

Lamotrigine effectively blocks synaptic transmission between nociceptive primary afferents and secondary sensory neurons in the rat superficial spinal dorsal horn

ZSÓFIA ANTAL, PÉTER SZÜCS, MIKLÓS ANTAL*

Department of Anatomy, Histology and Embryology, Medical and Health Science Center,
University of Debrecen, Debrecen, Hungary

*Corresponding author: Miklós Antal; Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical and Health Science Center, University of Debrecen, Nagyterdei krt 98, H-4032 Debrecen, Hungary; Phone: +36-52-255567; E-mail: antal@anat.med.unideb.hu

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Abstract: It has been demonstrated that in the superficial spinal dorsal horn, Lamotrigine, which is known to block voltage-sensitive Na⁺ and N-type Ca²⁺ channels, depresses neural activities evoked by sustained activation of nociceptive primary afferent fibres. In the present experiments, we study how Lamotrigine exerts its inhibitory effect on spinal nociceptive information-processing mechanisms. We show that Lamotrigine in an *in vitro* slice preparation effectively blocks synaptic transmission between primary afferents and secondary sensory neurons. Together with the robust increase in the failure rate and reduction in the amplitude of excitatory post-synaptic potentials (EPSPs) evoked by stimulation of nociceptive primary afferents, Lamotrigine causes a marked decrease in the number and amplitude of spontaneous EPSPs and a gradual shift of the resting membrane potential towards hyperpolarization. In addition, Lamotrigine treatment also changes the intrinsic firing pattern of superficial dorsal horn neurons. The results suggest that the effect of Lamotrigine on spinal nociceptive information-processing mechanisms is multiple: it depresses synaptic inputs from nociceptive primary afferents to secondary spinal sensory neurons and also weakens the intrinsic activities of nociceptive spinal neural circuits in the superficial spinal dorsal horn.

Keywords: Lamotrigine, synaptic transmission, nociceptive primary afferents, spinal cord, dorsal horn, *in vitro* electrophysiology

Introduction

A great deal of experimental evidence supports the idea that Lamotrigine, which is mostly used as an antiepileptic drug for certain forms of epilepsy [1, 2], elicits its effect through stabilization of neuronal membranes by blocking voltage-sensitive Na⁺ and N-type Ca²⁺ channels [3–5] and inhibiting the pre-synaptic release of glutamate [6].

It has also been demonstrated that Lamotrigine inhibits superficial dorsal horn neuronal activities evoked by sustained activation of nociceptive primary afferents [7, 8]. In clinical trials and animal pain models, Lamotrigine had no analgesic effect on acute thermal nociception but relieved chronic neuropathic pain of central and peripheral origin, suggesting that its analgesic effect depends on nerve injury-associated events [9, 10].

In the present experiments, we study how Lamotrigine exerts its inhibitory effect on spinal nociceptive information-processing mechanisms and investigate the effects of Lamotrigine on synaptic transmission between nociceptive primary afferents and spinal secondary sensory neurons in naive (non-neuropathic) animals.

Materials and Methods

Preparation of spinal cord slices

Experiments were carried out on P18–P34 Wistar rats (Charles Rivers Laboratories, Gödöllő, Hungary). Under deep ether anesthesia, the animals were decapitated and the spinal cords removed. A block of the spinal cord containing spinal segments L1–L5 was dissected, and 500–700-mm thick transverse slices with the dorsal roots (6–12 mm long) attached were cut with a Vibratome. The slices were incubated at room temperature in a chamber containing normal ACSF (NaCl, 126 mM; KCl, 3 mM; CaCl₂, 2 mM; MgCl₂, 2 mM; NaH₂PO₄, 1.3 mM; NaHCO₃, 26 mM; Glucose, 10 mM) for at least 1 h prior to recording.

Electrophysiological recording

In the recording chamber, slices were continuously perfused with ACSF (perfusion rate: 2–3 mL/min). Neurons in lamina II were visually selected under a Zeiss Axioskop

FS microscope equipped with a $\times 40$ DIC objective and recorded in whole-cell current-clamp or voltage-clamp configuration with an Axopatch-1D amplifier (Axon Instruments, Union City, CA, USA) by using 7–10 M Ω glass electrodes (Harvard Apparatus Ltd, Kent, UK). The intracellular solution in the electrodes contained (in mM) K-gluconate, 126; KCl, 4; ATP-Mg, 4; GTP- Na_2 , 0.3; Na_2 -phosphocreatine, 10; HEPES, 10 and 0.5% biocytin (Sigma, St. Louis, MO, USA) at pH 7.2.

