Effects of NO-Modulating Agents on the Development of Acute Painful Reaction in Rats

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Painful reaction of rats to intraperitoneal injections of L-arginine, Nω-nitro-L-arginine, and agmatine was studied on the model of formalin-induced inflammation. All drugs exhibited a dubious effect on the patterns of nociceptive behavior depending on the phase of painful reaction. The dynamics of nitrate/nitrite content in animal blood and serum indicated the presence of NO-dependent and NO-independent components in the mechanisms of pharmacological effects of these drugs.

Key Words: *nitrogen oxide; pain; analgesia*

The involvement of nitric oxide (NO) in painful reactions opens new prospects for the creation of new drugs with analgesic effects depending on the system of NO synthesis and metabolism. However, according to different studies of analgesic activities of NO-modulating drugs (primarily NO synthase inhibitors), performed on different experimental models, the effects largely depend on the substance, dose, and routes of its administration [9,10]. That is why not only the mechanisms, but also effects of NO in pain and drug analgesia remain unclear until present, while the opinions of scientists on the role of NOergic factor in the pathophysiology of pain are extremely contradictory [2,4,9]. We studied the course of painful reaction in rats after injections of drugs with opposite effects on the activity of L-arginine-NO system: L-arginine (NO synthesis precursor), Nω-nitro-L-arginine (L-NA; a nonselective inhibitor of NO-synthase), and agmatine, a bioactive amine, product of alternative metabolism of L-arginine. In order to evaluate more precisely the contribution of NOergic system into the development of pharmacological effect of these drugs, we compared the nociceptive behavior of animals with concomitant changes in the content of NO metabolites in the peripheral blood and cerebrospinal fluid.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (180-200 g) divided into groups of 5-6 animals. All experiments were carried out in accordance with ethical regulations of European Committee for Protection of Experimental Animals. The rats were kept under standard vivarium conditions at natural light with free access to water and food. As the half-life period for alimentary nitrates and nitrites is 6 h [3], the animals received no fodder for 12 h before the experiment. Decapitation and collection of biological material for analysis were carried out under Nembutal narcosis.

The model of tonic inflammatory pain was created by injection of 100 μl 4% formalin into the hind paw sole. Controls were injected with saline. Clinical manifestations of painful syndrome were evaluated by two characteristics of nociceptive behavior: duration of licking the damaged limb and total number of spontaneous jerking attacks. Behavioral activity of animals was evaluated by placing the animals in individual boxes (10×25×25 cm) with transparent walls and a mirror fixed to the floor at an angle of 45°. The parameters of painful reaction

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were recorded over 30 min after injection of formalin, the changes during the acute (minutes 1-10) and tonic phases of pain (minutes 10-30) were recorded separately.

Pharmacological activity of drugs was studied on rats injected intraperitoneally with L-arginine (NO synthesis precursor; 100 mg/kg), L-NA (NO synthase inhibitor; 50 mg/kg), or agmatine (10 mg/kg) 30 min before pain induction. The doses were based on the results of previous studies of pharmacokinetic and pharmacodynamic characteristics of these agents [2,5,9]. Activity of NO synthesis was evaluated by the nitrite/nitrate (NOx) concentrations in the peripheral blood and liquor, which were measured by spectrophotometry using Griess reagent [3] 10 and 30 min after pain induction. All results were evaluated using Student's *t* test.

RESULTS

Two phases are clearly distinguished in the structure of painful reaction to subcutaneous injection of formalin: acute painful reaction and tonic pain phase. The former is caused by direct effect of formalin on the pain receptors, while changes in animal behavior during phase 2 are caused by hyperalgesia processes developing due to sensitization of nociceptive and spinal neurons [7]. The nociceptive behavior of animals during acute and tonic pain is characterized by specific dynamic features (Fig. 1). Licking of the damaged paw predominates during phase 1, while during tonic phase both signs of painful reaction are augmenting. These changes are paralleled by consecutive shifts in the concentrations of NO metabolites in the peripheral blood and liquor (Fig. 2). The NOergic system in the nervous tissue is more sensitive to painful exposure, which is seen from more rapid (during phase 1) increment of NOx in the liquor, while in the serum clear-cut biochemical shifts are recorded only during phase 2 of pain.

