Nociceptive Thresholds of the Response to Lipopolysaccharide Injection into the Limbic Structures of the Brain in Rats

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Microinjections of LPS into the limbic structures of rat brain (dorsal hippocampus and caudal compartment of the cingulate fascicle) caused opposite effects on the nociceptive thresholds: injection into the dorsal hippocampus enhanced perception and reduced the emotional affective perception of pain, while injection into the cingulate fascicle reduced the perceptual and enhanced the emotional components of the nociceptive reaction. These results indicated specific involvement of these limbic structures in nociception modulation during induction of the immune response in CNS.

Key Words: *nociceptive thresholds; lipopolysaccharide; cingulate fascicle; hippocampus*

The immune system is involved in the regulation of painful reactions during acute phases of various diseases and plays an important role in the formation of chronic painful syndromes. The immune status may determine the emergence of severe pain in patients with cancer and HIV infection [11]. The mechanisms of pain in inflammation and other pathological processes involving the immune reactions are sufficiently well studied. On the other hand, immunity changes may act as the initiatory factor determining some symptoms of the disease, including pain of unknown etiology. The origin of this pain or mechanisms of painful sensitization under conditions of primary modulation of the immune processes remain unclear.

We have previously shown that immunity stimulation by immunophane led to the development of hyperalgesia in rats. This treatment significantly reduced vocalization threshold (VT) in animals, a criterion of emotional reaction to nociceptive electrocutaneous stimulation. This sensitization partially explained the origin of pain of unknown etiology, the primary shifts in the immunity underlying its emergence [3]. A relationship between the immune status and pain threshold was detected [4]. However, the origin of pain remained unclear; it could be caused by initial changes in the immune processes with the involvement of brain structures. Pain perception and emotional evaluation are components of functional activities of the cerebral cortical and limbic structures. The involvement of the cingulate cortex and cingulate fascicle of the brain in the perceptual and emotional components of nociception have been revealed [2,8,12]. The contribution of the hippocampus to mechanisms of nociception (mainly the formation of the affective and emotional component of pain) has been persuasively demonstrated [14]. In addition, the hippocampus plays an important role in regulation of immune reactions. Stimulation or local damage to the dorsal hippocampal structures stimulate or inhibit, respectively, the immune response to bacterial antigens. Damage and hyperstimulation of the dorsal hippocampus may lead to complete discontinuation of antibody production in response to bacteria [6].

Despite numerous studies in this field, our knowledge of the central mechanisms of immunity-dependent regulation of pain remains insufficient. Therefore, studies of the central immunity-dependent mechanisms of nociception with participation of the limbic structures are important and perspective trends of experimental research. Lipopolysaccharides have been used for studies of nociceptive reactions under conditions of

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immune processes stimulation. Due to LPS presence in bacterial cell membranes, they are typical antigens and stimulants of immune reactions. Lipopolysaccharides stimulate and suppress the immune response [5]. They modulate the main effector cells of the immune system and promote the development of immune disorders. Injection of LPS is a natural model of immune processes stimulation, including those in the brain structures, where toll-like receptors located on various immunocompetent cells (astrocytes and glia in the CNS) play the key role in congenital immunity stimulation. After binding to the pathogen, toll-like receptors transmit the intracellular signal to stimulation of cytokine synthesis and stimulate the specific combinations of cytokines and co-stimulatory factors, this eventually determining the type and efficiency of the unfolding acquired immune response [9].

Russian drug pyrogenal is *Pseudomonas aeruginosa* cell LPS [7]. Pyrogenal is used to induce the immune response in healthy animals and to study its effects on painful sensitivity.

We studied the immune mechanisms of regulation of nociceptive reactions in rats in response to local injection of LPS into the limbic structures.

MATERIALS AND METHODS

Experiments were carried out on 40 male Wistar rats (250-300 g). The animals were handled in accordance with the Regulations for Studies with the Use of Experimental Animals, approved by the Ethic Committee of P. K. Anokhin Institute of Physiology (Protocol No. 1 of 3.09.2005), regulations of the World Society for Protection of Animals (WSPA), and European Convention for Protection of Experimental Animals.

Pyrogenal was injected into the caudal part of the cingulate fascicle – the site of formation of anatomic bonds between different structures of mammalian brain. The drug was also injected into CA1 field of the dorsal hippocampus (its functional activity determining the immune response realization).

Pyrogenal or saline (5 μl) was injected into the studied brain structures of animals with Hamilton's syringe fixed in a special injector. The LPS dose was 0.05 μg. Four groups were formed. Rats of control groups 1 (n=12) and 2 (n=10) were injected with saline into the dorsal hippocampus or caudal part of the cerebral cingulate fascicle, respectively. Animals of experimental groups 3 (n=7) and 4 (n=11) were injected with LPS into the respective brain structures. Scalping and microinjections were carried out using TSE systems for small laboratory animals. Pyrogenal or saline were injected according to the Atlas [15] coordinates into the dorsal hippocampus (AP=-5 mm, L=+2.5 mm, H=2.5-3 mm) or into the caudal part of the cingulate fascicle (AP=-4.5 mm, L=+1 mm, H=1.5 mm).