Firing patterns of neurons were obtained by applying 800-ms long incrementing current steps ranging from –30 to 130 pA. The dorsal root was stimulated via a suction electrode at 0.2 and 1 Hz with stimulation strength of 450–2000 μA (Fig. 1A). The stimulation strength was always set at a value that was 1.2 times higher than the threshold capable of eliciting excitatory post-synaptic potentials (EPSPs) in the recorded post-synaptic neuron. To test the monosynaptic nature of the connection, we used the criteria of Kawasaki et al. [11]. Conduction velocity of the stimulated primary afferent was calculated by dividing the length of the dorsal root by the delay between the stimulation artifact and the onset of the evoked EPSP. Primary afferents were considered to be C- and A-delta fibres if the conduction velocities were in the range of 0.3–0.8 m/s and 3.8–15 m/s, respectively. Amplitude of the evoked EPSP was measured, along with the baseline membrane potential. Failure rates were calculated for 10 consecutive trials by counting the number of trials in which the electrical stimulation of the dorsal root failed to evoke an EPSP in the post-synaptic neuron. Spontaneous EPSPs and excitatory post-synaptic currents (EPSCs) that reflect network activity of neural circuits in the dorsal horn were detected, and their amplitudes were plotted against time. All recordings were performed at room temperature. Data were filtered at 5 kHz, recorded on the hard disk of a computer and analyzed off-line after the recording session.

Lamotrigine application

Lamotrigine (Sequoia Research Products, UK) was applied to the bath in 100 and 200 μM concentrations [6] for 10–15 min.

Morphological analysis of the recorded neurons

To reveal the laminar localization and morphological characteristics of the recorded neurons, after the recording session the slices were processed further for morphological analysis. Following fixation in 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (pH 7.4), serial sections were cut from the slices at 60 μm with a Vibratome. To reveal biocytin that diffused into the cell during the physiological recording, the sections were treated with streptavidin conjugated with Alexa-532 fluorochrom. The sections were mounted, covered with Vectashield and investigated in an Olympus 1000 confocal microscope.

