Effect of Lamotrigin on the Development of Neurogenic Pain Syndrome in Rats

M. L. Kukushkin, E. I. Danilova, V. N. Grafova, V. S. Smirnova, V. K. Reshetnyak

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Analgesic activity of a new anticonvulsive agent lamotrigin was studied on the model of neurogenic pain syndrome produced in rats by penicillin applied to the dorsal surface of the spinal cord and by dissection of the sciatic nerve. Lamotrigin was shown to have a profound analgesic activity. It can be used as an efficient prophylactic agent for prevention of chronic pain syndromes by suppression of the generators of pathologically enhanced excitation in the nociceptive structures which are the pathophysiological basis of the chronic pain syndromes.

Key Words: *lamotrigin; neurogenic pain syndromes; nociceptive neurons*

Neurogenic pain syndromes appear due to damage to peripheral or central structures of the nervous system that participate in conduction of nociceptive signal [7]. They are characterized by prolonged painful seizures which cannot be arrested by conventional analgesics [2,3]. The pathophysiological basis of neurogenic pain syndromes is formation of the generators of pathologically enhanced excitation which are the aggregates of interacting hyperactive neurons producing an anomalous impulse traffic in the structures responsible for regulation of pain sensitivity. Formation of such generators results from suppression of inhibitory reactions in the central nervous system structures, which is mediated by glycin and γ-aminobutyric acid [2] and enhancement of the effect of excitatory amino acids on the nociceptive neurons via NDMA and non-NDMA receptors [7, 11]. Logically, the treatment of the neurogenic pain syndromes by administration of antiepileptic drugs (carbamazepine, phenytonin, clonazepam, diazepam, etc.), which eliminate the pathological hyperactivity of nociceptive neurons, is substantiated by pathogenic reasons [10].

In this work we study the effect of a novel antiepileptic preparation lamotrigin (LT) on the development of neurogenic pain syndromes, which were elicited by application of a convulsive agent (penicillin) to the dorsal surface of the spinal cord as well as by transection of the sciatic nerve.

MATERIALS AND METHODS

Experiments were carried out on 73 outbred albino rats weighing 180-200 g. The ethic requirements of International Association of Study of Pain were adhered to in the experiments. The spinal pain syndrome (SPS) was produced with the help of benzyl penicillin sodium salt dissolved in 1% agar, which was applied under ether narcosis unilaterally to the dorsal surface of the lumbar segments in the spinal cord (L_{IV}-L_V). After application of the convulsant, the wound was sutured, and the animal was placed into a glass cage for observation. The degree of SPS was evaluated by a 3-point scale [1].

The neuropathy pain syndrome (NPS) was produced by transecting of the sciatic nerve under ether narcosis at the level of the popliteal fossa and placing

Laboratory of Physiology of Pain, Institute of General Pathology and Pathological Physiology, Russian Academy of Medical Sciences, Moscow

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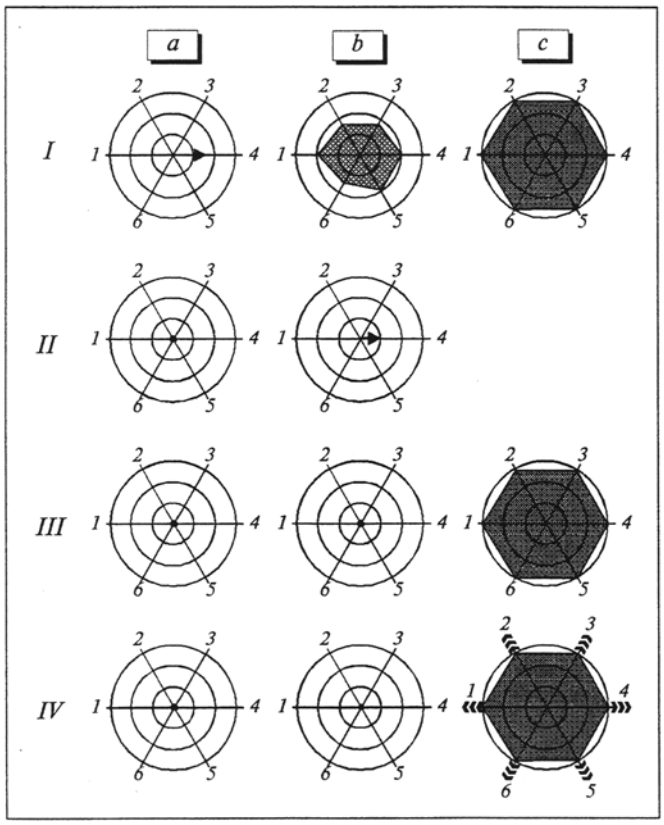