Blockade of NO synthesis with L-NA significantly modified the pattern of animal reaction to tissue injury. The analgesic effect of the drug was more pronounced for symptoms supported by the suprasegmentary motor systems: the duration of licking of the damaged paw decreased throughout the entire period of observation: by 2.34 times during phase 1 and by 3.31 times during phase 2. However, the pronociceptive effect of the drug manifested during the tonic pain period, which was seen from activation of spinal nociceptive reflexes: the number of spontaneous jerking attacks increased 2-fold in comparison with that in animals receiving no drug. Measurements of NO metabolites in rats injected with L-NA revealed significant synchronous reduction of their levels in the peripheral blood and liquor in comparison with pharmacologically intact group. It is noteworthy that NO content in biological liquids remained stable throughout the entire period of observation, despite alteration of pain-associated behavior pattern during phase 2 of pain. The totality of physiological and biochemical parameters indicates that the analgesic effects of L-NA are determined by reduced production of NO and can be realized by direct modification of the severity of inflammatory process in damaged tissues and by modification of activities of spinal and cerebral neuronal structures. The mechanisms underlying the pronociceptive effects of NO synthase inhibitor used in the study deserve further investigation.

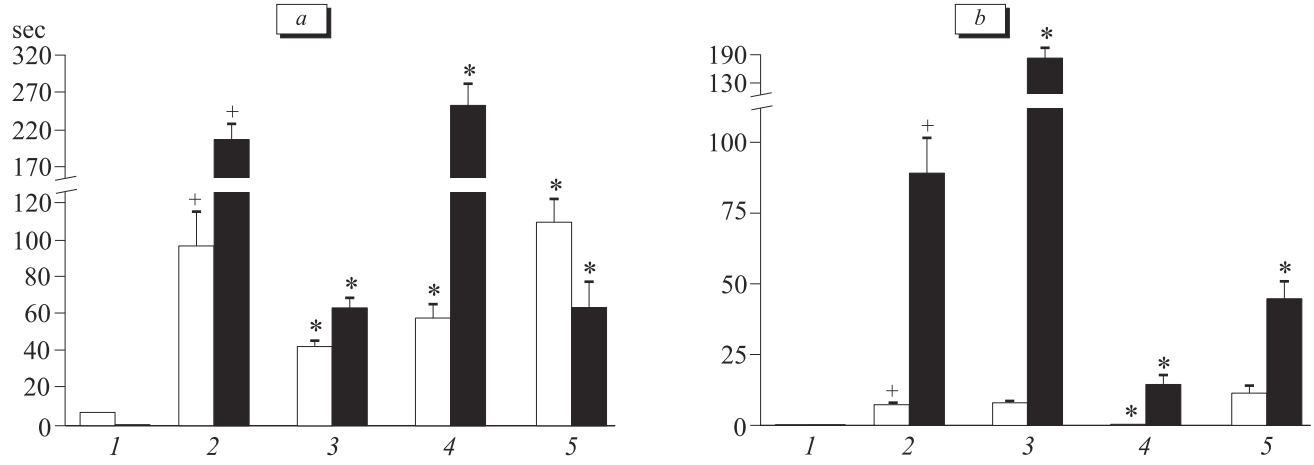

Fig. 1. Manifestation of painful reaction signs in rats after injection of drugs modifying activity of L-arginine-NO system. *a*) duration of licking reaction; *b*) number of spontaneous jerking in damaged paw and entire body. Here and in Fig. 2: light bars: phase 1; dark bars: phase 2 of pain. *1*) control; *2*) pain; *3*) L-NA; *4*) L-arginine; *5*) agmatine. $p<0.05$ compared to: +control; *pharmacologically intact group (pain).