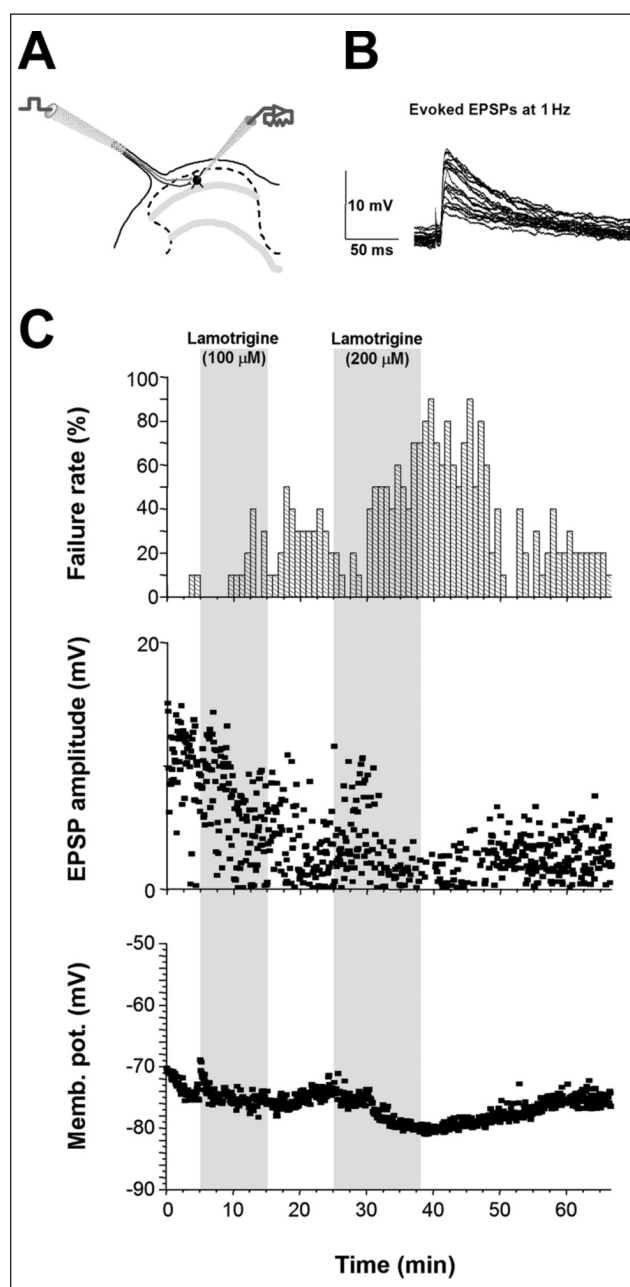


Fig. 1. Effect of Lamotrigine on EPSPs evoked by primary afferent stimulation. A: Schematic drawing showing the recording configuration. B: Cumulative records of evoked EPSPs. Conduction velocity, calculated from the latency of the response, suggests that this neuron received input from C primary afferent fibres. C: Failure rate (top), EPSP amplitude (middle) and membrane potential (bottom) change along the course of the experiment. During the first application of Lamotrigine (100 μM), failure rate increased to ~50%, while EPSP amplitudes and the membrane potential of the recorded neuron decreased gradually. At the end of the first washout period (overlapping with the initial 5 min of the repeated drug application period), the changes were slightly reversed. The second application of the drug (200 μM) almost completely abolished evoked EPSPs and reduced the amplitude of the ones that were still successfully evoked. Membrane potential of the neuron was further hyperpolarized. A gradual decrease in the EPSP amplitude was observed throughout the experiment

Results

EPSPs evoked by dorsal root stimulation

Whole-cell recordings were performed from 25 substantia gelatinosa neurons. The average resting membrane potential and input resistance of the neurons were -60.3 ± 1.3 mV and 662.2 ± 88.6 M Ω , respectively (mean \pm SEM). Stable recordings for the course of the pharmacological protocol were obtained from eight superficial dorsal horn neurons. Monosynaptic EPSPs evoked by electrical stimulation of the dorsal root could be detected in five of the eight recorded neurons. Based on their conduction velocity, one of the five neurons received A-delta input, whereas the other four were excited by C-fibres. The amplitudes of the evoked EPSPs varied in the range of 5–15 mV regardless of whether the neurons were excited by A-delta or C fibre stimulation (Fig. 1B). Application of 100 μ M Lamotrigine caused a remarkable variability in the amplitudes of the evoked EPSPs (with a slight reduction in the average value) and a small increase in the failure rate (Figs 1C and 2). After doubling the concentration of Lamotrigine to 200 μ M, EPSP amplitudes dropped dramatically and the failure rate increased strongly (Figs 1C and 2), so that evoked EPSPs were completely abolished in three neurons (data not shown). The onset of this effect during the wash-in of the drug varied between experiments. During washout, the evoked EPSPs reappeared in an abrupt way, although their amplitudes never reached the values recorded in the control period of the experiment. This might partially be due to the small, gradual decrease in the evoked EPSP amplitude, possibly caused by repeated electrical stimulation and the continuous depletion of transmitter molecules from the pre-synaptic terminals, which was observed throughout the experiments. In addition, a gradual, small, reversible hyperpolarization of the post-synaptic membrane was also detected during Lamotrigine application (Fig. 1C).

Spontaneous EPSPs and EPSCs

All recorded slices showed remarkably strong spontaneous activity. The amplitude of the spontaneous EPSPs and EPSCs occurring in the recorded neuron varied between 0.5 and 6 mV and 5 and 60 pA, respectively. Occasionally, larger spontaneous EPSPs were also observed. Application of Lamotrigine in 100 μ M concentration caused negligible changes in the amplitude and number of spontaneous events (Fig. 3A). However, Lamotrigine in 200 μ M concentration almost completely eliminated large-amplitude spontaneous EPSCs and left only small-amplitude (<10 pA) spontaneous events intact (Fig. 3B). The wash-in time needed to reach this effect seemed to be longer in neurons that showed weaker spontaneous activity in the initial control period. In addition, a grad-

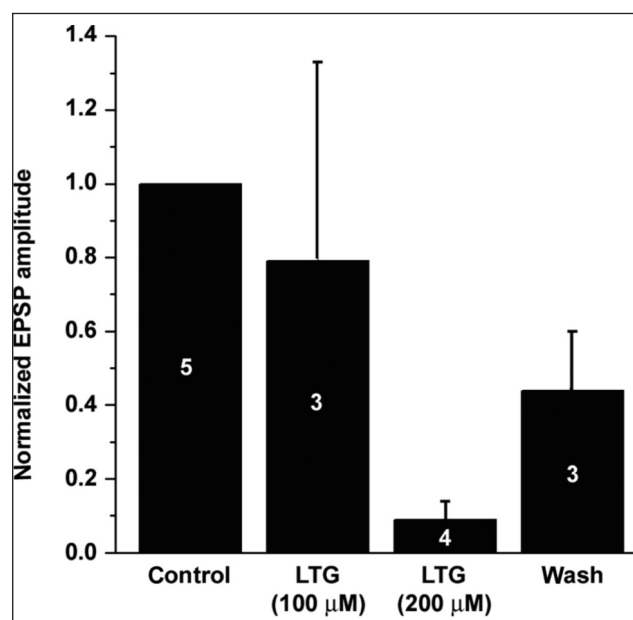


Fig. 2. Histogram showing the effect of Lamotrigine on evoked EPSP amplitudes. Application of 100 μ M Lamotrigine caused a remarkable variability in the amplitudes of the evoked EPSPs with a slight reduction in the average value. Higher dose of the drug (200 μ M), however, reduced the amplitude of EPSPs dramatically and this effect could be partially reversed with washout of the substance. Data presented in the histogram have been normalized to the control value, which was taken as 1.0. Numbers of neurons recorded in the various experimental paradigms are indicated in the columns. LTG = Lamotrigine

ually appearing small outward current was regularly observed during Lamotrigine application (Fig. 3B).

