Effects of L-Arginine on Various Types of Pain Sensitivity

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Intraperitoneal injection of L-arginine to male Wistar rats 12 min before the start of the experiment produced a nociceptive effect on models of electrocutaneous stimulation of the tail or hot-plate test and increased nociceptive behavior due to high sensitivity of supraspinal nociceptive structures to this compound. The nociceptive effect of this amino acid was more pronounced and persistent under conditions of electrocutaneous stimulation.

Key Words: *L-arginine; pain; electrocutaneous stimulation; thermal pain stimulation*

Study of the neurotropic effects of 20 analogues of hypothalamic-pituitary peptides (N-terminal fragments of adrenocorticotropic hormone, gonadotropin-releasing hormone, and enkephalins) and tamoxifen showed that systemic administration of peptides containing L-arginine residue increases fear response and affective aggression in rats exposed to unavoidable electrostimulation. The test peptides also exhibited nociceptive activity [1,3,4,12]. Further studies showed that this amino acid plays an important role in the regulation of pain sensitivity. Different modes of treatment with L-arginine in different doses were tested on a variety of pain models. The influence of L-arginine on pain sensitivity is related to the fact that this compound serves as a precursor of nitric oxide (NO) and dipeptide kyotorphin [7]. However, the mechanisms of neurotropic activity of L-arginine remain unknown.

Here we studied the effects of L-arginine on various types of pain sensitivity in rats exposed to electrocutaneous and thermal stimulation.

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MATERIALS AND METHODS

Experiments were performed on 220 male Wistar rats weighing 180-200 g. The animals were divided into groups (10 rats each). L-Arginine in doses of 0.05, 0.15, 0.5, 1.5, 5, 15, 50, 150, 450, and 1500 μg/kg was dissolved in physiological saline and injected intraperitoneally 12 min before the start of the experiment. Control animals received physiological saline (1 ml/kg).

For evaluation of electrocutaneous pain threshold in rats, electrical stimulation was delivered through plate electrodes placed on the base of the tail. The pain response was assayed by the threshold (mA) of behavioral activity reflecting pain sensitivity (tail stretching and head torn to the electrode) and emotional and affective reactions to pain (rotation, vocalization, and biting of the electrode). Each freely moving animal was tested 2 times at a 1-min interval [6].

For evaluation of thermal pain sensitivity, the animals were placed on a plate with a restricting glass cylinder. The plate was heated to 55°C. Four trials (1 min) were performed at a 1-min interval. Pain sensitivity was estimated by the latency (sec) of paw licking (trials 1 and 2) and jumping-out to the edge of a restricting cylinder (trials 3 and 4) [5].

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The results were analyzed by Student's *t* test.

RESULTS

Under control conditions the tail stretching response, head turns, rotation, vocalization, and biting of the electrode were observed upon electrocutaneous stimulation with 0.73±0.07, 0.76±0.03, 1.31±0.04, 1.27±0.05, and 1.14±0.05 mA, respectively. L-Arginine increased pain sensitivity and degree of pain behavior (Fig. 1). The defense response and aggression depended strongly on the dose of L-arginine. The pain threshold (tail-flick latency reflecting the pain response) decreased by 12-15% after administration of L-arginine in low doses of 0.05, 0.15, and 0.5 μg/kg ($p<0.01$). The threshold for biting of the electrode, which reflects the defense response and aggression, decreased by 14-23% ($p<0.05$). L-arginine in higher doses produced a more pronounced nociceptive effect. The pain threshold decreased to 25-37% ($p<0.001$). Changes in the defense response and aggression were less significant under these conditions. The thresholds of the pain response and affective and defensive behavior decreased similarly for L-arginine doses of 50, 150, and 450 μg/kg. The t maximum dose (1500 μg/kg) produced minimum nociceptive and defensive effect.