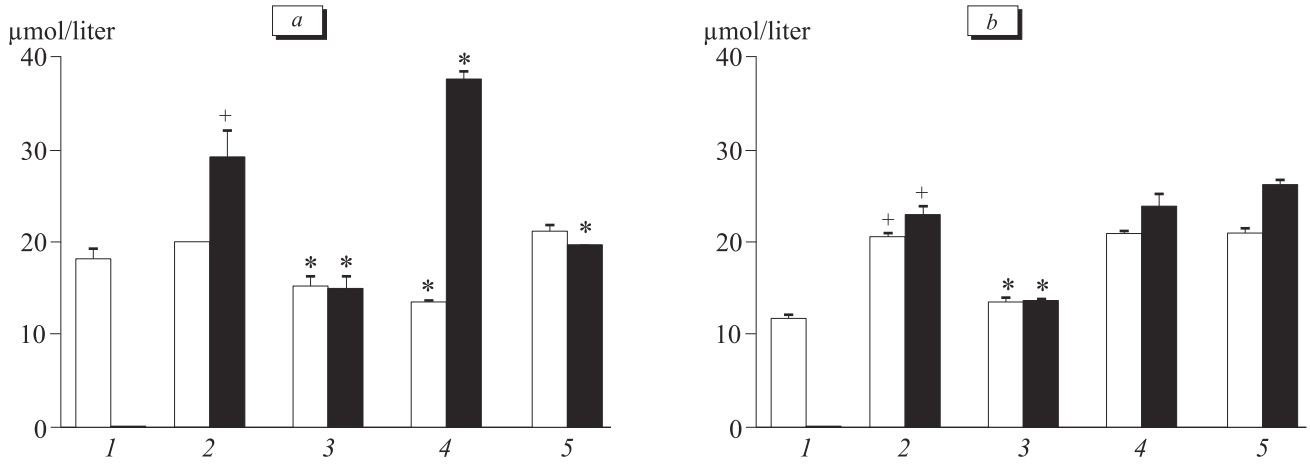

Fig. 2. Concentrations of NO metabolites (1 μmol/liter) in the peripheral blood (*a*) and liquor (*b*) of animals during pain and its pharmacological correction with substances modulating activity of L-arginine—NO system.

Premedication of rats with L-arginine led to development of a biphasic reaction to subsequent painful stimulation. A clear-cut analgesic effect of the drug was observed during the first 10 min: both signs of the nociceptive reaction were inhibited (Fig. 1). However, during the tonic pain phase the proalgesic effect predominated, which was seen from significant prolongation of the time of damaged paw licking, though spontaneous painful attacks during this period were significantly less severe than in rats with untreated pain. It seems that the effect of L-arginine is not always and not completely mediated by the effect of produced NO. Indeed, the dynamics of NOx biochemical shifts corresponded to the direction of the pharmacological effect of the drug only in the blood: decreased during the analgesic phase and increased significantly during the algogenic phase. On the other hand, the concentration of NO metabolites in the liquor corresponded to the parameters in pharmacologically intact animals. The pronociceptive effect of L-arginine during the tonic phase of pain in this case can be explained by production of excessive amount of nitroxide at the site of injury. The development of analgesic effect of the drug during the acute phase is obviously caused by activation of the metabolic pathways competitively utilizing L-arginine for substrate and leading to the synthesis of two endogenous analgesic substances, kyotorphin dipeptide [6] and agmatine [1].

In order to verify this hypothesis, we studied animal reactions after injection of agmatine (its analgesic activity was previously demonstrated on the model of chronic neuropathic pain [2]). The analgesic effect of the drug under conditions of acute inflammation was realized only during phase 2. During this period, the pain symptoms are suppressed against the background of significantly reduced concentrations of NO metabolites in the peripheral blood and their elevated concentrations in the liquor in comparison with pharmacologically intact animals (Fig. 1, 2). During phase 1 of pain, the nociceptive behavior pattern and biochemical shifts in the serum and cerebrospinal fluid did not differ from those in rats with untreated pain. Today the analgesic effect of agmatine is attributed to its effects on activities of imidazoline, α_2-adrenergic, and NMDA-glutamate receptors [2]. Our results suggest the presence of NO-dependent component in the structure of analgesic effect of the drug, realized during the tonic pain phase at the expense of NO-synthase inactivation in damaged tissues. Opposite changes in NOx concentrations in the peripheral blood and liquor during this phase are presumably explained by differentiated effects of agmatine on activities of endothelial, neuronal, and inducible NO-synthase forms [1].

Hence, acute inflammatory pain is associated with pronounced changes in endogenous production of NO, while the dynamics of NOergic activity in the plasma and cerebrospinal fluid corresponds to the stage of painful reaction. On the other hand, the type of changes in NO synthesis under conditions of pharmacological correction of pain by drugs modulating the L-arginine—NO system not always reflects the severity of painful syndrome and depth of forming analgesia. This indicates an intricate ambiguous role of NO in the system of integrative painful reaction, where its pro- or antinociceptive effect is determined by the stage of pain process and its local concentration in damaged tissues and nerve centers of segmentary and suprasegmentary levels.

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