organization of nerve elements [2,3]. It can be hypothesized that L-lysine plays an independent role in brain functioning, because recent studies demonstrated its effects on some neurotransmitter systems. This amino acid acts as a depressant with anticonvulsive action realized through increasing of the affinity of the GABA-benzodiazepine receptor complex in CNS [4]. The role of the neurotransmitter or neuromodulator in the central GABA-ergic inhibitory systems is attributed to pipecolic acid (the main metabolite of L-lysine in brain tissue) [5]. Radioligand binding assay showed that L-lysine can also act as a partial antagonist of serotonin receptors [9].

Our aim was to detect the effects of L-lysine on the formation of rat behavior caused by painful electrocutaneous stimulation.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (180-200 g) kept under standard vivarium conditions. The animals were divided into groups, 10 per group. L-Lysine (ICN) was dissolved in saline and injected intraperitoneally in doses of 0.15, 0.5, 1.5, 5.0, 15.0, and 50.0 μg/kg 12 min before the experiment. Controls were injected with an equivalent volume of saline.

Several models of behavior caused by painful stimulation were studied.

Electrocutaneous stimulation was modeled by applying plate electrodes to the tail base; the threshold (mA) reactions to painful stimulation were recorded in succession. The level of pain sensitivity (reaction of the tail strain and head turning to the electrodes) and threshold levels of emotional affective behavior components (vocalization, rotation, biting of electrodes) were evaluated. Experiments were carried out under conditions of free behavior in the cage.

Aggressive defense behavior under conditions of inevitable electrical stimulation of rat's paws was modeled by placing two rats in a cage with electrified grid floor. Gradually increasing (1 V/sec) alternating current was applied to the floor and threshold values of developing behavioral components (starting, vocalization, rearing, running, battle) and number of fighting reactions (% of tests) at certain voltage (70 V) were measured. Stimulation was

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discontinued after the start of fighting or after reaching the voltage threshold.

Two sessions at 1-min interval were carried out in each test.

The results were evaluated using Student's *t* test.

RESULTS

The threshold values of behavioral components in response to electrocutaneous stimulation of the tail were as follows: 0.78±0.04 mA for tail strain reaction, 1.10±0.09 mA for head turning, 0.95±0.11 mA for vocalization, 1.49±0.06 mA for rotation, and 1.51±0.07 mA for electrode biting. L-Lysine significantly (p<0.050-0.001) decreased the threshold values for all components of the pain-provoked behavior (except rotation reaction), in other words, exhibited a dose-dependent algic effect (Fig. 1). After injection of the drug in doses of 0.15 and 0.5 μg/kg, the threshold values of pain reactions decreased by 16-28% in comparison with the control values, which was more pronounced than changes in components of emotional affective behavior (15-20% reduction). The algic effect of L-lysine in doses of 5.0 and 15.0 μg/kg led to virtually the same results. The maximum effect of L-lysine was observed after its injection in doses of 1.5 and 50.0 μg/kg. The significant difference between the effects of these doses is worthy of note. L-Lysine in a dose of 1.5 μg/kg more intensely stimulated pain sensitivity than emotional affective reactions (the difference in reduction of threshold values reached 10-19%), while the dose of 50.0 μg/kg led to a significant potentiation of all components of the studied behavior.

The dose dependence of the effect of L-lysine on the aggressive and defense behavior of rats subjected to electrical painful stimulation of the paws was described by an U-shaped relationship (Fig. 2). Control values for behavioral parameters were: 28.4±2.0 V for starting reaction, 35.8±1.6 V for vocalization, 31.0±3.2 V for rearing, 47.7±1.4 V for running, and 56.6±3.1 V for fighting reaction. Injection of the amino acid in the middle doses (1.5, 5.0, and 15.0 μg/kg) resulted in reduction of the threshold values of behavioral painful components to 18% of the control, but statistically significant increase in pain sensitivity was observed only after injection of L-lysine in a dose of 1.5 μg/kg. Increased aggression manifested in higher incidence of fights (by 20-50% *vs.* control). The effect of 5.0 μg/kg L-lysine was statistically significant and opposite to that produced by 0.5 and 50.0 μg/kg L-lysine (*i.e.* analgesic and antiaggressogenic). Threshold values of pain characteristics increased by 6-33% *vs.* control, of defense behavior characteristics by 20-26% (p<0.050-0.001). Changes in the threshold of fighting reactions were not unidirectional and did not reach the level of statistic significance, though the incidence of attacks decreased significantly (to 10%).

These forms of behavior were caused by virtually equivalent (by the strength of current) painful stimulation, though in situations of different biological significance, which determined their characteristics. Defense behavior was pronounced in response to electrocutaneous stimulation of the tail, while the reaction of "attacking" a physical object (source of pain), *i.e.* biting the electrodes, can be regarded as a manifestation of affective defense. In this situation, painful spinal tail tension reflex was more sensitive to L-lysine. The increase in amino acid dose still more activated the supraspinal structures involved in the formation of defense behavior.