L-Arginine in various doses (except 15 and 5 μg/kg in trials 1 and 2, respectively) decreased the latency of pain response (paw licking, Table 1). Therefore, systemic administration of L-arginine has a nociceptive effect under conditions of thermal stimulation. The thermal pain sensitivity (paw licking) in trial 2 was lower than in trial 1 (Fig. 2). These changes were more pronounced in trial 3. The latency of jumping-out (affective and emo-

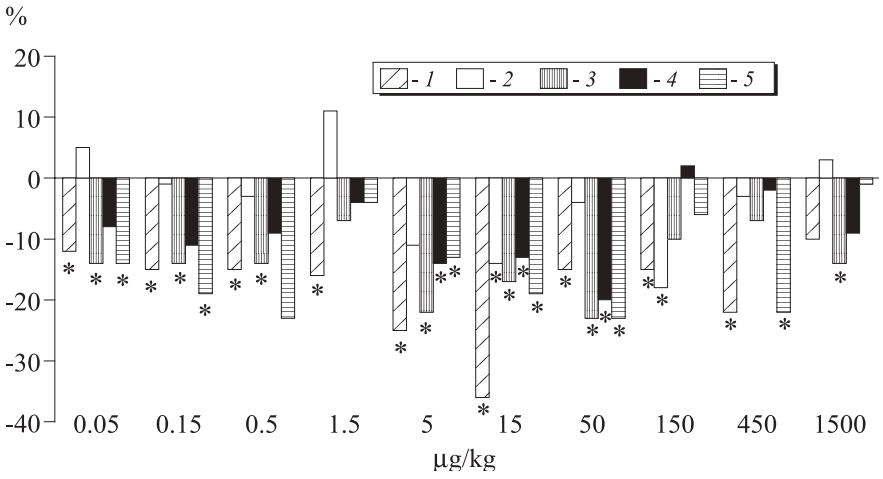

Fig. 1. Pain response thresholds during electrocutaneous stimulation of the tail. Changes in the thresholds of tail stretching (*1*), head rotation toward the electrode (*2*), body rotation (*3*), vocalization (*4*), and biting of the electrode (*5*, % of the control). Here and in Figs. 2 and 3: *$p<0.05$ compared to the control.

TABLE 1. Latency of the Pain Responses in Rats Subjected to the Hot-Plate Test (sec, *M*±*m*)

Group	Paw licking		Jumping-out	
	trial 1	trial 2	trial 3	trial 4
Control	11.3±1.6	19.0±2.4	12.6±2.2	17.1±3.3
L-Arginine, μg/kg				
0.05	9.6±0.9	12.7±2.2	11.8±1.5	17.0±3.7
0.15	9.2±1.1	15.7±2.3	18.9±4.8	11.0±2.4
0.5	7.5±1.0*	14.6±2.0	17.4±2.8	15.4±2.5
1.5	9.9±0.8	13.3±2.3	24.7±4.8*	15.0±4.0
5	10.6±1.4	22.0±2.7	20.5±5.0	16.2±2.3
15	12.1±1.5	17.4±2.6	28.8±5.7*	21.8±4.8
50	8.3±1.0	11.4±2.5*	24.9±4.9*	18.0±3.1
150	9.7±1.3	12.0±1.7*	19.6±5.5	10.7±1.7
450	10.0±1.3	12.1±2.0*	27.1±5.7*	18.4±3.1
1500	8.4±0.7	16.5±3.1	24.7±4.8*	19.1±4.1

Note. *$p<0.05$ compared to the control.

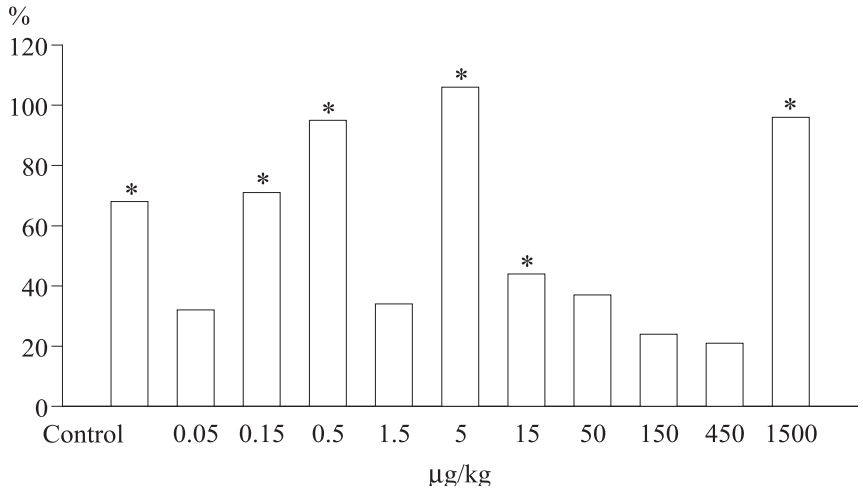

Fig. 2. Latency of paw licking in trial 2 (% of trial 1).

