Autoantibodies against Glutamate, γ-Aminobutyric Acid, and Norepinephrine in Mechanisms of Neuropathic Pain Syndrome

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The production and role of autoantibodies against neurotransmitters glutamate, γ-aminobutyric acid, and norepinephrine were studied in rats with experimental neuropathic pain syndrome induced by sciatic nerve transection. Nerve transection in rats was followed by behavioral reaction of autotomy (self-mutilation of the operated limb), which often accompanies neuropathic pain syndrome. The development of neuropathic pain syndrome was accompanied by increased production of autoantibodies against glutamate, γ-aminobutyric acid, and norepinephrine. A negative correlation was found between the amount of autoantibodies against neurotransmitters and severity of neuropathic pain syndrome. Our results suggest that antibodies against glutamate and norepinephrine exhibit protective activity.

Key Words: *neuropathic pain syndrome; antibodies against neurotransmitters; glutamate; γ-aminobutyric acid*

Published data and results of our previous studies indicate that immune factors play an important role in the pathogenesis of neuropathic pain syndromes. Exogenous hyperalgesic factors (damage, stimulation, *etc*.) stimulate the immune system to produce specific algogens, including proinflammatory cytokines interleukin-2 (IL-2), IL-6, and tumor necrosis factor-α. They activate nociceptive neurons of the spinal cord and brain [14]. Increased production of autoantibodies against serotonin and catecholamines was observed in animals with neuropathic pain syndrome. Previous experiments showed that immunization of animals with neurotransmitter-protein conjugates produced a pronociceptive effect [3,4]. The excitatory neurotransmitter glutamate (Glu) and inhibitory neurotransmitter γ-aminobutyric acid (GABA) play an important role in the mechanisms of nociception [8,12,13].

Here we studied production and role of autoantibodies against Glu, GABA, and norepinephrine (NE) in experimental neuropathic pain syndrome.

MATERIALS AND METHODS

Experiments performed on 50 male Wistar (220-250 g) according to the rules of researches with animals (Ethics Committee of the International Association for the Study of Pain). The rats were maintained in a vivarium under standard conditions and natural light/dark cycle and had free access to water and food. The animals were divided into 3 groups: group 1, intact rats (n=10); group 2, sham-operated rats (n=10); and group 3, treated rats (n=30) with neuropathic pain syndrome. Neuropathic pain was induced by sciatic nerve transection an the hind-limb. The development of pathological pain syn-

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drome was verified by autotomy (self-mutilation of digits of the operated limb). The severity of pathological changes was estimated by the standard scale for foot tissue injury in the operated paw [10]. A comparative study was performed with sham-operated animals (pathophysiological control, group 2): incision of the skin and soft tissues, exposure of the nerve, and layer-by-layer closure of the wound. The state of animals was monitored daily over 28 days after surgery. Autoantibodies against neurotransmitters in blood plasma from group 1 and 2 animals were assayed on days 14 and 28 after surgery. ELISA was performed on polystyrene plates sensitized with the corresponding test antigens. The test antigens (Glu-BSA, GABA-BSA, and NE-BSA) were synthesized as described elsewhere [1,3,4]. The amount of autoantibodies was estimated from the optical density of blood plasma at 495 nm (MINI-READER, DYNATECH) and expressed in arbitrary units of activity. The parameter K was calculated as the ratio of the optical density of blood plasma from each treated animal to the mean optical density of blood plasma from intact rats. Autoantibodies were present at $K>1$. Blood plasma from treated rats was assayed for autoantibodies against the carrier protein (BSA). Anti-BSA autoantibodies were not found.

The results were analyzed by unpaired parametric Student's t test and Fischer test.

RESULTS

Sciatic nerve transection was followed by the development of neuropathic pain syndrome in 23 of 30 animals of the treatment group. On day 14 after surgery, autotomy was observed in 22 of 30 rats of group 3. The severity of pain syndrome was 5.90±0.79 points (Fig. 1). Autotomy (7.87±0.94 points) was revealed in 23 rats (76.6%) on day 28 after surgery. Autotomy was absent in group 2 animals.

Immunological testing produced a surprising result. On day 14 after surgery, autoantibodies against neurotransmitters were found in treated and sham-operated rats (60-75% animals, Table 1). The production of autoantibodies against Glu and GABA significantly decreased on day 28 after surgery (except for anti-NE autoantibodies). The production of anti-GABA autoantibodies decreased most significantly in group 3 animals (Table 1).

The frequency of detection of autoantibodies was compared in animals with different severity of neuropathic pain syndrome to estimate the pathogenetic relationship between anti-neurotransmitter autoantibodies and pain. Table 2 shows the frequency of detection of autoantibodies against neurotransmitters in animals with different severity of the pain syndrome (Table 2). Autoantibody production was maximum in animals with the lowest degree of pathological changes. Autoantibodies against neurotransmitters were revealed in 91.7% animals with pain syndrome of 1-5 points. However, the frequency of detection of autoantibodies against Glu and GABA was much lower in rats with the highest score of autotomy (6-11 points). The frequency of detection of anti-Glu antibodies decreased by 1.9 times by the end of the study. Anti-GABA autoantibodies were not detected in animals without autotomy. Production of anti-NE autoantibodies remained unchanged in this period, which is prob-