Painful stimulation of paws on the electrified floor in two animals simultaneously (under condi-

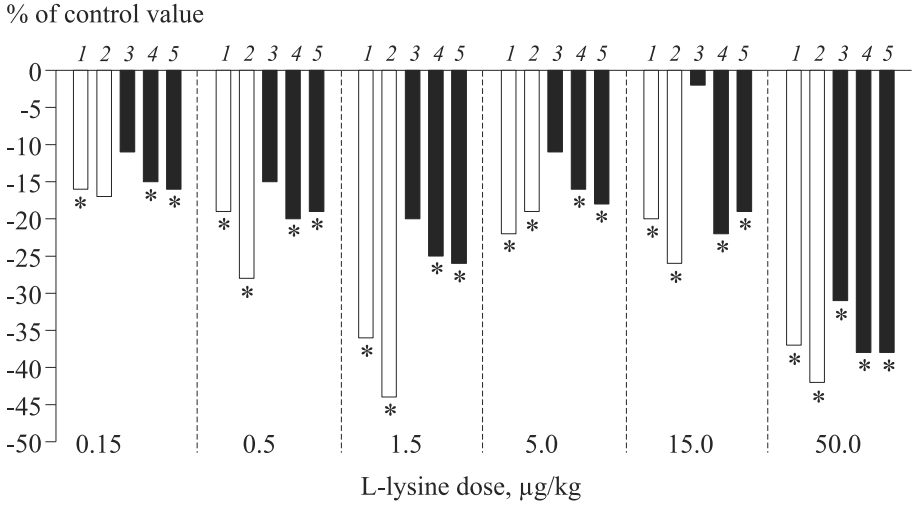

Fig. 1. Dynamics of painful reaction threshold in rats subjected to electrocutaneous stimulation. Light bars: manifestation of pain sensitivity; dark bars: behavioral manifestations. *1*) tail strain; *2*) head turn to electrodes; *3*) rotation; *4*) vocalization; *5*) biting the electrodes. Here and in Fig. 2: *p<0.050-0.001 compared to the control group.

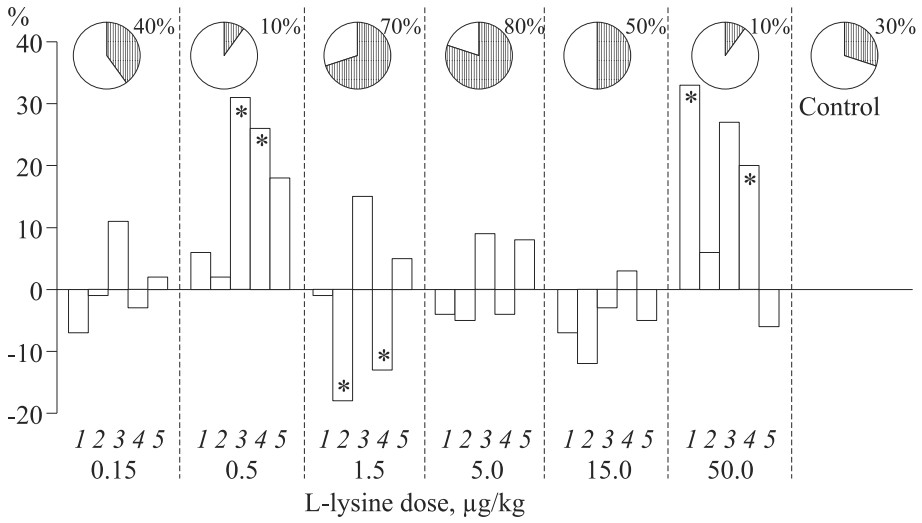

Fig. 2. Time course of parameters of aggressive/defense reaction of rats. *1*) starting; *2*) vocalization; *3*) rearing; *4*) running; *5*) fights. Circular diagrams: percent of fighting reactions (dark segment) in the total number of trials.

tions of probable intercourse and perception of visual and olfactory stimuli) led to appearance of painful and defense reactions which result from inevitable stimulation and eventuate in consumatory aggression fits, presenting as species-typical attacks against the other animal. In this situation, the effect of L-lysine can be stimulatory or inhibitory, depending on the dose.

The neurochemical mechanisms of defense and aggressive behavior are different and depend on activities of certain neurotransmitter system, such as serotonin-, catecholamine-, choline-, and GABA-ergic systems [1]. The limbic, striatal, and cortical functions providing integration of the aggression and defense mechanisms are maintained and regulated through these systems. It can be hypothesized that the effects of L-lysine can be mediated through changed activities and interactions of the mediator systems with certain neuroanatomical localization. This hypothesis is supported by the data on decreased release of serotonin in the central nucleus of the amygdala [6] and norepinephrine in the ventromedial hypothalamus [8]. The amino acid can exhibit the effects of a competitive antagonist of serotonin, which were observed *in vitro* on guinea pig ileum [7]. Hence, L-lysine modulates the cerebral structures playing an important role in the formation of agonistic behavior.

The effect of L-lysine in the test of aggressive/defense behavior can be based on the dose-effect regularity characteristic of the neurotransmitter system reactions. GABA and benzodiazepines in low doses and the minimum reduction in serotonin concentration in the brain as a rule stimulate the intraspecies aggression in animals, but suppress it with increasing the doses [1]. In our study, this regularity could manifest by the algic and aggressogenic effects of L-lysine in doses of 1.5, 5.0, and 15.0 μg/kg. Opposite effects were observed after administration of the drug in the maximum dose (50.0 μg/kg). Activation of defense behavior and suppression of aggression after injection of the amino acid in a dose of 0.5 μg/kg could result from its effect of a serotonin receptor antagonist (blocking of these receptors, particularly under conditions of electrical painful stimulation, provides the defense behavior), while cholinergic and adrenergic mechanisms are essential for manifestations of aggression and attack [1]. Presumably, electrical painful stimulation of the tail also leads to the formation of interactions between the neurotransmitter systems with predomination of the defense behavior.

Our results confirm that the biological significance of the situation (but not the intensity of painful stimulation) determines the supraspinal integrative interactions.

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