The perceptual component of nociception in rats was evaluated by the tail-flick method (the latent period of the tail hardening reaction (LP THR) to photothermal stimulation). The measurements were carried out on a Tail-Flick Analgesia Meter 0104-301M (Columbus Instruments). The animals were immobilized in plastic boxes. The nociceptive stimulus was presented 5 times at 3-5-min intervals and the mean LP THR was estimated (sec). The emotional component of nociception was evaluated by the rat VT (expressed in mA) in response to electrocutaneous stimulation of the tail, carried out with metal ring electrodes and SEN-3201 electrostimulator (Nihon Kohden). Electrostimulation parameters were as follows: 10 Hz frequency, 0.5 msec pulse duration. The strength of current (0.25-1 mA) was gradually increased until manifestation of vocalization reaction (squeak).

The basal values of the studied parameters were recorded after adaptation of animals in plastic boxes for 30 min. The rats were then narcotized with Nembutal (30 mg/kg intraperitoneally) and scalped. Pyrogenal or saline were injected through the trephination holes. The nociceptive thresholds were repeatedly recorded 7 days after the microinjections.

The data were processed by the standard statistical methods (Excel software) using parametrical tests. The significance of differences in the parameters was evaluated by Student's test. For small samples, the statistical hypotheses were verified using nonparametric Z test.

RESULTS

Registration of the initial nociceptive thresholds in rats showed a great variability thereof in a group: LP THR of 1.7-3.4 sec and VT 02-1.0 mA. Therefore, the mean initial nociceptive threshold values were taken for 100% for further analysis and curve plotting. Changes in the recorded values in response to drug injection were expressed in percent of the basal values.

Injections of saline into the dorsal hippocampus and caudal part of the cingulate fascicle of control animals caused no appreciable changes in the studied nociception parameters. Injection of saline increased LP THR in group 1 by 2% (from 2.34 to 2.39 sec; Fig. 1, *a*) and in group 2 by 4% (from 2.89 to 3.0 sec; Fig. 1, *b*). VT increased by 6% (from 0.7 to 0.74 mA; Fig. 2, *a*) in response to saline injection into the hippocampus and decreased negligibly by 11% (from 0.84 to 0.75 mA; Fig. 2, *b*) after its injection into the cingulate fascicle.

Local injection of LPS into the dorsal hippocampus led to a significant reduction of LP THR by 10% (from 3.07 to 2.77 sec; Fig. 1, *a*). Injection of pyroge-

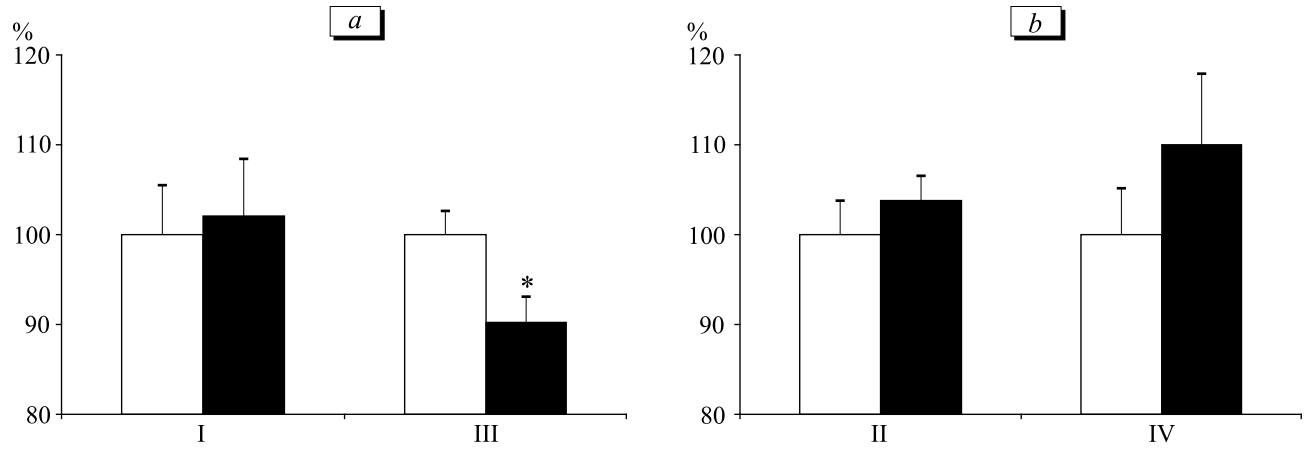

Fig. 1. Tail-flick test (LP THR) in rats before (light bars) and after (dark bars) injection of saline (I, II) or pyrogenal (III, IV) into the dorsal hippocampus (*a*) and cingulate fascicle (*b*). Here and in Fig. 2: I: group 1; II: group 2; III: group 3; and IV: group 4. *$p<0.05$ in comparison with the initial value.

