Oral Administration of L-Arginine, a Nitric Oxide Precursor, Decreases Nociceptive Sensitivity in Rats

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Tail-flick test was used to evaluate the effect of orally administered L-arginine on nociceptive sensitivity of albino rats, which produced a) analgesia at 30 min postadministration lasting about 1.5 h (100 mg/kg); b) short-term analgesia (50 mg/kg); and c) no analgesic effect (250 mg/kg). D-Arginine (100 mg/kg) did not affect the nociceptive sensitivity. A significant NO increase took place in cerebral cortex at 30 min postadministration of L-arginine in the given doses. At 1 h postadministration of L-arginine in doses of 50 and 250 mg/kg, cortical NO content was lower than that in control animals. Analgesic effect of L-arginine is presumably related to additional synthesis of NO. This effect seems to be not directly produced by NO, but is realized via other transmitter systems.

Key Words: *nociceptive sensitivity; nitric oxide (NO); arginine*

Physiological processes in living organisms are controlled by hormonal and neurotransmitter systems with participation of second messengers and neuromodulators. A recently found second messenger, nitric monoxide (NO), is an endogenous activator of guanylate cyclase. In addition, it modulates secretion of a number of transmitters and hormones. NO regulates vascular tone, cardiac activity, and immunological status [12]; it acts as a retrograde messenger in the nervous system subdivisions related to fixation and reproduction of the memory trace [6]. Recent data describe animal behavior under conditions of heterodirected influences on NO synthesis [5,7]. As a rule, such works study the effects of NO synthesis inhibitors, but not the effects of its endogenous precursor, L-arginine (L-Arg). Previously, we showed that oral administration of L-Arg increases motor activity of albino rats [1] and deteriorates food-procuring conditioning in intricate labyrinth experiments [3]. Here we study analgesic activity of orally administered L-Arg.

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MATERIALS AND METHODS

Experiments were carried out on random bred male albino rats weighing 220-270 g. L-Arg was administered orally in a volume of 0.8 ml and doses of 50 (n=22), 100 (n=41), and 250 mg/kg (n=17). Control rats received an equal volume of distilled water (n=51). To test a possible relation of NO increase to the effects of L-Arg, we also studied the effects of its stereoisomer D-Arg which is not a substrate of NO-synthetase and, therefore, cannot lead to NO synthesis. D-Arg was administered orally in a dose of 100 mg/kg (n=27). Analgesic effects of the preparations were evaluated in the tail-flick test using hot water (56°C). The latent period of tail-flick was determined before administration of the preparations, and every 10 min during the following 2 h. The results were subjected to statistical analysis by standard methods. The analgesia coefficient (A) was calculated from the formula: $A=(T_t-T_c/30-T_c)\times 100$, where T_t is the latent period of tail-flick in the test group, T_c is the analogous value for control group, 30 sec is the maximum period of noxious stimulation [8]. NO content was determined in the cerebral subdivisions: in cerebral hemispheres,

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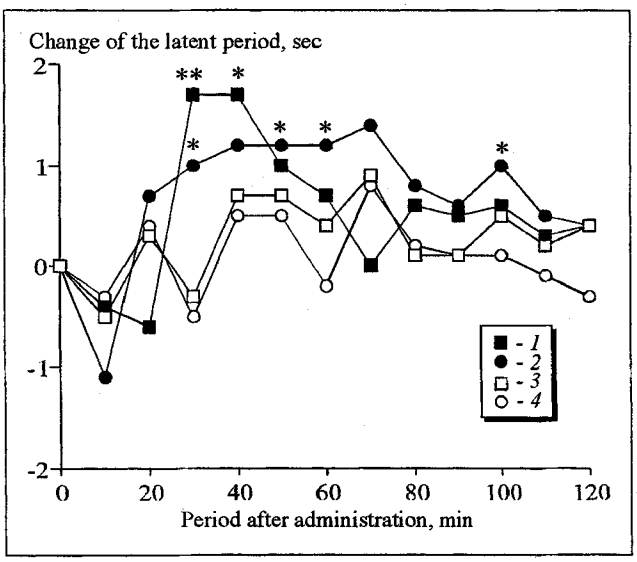