Fig. 1. The effects of lamotrigin during spinal pain syndrome. Spinal cord application of (*I*) penicillin; (*II, a*) Lamotrigin, 10 mg/ml; (*II, b*) lamotrigin, 25 mg/ml; (*III*) penicillin+lamotrigin, 10 mg/ml and (*IV*) penicillin+lamotrigin, 25 mg/ml. Arrow indicates: *a*) response to 1.5 point provocation, spontaneous pain seizures absent; *b*) response to 1.0 point provocation, spontaneous pain seizures absent; *c*) enhancement of the pain syndrome signs above the control. Penicillin dosage (*I, III,* and *IV*), *a*) 3000 U, *b*) 8000 U, and *c*) 15000 U. *1*) Number of seizures in 1 min; *2*) duration of a single seizure, sec; *3*) interval between seizures, sec; *4*) response to provocation, points; *5*) vocalization: weak short squeak, 1 point; shrill scream during entire seizure, 3 points; *6*) motor activity: 1-2 runs during a seizure, 2 points; persistent running and jumping in the cage during entire seizure, 3 points. The degree of signs is represented in a 3-point scale counted from the center of diagrams and marked with the respective circles: no signs, 0 point; light degree, 1 point; middle degree, 2 points; severe degree, 3 points.

the central end of it into a polyethylene capsule. The syndrome was assessed according to the moment of appearance of autotomies in the operated paw, which were quantified by an 11-point scale [5].

SPS was evoked by a single application of agar plate with LT (Lamictal, Galaxo-Wellcome, 10 and 25 mg/ml) and penicillin to the dorsal surface of the spinal cord (segments L_{IV}-L_V). In the control rats a penicillin-free agar plate was applied to the same region.

In NPS rats LT was administered 2 times a day according to the next scheme: 1st week, 10 mg/ml, 2nd week, 20 mg/ml, 3rd and 4th weeks, 30 mg/ml. The drug was given both preventively (from the 1st day after transection of sciatic nerve) and with the therapeutic aim (from the 12th day after operation). The hot plate and tail flick tests were used to study changes in the nociception thresholds in NPS rats. The results were statistically analyzed using Student's *t* test for small samples.

RESULTS

Application of penicillin to the dorsal surface of spinal cord lumbar segments caused behavioral nocifensive reaction dependent on the convulsant dose. The paw jerking reaction appeared 25-30 min after application of 3000 U penicillin to segments L_{IV}-L_V, which was accompanied by vocalization in response to light tactile stimulation applied to the femoral dorsal surface, i.e., the rats demonstrated mechanical allodynia (Fig. 1, *I, a*). Tactile stimulation of other parts of the body did not produce nocifensive reaction. An increase in penicillin dose up to 8000 U resulted in anxiety 20 min after application, which was manifested in vocalization and persistent running. The rats licked the region of the leg corresponding to the zone of convulsant application. Tactile stimulation of this region caused a 2-point nociceptive seizure (Fig. 1, *I, b*). Tactile stimulation of other parts of the body resulted in motor anxiety and vocalization. Application of penicillin in a dose of 15000 U led to a 3-point nociceptive syndrome accompanied by strong motor excitation and marked vocalization. The rats licked and gnawed the region of the leg corresponding to the nociceptive zone projection (Fig. 1, *I, c*). The paroxysmal nature of nocifensive reaction was clearly demonstrated. The seizures appeared spontaneously; they could be also elicited by tactile stimulation of any part of the body or by sharp sound.