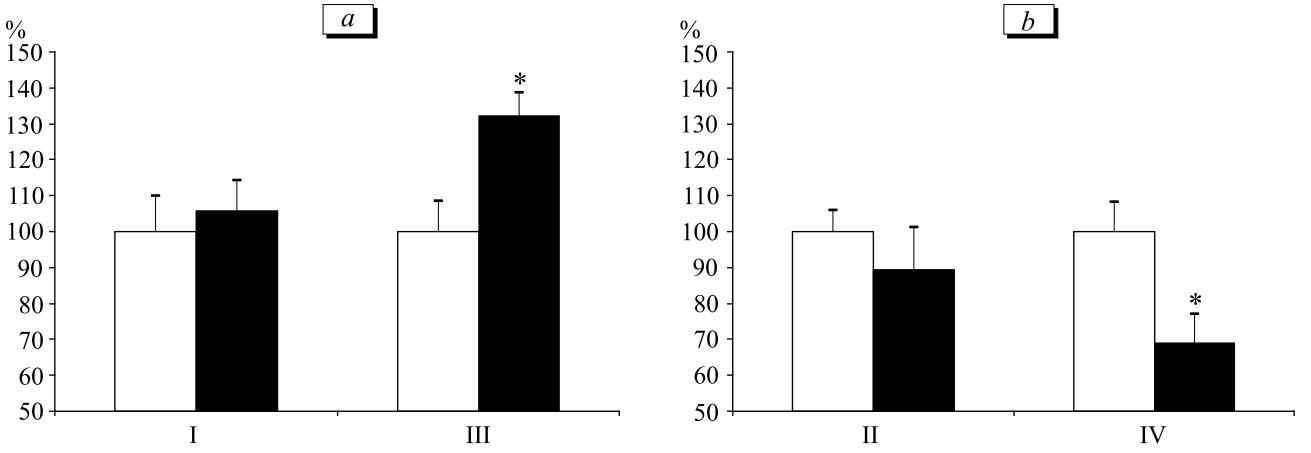

Fig. 2. Vocalization threshold in rats before (light bars) and after (dark bars) injection of saline (I, II) or pyrogenal (III, IV) into the dorsal hippocampus (*a*) and cingulate fascicle (*b*).

nal into the cingulate fascicle caused an 11% increase of this parameter (from 2.63 to 2.92 sec; Fig. 1, *b*). Evaluation of emotional perception showed that injection of LPS into the dorsal hippocampus increased VT significantly, by 32% (from 0.56 to 0.74 mA; Fig. 2, *a*), while its injection into the cingulate fascicle reduced the parameter by 32% (from 0.61 to 0.42 mA; Fig. 2, *b*).

Our results confirmed significant changes in the nociceptive thresholds in response to central injection of LPS. The modulatory effects of the studied limbic structures on nociception parameters were somewhat reciprocal. The data on the afferent/efferent bonds and on differences in neurotransmission in these compartments of the limbic system and their astrocytic and microglia environment could presumably explain these facts. Astrocytes and microglia (the main immunocompetent cells of the CNS) could be directly involved in painful reaction regulation. Stimulated astrocytes regulated through cytokines virtually all types of stimulation neurtransmission: dopamine-, serotonin-, norepinephrine, cholin-, and opioidergic. They produced opioid peptides, contained μ-, δ-, and κ-opioid receptors, increased the proenkephalin synthesis in response to cytokines. It was shown that IL could stimulate the cerebral opioid mechanisms of the brain, which, in turn, modulated activities of the peripheral cytokines and hence, opioid production. Astrocyte effects on the mechanisms of glutamatergic synaptic transmission were lasting; they regulated glycine and GABA content in the synaptic fissure via the inverse capture mechanism. Under certain conditions, astrocytes released glutamate, increasing the neuronal excitability [1].

The studied limbic structures are anatomically and neurochemically complex formations. The cingulate fascicle includes various groups of transmitting fibrils, ascending from the parietal and frontal cortex,

cingulate girus (areas 23 and 24), frontal and laterodorsal thalamic nuclei. The cingulate fascicle contains the cortico-thalamic fibrils and associative fibrils to all limbic structures. Hence, the cingulate fascicle includes thalamic, cingulate, and associative fibrils, their terminals have serotonin-, glutamate-, and opioidergic projections. In addition, the hippocampus is a polyfunctional structure involved in the training, memory, attention, and pain processes [13,14]. Neurotransmitters (acetylcholine, opioid peptides and GABA) contribute to the hippocampal modulatory effects on nociception [10]. Intricate interactions between the mediator systems and LPS-stimulated neuroglia may cause opposite modulatory effects of these structures on nociception parameters, which presumably indicates their specific involvement in the development of the immune component of total systems nociceptive reaction.

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