Fig. 1. Changes in the latent period of tail-flick in white rats after oral administration of L-arginine in a dose of 50 (*1*), 100 (*2*), and 250 mg/kg (*3*), and in control (*4*). Here and in Figs. 2 and 3: *$p<0.05$ and **$p<0.01$ in comparison with the control group.

cerebellum, and subcortex, including basal ganglia, diencephalon, and limbic structures. To this end we used a method based on selective entrainment of Fe^{2+} complexes of NO with diethyldithiocarbamate (DETC) [2].

RESULTS

Administration of L-Arg at 50 and 100 mg/kg resulted in an increase in the tail-flick latency, i.e., a decrease in nociceptive sensitivity (Fig. 1). In this test the effect of 50 mg/kg L-Arg was shorter. At 250 mg/kg L-Arg had no analgesic effect.

For detailed study of analgesic effects the animals were divided into two subgroups: with initially higher and lower nociceptive sensitivity (latent period of tail-flick shorter or longer than 7 sec, respectively). Figure 2 shows that analgesic effect depends on the initial nociceptive sensitivity. After administration of 50 mg/kg L-Arg, analgesia was observed in rats with initially low nociceptive sensitivity. A certain hyperalgesia occurred in rats with initially higher sensitivity. When L-Arg was administered in a dose of 100 mg/kg, analgesia was observed in both groups, and this effect was much longer (up to 120 min after administration) in rats with an initially higher nociceptive sensitivity.

D-Arg (100 mg/kg) did not affect the latent period of tail-flick in rats with either low or high initial nociceptive sensitivity.

Figures 1 and 2 show that analgesia arose 30 min after administration of L-Arg. To check whether the observed analgesic phenomena were related to NO excess, we measured NO content in the brain. Thirty minutes after administration of L-Arg in every dose tested (n=10 in each group), a significant increase in NO was observed in the cortex (Fig. 3). We found no significant changes in the subcortex and cerebellum. One hour after administration of L-Arg in doses of 50 and 250 mg/kg, a decrease in cerebral NO was observed in comparison with the control group (n=10 in the test groups, and n=7 in the control). Presumably, this decrease is caused by a feedback inhibition of NO-synthetase. Administration of either authentic NO or the substances inducing spontaneous NO release led to a decrease in the activity of NO-synthetase. By contrast, the NO-binding agents pre-