Unilateral application of LT to the dorsal surface of lumbar segments in a dose of 10 mg/ml in the control (intact) rats did not produce behavioral reactions (Fig. 1, *II, a*). An increase of LT dose to 25 mg/ml caused allodynia in the ipsilateral hind leg in 60% rats (Fig. 1, *II, b*).

Combined application of LT (10 or 25 mg/ml) with penicillin (3000 or 8000 U) prevented the development of SPS (Fig. 1, *III, a, b*; *IV, a, b*). In combination with 15000 U penicillin, LT (10 mg/ml) augmented SPS (Fig. 1, *IV, c*).

In 2 out of 10 rats with transected sciatic nerve (control), the appearance of autotomies indicating the development of NPS was observed on day 4 after operation. n postoperation day 12, the autotomies were observed in 6 rats, and on postoperation day 30, NPS was documented in all rats. The NPS development was accompanied by a decrement of noci-

ception thresholds in the hot plate and tail flick tests (Table 1) and by an increase in the intensity of autotomies (Fig. 2).

Administration of LT immediately after transection of the sciatic nerve (1st test group) changed drastically the dynamics of NPS development. The autotomies in the 1st group rats appeared on day 5 after transection, 4 rats demonstrated NPS on day 12, 6 on day 20, and only 2 out of 10 rats had autotomies on day 50 after operation. Preventive administration of LT not only stave off the development of NPS in 40% rats, but also decreased markedly the intensity of autotomies, which was significantly lower than in the control (Fig. 2). In this case there was no decrement in nociceptive thresholds (Table 1).

To study the therapeutic effect of LT, 10 rats with transected sciatic nerve were chosen (2nd test group), which had 2-3 point autotomies on postoperation day 12. It is noteworthy that NPS dynamics in the second group did not significantly differ from the control up to day 30, i.e., during the entire period of administration of the drug. In the 2nd group, LT affected neither intensity of the autotomies (Fig. 2) nor the decrement of nociceptive thresholds (Table 1). After cancellation of LT, the intensity of autotomies in the 2nd group was larger than in control (Fig. 2).

These data show that LT effectively prevents the development of neurogenic pain syndromes. In the SPS model the analgesic effect of LT was observed when penicillin was applied to the dorsal surface of the spinal cord in doses of 3000 and 8000 U, but when the concentration of the convulsant was 15000 U, the analgesic effect was not observed.

Taking into consideration the fact that penicillin disturbs GABA-mediated inhibition when applied to the dorsal surface of the spinal cord [2,8], it can be suggested that the development of SPS resulted from

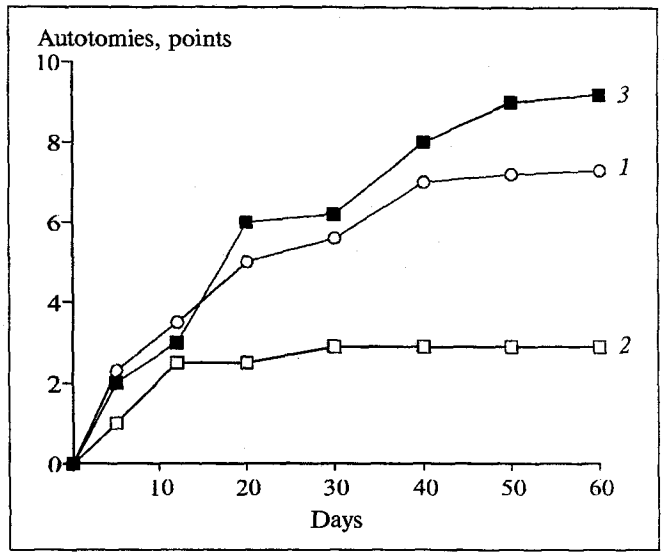

Fig. 2. Effects of lamotrigin during neuropathic pain syndrome. *1*) control; lamotrigin from 2) postoperation day 1 and 3) post operation day 12.