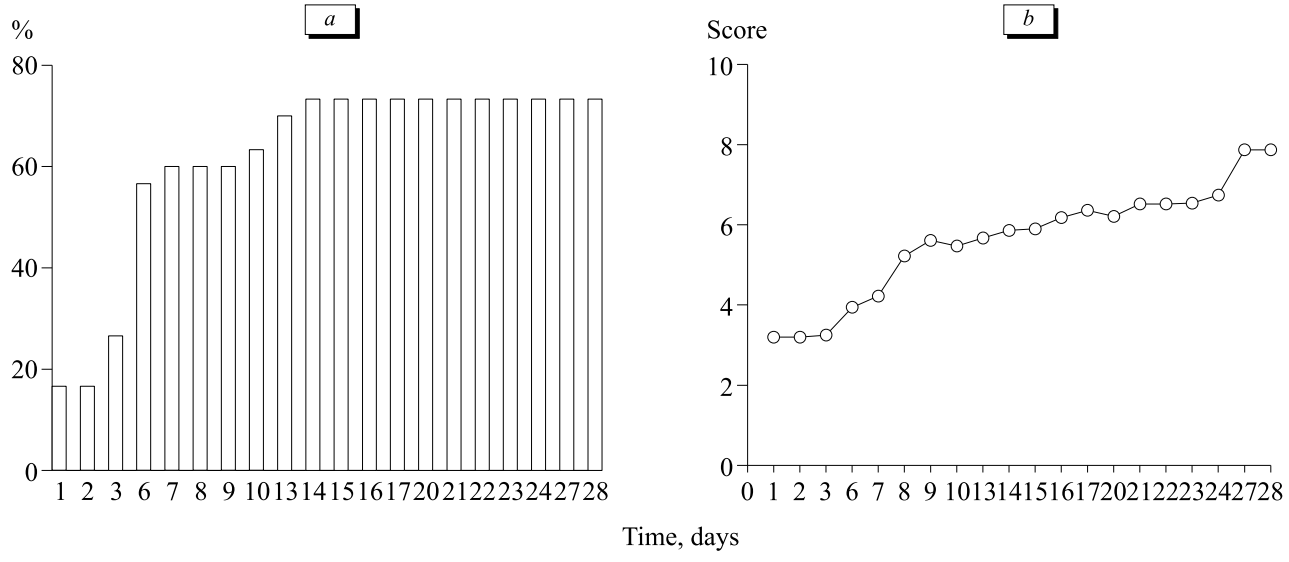

Fig. 1. Development of pain syndrome in rats: animals with autotomy (*a*); and severity of pain syndrome (*b*).

TABLE 1. Frequency of Autoantibodies against Neurotransmitters during Experimental Neuropathic Pain Syndrome

Group	Anti-Glu autoantibodies		Anti-GABA autoantibodies		Anti-NE autoantibodies	
	day 14	day 28	day 14	day 28	day 14	day 28
Treatment						
frequency, %	64.5**	41.9*+	66.7**	17.2+	73.3**	60.0*
K, arb. units	1.46±0.06*	1.45±0.09*	1.45±0.06*	1.55±0.02*	1.56±0.09*	1.57±0.10*
Sham operation						
frequency, %	60**	30	60**	30	50*	50*
K, arb. units	1.41±0.06*	1.5±0.2*	1.78±0.27*	1.58±0.19*	1.5±0.2*	1.54±0.12*

Note. *p<0.05 and **p<0.001 compared to intact animals; +p<0.05 compared to the previous test.

TABLE 2. Frequency of Autoantibodies against Neurotransmitters during Experimental Neuropathic Pain Syndrome (Depending on the Severity of Pain Syndrome, Autotomy)

Autoantibodies	Severity of autotomy, points					
	0		1-5		6-11	
	number	frequency, %	number	frequency, %	number	frequency, %
Day 14 after surgery						
against Glu	9	55.5	12	91.7	9	33.3+
against GABA	9	44.4	12	91.7	9	55.5+
against NE	9	55.5	12	91.7	9	77.8
Day 28 after surgery						
against Glu	7	28.6	9	55.5*	14	42.8
against GABA	7	0**	9	33.3**	14	14.2*
against NE	7	85.7	9	66.6	14	57.1

Note. *p<0.05 and **p<0.001 compared to the previous test (dynamics); +p<0.001 compared to the severity of 1-5-point pain syndrome.

ably related to the stress component of neuropathic pain syndrome [8,11].

The following phenomena require further detailed investigations: production of autoantibodies against neurotransmitters during nerve damage; presence of autoantibodies against neurotransmitters in rats without neuropathic pain syndrome; and increased synthesis of autoantibodies against Glu, GABA, and NE in sham-operated animals. Production of autoantibodies against neurotransmitters is associated with the appearance of endogenous conjugated antigens (neurotransmitter and protein) due to dysfunction of brain neurotransmitter systems under conditions of nociceptive stimulation [5,6,7]. It should be emphasized that production of autoantibodies cannot differ in animals without pain syndrome. The animals of all groups (pain syndrome, no pain syndrome, and sham operation) are characterized by operative trauma, which induces nociceptive afferentation. It results in similar neurochemical changes in the corresponding brain structures (hypothalamus, thalamus, somatosensory cortex, and other central regulators of neuroimmune interactions).

A negative correlation was found between autoantibody production and severity of neuropathic pain syndrome (Table 2). These data suggest that autoantibodies against Glu and NE produce a protective effect. We revealed reduced production of anti-GABA autoantibodies increasing (accoding to our findings) the severity of neurogenic pain syndrome [9]. Our results are consistent with published data that local (intrathecal) application of anti-Glu antibodies has a protective effect under conditions of spinal pain syndrome [9]. Hence, production of autoantibodies against neurotransmitters serves as a mechanism of regulation of nociceptive afferentation during trauma. Examination of patients with osteochondrosis of the lumbar spine and pain syndrome revealed a protective role of anti-Glu auto-

antibodies. The frequency of these antibodies was highest during pain syndrome of short duration and low severity [2]. The role of autoantibodies against neurotransmitters in the pathogenesis of neuropathic pain in humans requires further investigations.

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