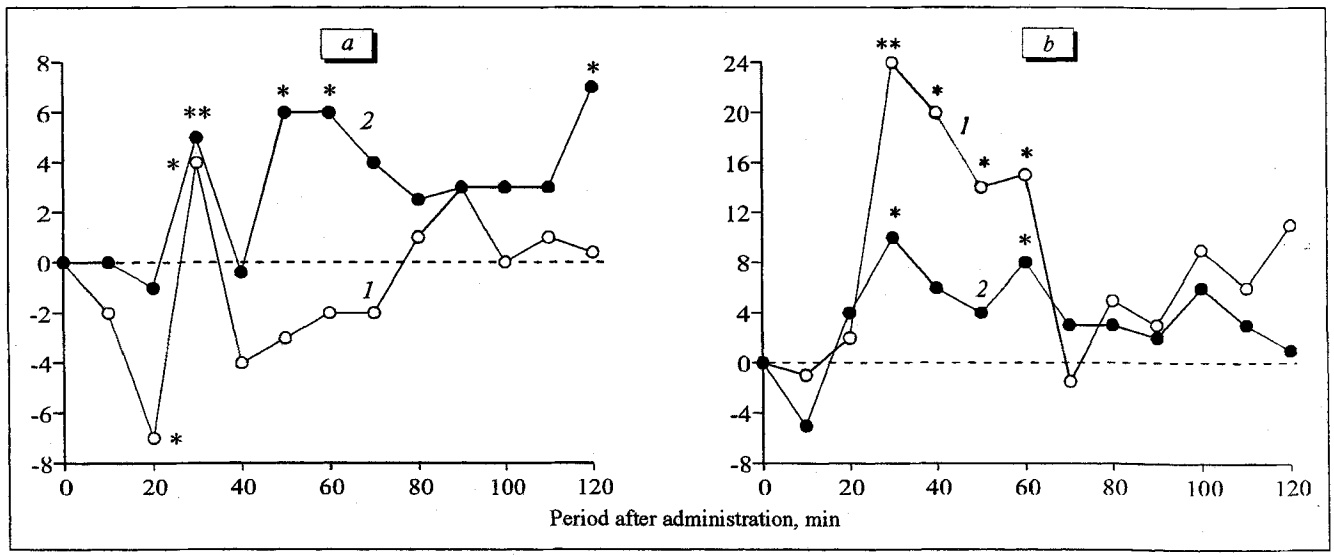

Fig. 2. The analgesia coefficient (%) in rats with initially higher (*a*) and lower (*b*) nociceptive sensitivity after administration of L-arginine in doses of 50 (*1*) and 100 mg/kg (2). Analgesia coefficient in the control group is taken for zero. *a*: control n=17; *1*) n=13; 2) n=16; *b*: control, n=27; *1*) n=9; 2) n=25.

vented inhibition of NO-synthetase activity [11]. Therefore, the analgesic effect observed after administration of L-Arg arose during the period characterized by enhanced level of NO in cerebral hemispheres. However, nociceptive sensitivity was reduced even 1 h postadministration of L-Arg, when NO level decreased. One can conclude that enhanced NO in rat cerebral hemispheres is accompanied by a decrease in nociceptive sensitivity. The fact that D-Arg does not affect the nociceptive sensitivity in rats supports the suggestion that NO, which is synthesized from L-Arg, modulates nociceptive sensitivity.

These data agree with those on the effect of L-Arg on nociceptive sensitivity. Even 10 min after intracranial injection of L-Arg in a low dose (1 nM), a 35-min analgesia was observed. Higher doses (10-1000 nM) resulted in a rapid development of analgesia, which lasted 1 h. The decrease in nociceptive sensitivity, induced by L-Arg, was prevented completely by preliminary administration of the NO synthesis blocker nitro-L-arginine methyl ester (L-NAME) [8]. In the formalin test, intraperitoneal injection of L-NAME in the doses of 1-100 mg/kg resulted in antinociception [4]. There is direct neurophysiological evidence that L-NAME blocks the propagation of nociceptive signals in the dorsal horn of the rat spinal cord, induced by thermal, mechanical, or chemical noxious stimuli [9,10].

It is known that NO modulates the release of a number of physiologically active substances, including transmitters that participate in nociceptive traffic. One of these agents is substance P [10]. Thus, being a second messenger, NO affects the nociceptive sensitivity indirectly, via interaction with various transmitter systems.

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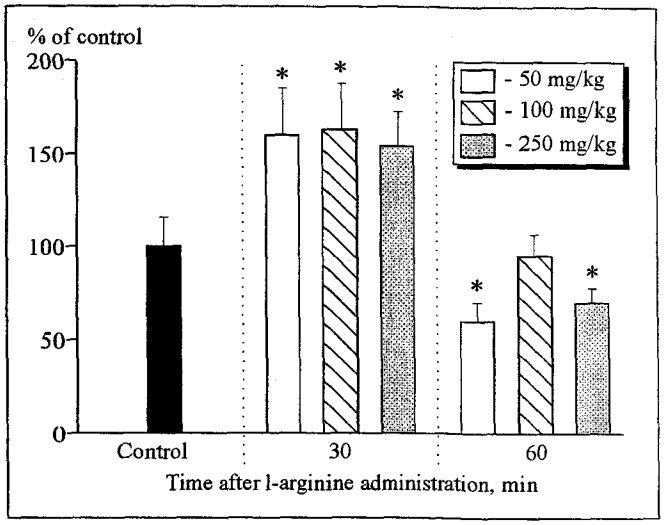

Fig. 3. The number of NO—DETC complexes in cerebral cortex after oral administration of L-arginine in varied doses.

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