hyperactivation of the broad dynamic range nociceptive neurons caused by augmentation of the excitatory influence of the low-threshold A-β afferents. This is substantiated by appearance of allodynia, i.e., nocifensive behavior in response to tactile stimulation in the corresponding cutaneous regions after application of penicillin to the dorsal surface of the spinal cord. Consequently, the analgesic effect of LT, which controls the extra release of excitatory amino acids (glutamate predominantly) due to blockage of voltage-dependent Na channels at the neural presynaptic membranes [9,13,14] is most probably related to restriction of excitatory influence of A-β afferents on the broad dynamic range neurons in the dorsal horns of spinal cord.

The inefficiency of LT in rats with SPS produced by 15000 U penicillin applied to the dorsal surface of the spinal cord resulted from extreme

TABLE 1. Changes in Pain Sensitivity Thresholds during Neuropathic Pain Syndrome under the Effect of Lamotrigin (*M*±*m*)

Days	Hot plate test			Tail flick test		
	control	LT from day 1 of operation	LT from day 12 of operation	control	LT from day 1 of operation	LT from day 12 of operation
Prior to operation	8.8±1.3	9.0±1.0	8.8±1.1	4.2±0.6	4.0±0.7	4.0±0.5
5	7.0±0.9	8.8±1.3	6.4±1.0*	2.8±0.4*	2.7±0.5*	3.1±0.6
12	6.6±0.9*	8.4±1.0	5.6±1.0*	2.7±0.4*	2.8±0.4*	2.4±0.3*
20	6.0±0.8*	8.2±0.9	5.6±0.9*	2.9±0.6*	2.9±0.7*	2.5±0.4*
30	5.1±0.8*	8.4±1.1	7.0±1.0*	2.9±0.5*	3.2±0.5	2.8±0.7*
40	7.0±1.2	8.2±1.0	8.0±1.4	3.8±0.7	3.2±0.6	3.9±1.0
50	7.2±1.1	8.2±1.1	8.2±1.3	3.8±0.4	3.2±0.5	3.8±0.4
60	7.6±0.9	8.8±1.0	8.0±1.1	4.0±0.8	3.8±0.5	3.5±0.6

Note. *$p<0.05$ relative to pain thresholds prior to operation.

power of generator of pathologically enhanced excitation, which sensitizes nociceptive neurons in the thalamus and sensorimotor cortex [4,12], so that the nociceptive paroxysms in animals could be provoked virtually from tactile stimulation of any part of the body or by sound signals [6].

The appearance of allodynia after application of LT in a dose of 25 mg/ml to the spinal cord, as well as augmentation at this LT dose of the nocifensive paroxysms provoked by 15000 U penicillin could be related to LT-caused decrease in the release of monoamines (5-hydroxytriptamine, dopamine, and norepinephrine) and enkephalins, i.e., neurotransmitters that mediate the descending inhibition of nociceptive neurons in the dorsal horns of the spinal cord [6].

Prophylactic effect of LT was observed in rats with NPS. Administration of LT from the 1st day of transection of the sciatic nerve not only drastically moderated NPS, but decreased markedly the number of animals with autotomies, while there was no analgesic effect in rats given LT after development of NPS. It favors the hypothesis that the excitatory amino acids released from presynaptic terminals of primary afferents are the key link in provoking pathological synaptic, non-synaptic, and intraneuronal processes. They lead to formation of generator of pathologically enhanced excitation in dorsal horns of the spinal cord, thereby inflicting severe disturbance in the work of neural ensembles [4].

Our data agree with the results of other researchers [15] who demonstrated analgesic effect of LT in the models of acute and chronic hyperalgesia caused by subcutaneous application of prostaglandin E_2.

From our findings it can be concluded that LT can be used as an efficient preventive agent blocking hyperactivity of nociceptive neurons caused by various peripheral damage, thereby arresting formation of the generators of pathologically enhanced excitation, which are the pathophysiological basis of the chronic pain syndromes in the structures of the nociceptive system.

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