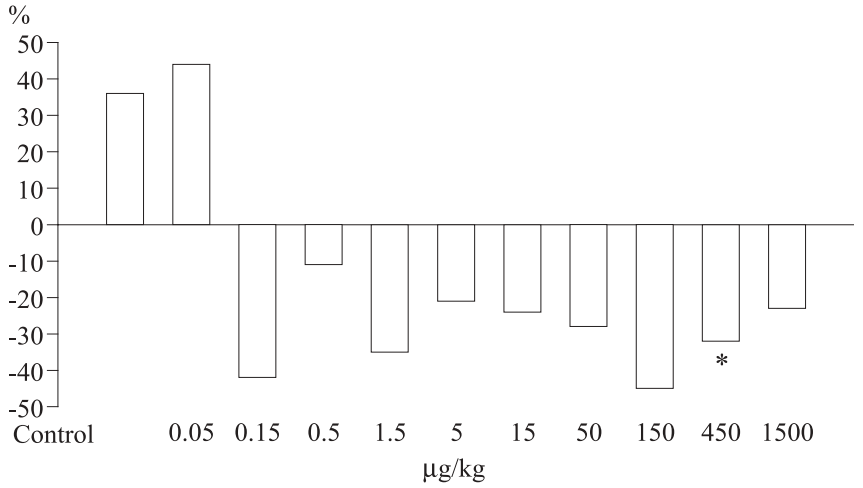

Fig. 3. Latency of jumping-out in trial 4 (% of trial 3).

tional pain behavior) increased by 96-126% compared to the control. The observed changes were statistically significant after treatment with L-arginine in various doses. However, the effects of the test substance were unstable and even opposite in trial 4 (compared to control animals). The avoidance latency in trial 4 was lower than in trial 3 (Fig. 3). These changes reflect an increase in the pain behavior.

Manifestations of the pain behavior depend on total locomotor activity of animals. We previously studied the effect of L-arginine in low doses on vertical orientation activity, grooming, and emotional state in the open field.

Our results indicate that systemic administration of L-arginine in specified doses has the nociceptive effect on the model of electrocutaneous and thermal sensitivity. The test substance significantly modulated the pain behavior. This amino acid had a strong and persistent nociceptive effect on the tail-flick behavior during electrocutaneous stimulation.

Electrocutaneous stimulation of the tail was applied to evaluate the effect of L-arginine at the spinal (spinal pain response) and supraspinal level (behavioral response to pain). The supraspinal integrative mechanisms were more sensitive to L-arginine. After administration of L-arginine in very low doses (0.15 and 0.5 μg/kg) a decrease in the threshold for biting of the electrode was more significant compared to the increase in the pain threshold. L-Arginine in higher doses had a greater effect at the spinal level. These effects are probably related to facilitation of pain transmission with NO. The formation of NO from L-arginine is accompanied by glutamate-induced activation of NMDA receptors [9]. Increased production of NO can result from an increase in the dose of this amino acid. The nociceptive effect decreased with increasing the dose of L-arginine, which was probably associated with activation of kyotorphin synthase. This enzyme has lower affinity for L-arginine and is involved in metabolism in the presence og higher doses of this of the amino acid [7].

Systemic administration of L-arginine is followed by various nociceptive effects during thermal stimulation. It is probably related to complexity of

the hot-plate test. Rat behavior in this test results from the interaction between spinal and supraspinal mechanisms. The delayed avoidance response in trial 3 is partly associated with impaired orientation reaction after its short-term increase induced by L-arginine. Acceleration of the avoidance behavior in trial 4 can be due to improvement of learning under the influence of L-arginine. Our hypothesis is supported by published data that the amino acid improves memory under adverse conditions [11] and promotes learning [10].

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