Firing pattern of the recorded neurons

Lamotrigine application caused a marked change in the firing pattern of the recorded neurons (Fig. 3C). Under the influence of Lamotrigine, the firing pattern of the recorded neurons became more and more phasic, showing prominent amplitude accommodation. Finally, the neurons reached a state in which they only fired a single action potential at the beginning of the depolarizing current test pulse and were not capable of producing further spikes without a repolarization to the resting membrane potential. This effect of the drug could not be reversed even after a long 10–30 min washout period (data not shown).

Morphology of recorded neurons

Of the eight recorded neurons, biocytin labeling was successful only in one case. The labeled neuron presented the characteristic dendritic arborization pattern of stalked cells [12] (Fig. 3D).

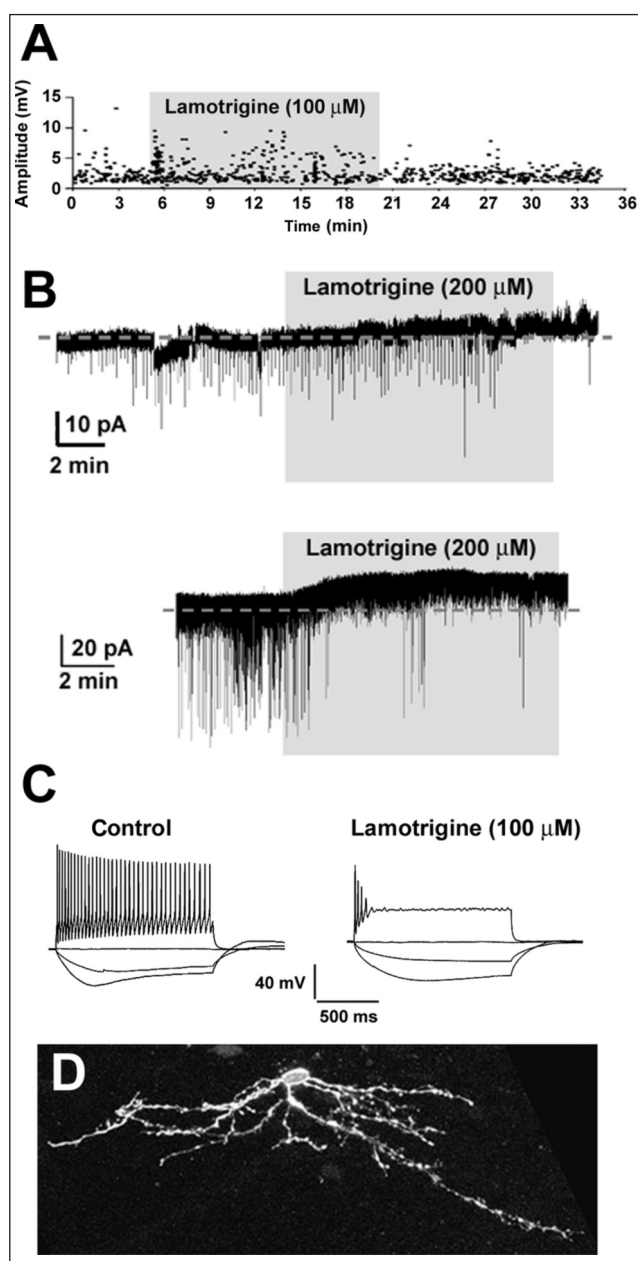


Fig. 3. Effect of Lamotrigine on the spontaneous EPSPs and firing pattern of superficial dorsal horn neurons. **A:** Lamotrigine in 100 μM concentration only slightly decreased the amplitude and frequency of spontaneous EPSPs (RMP: -65 mV, P19). **B:** Spontaneous EPSCs of two different neurons (top: RMP: -62 mV, P21; bottom: RMP: -59 mV, P22). Application of Lamotrigine in 200 μM reduced both the amplitude and the number of spontaneous EPSCs. Note that the wash-in times needed to reach the effect of Lamotrigine were different in the two neurons. **C:** Firing patterns of the substantia gelatinosa neuron in **A** before (control) and after Lamotrigine (100 μM) application. The firing pattern was converted to phasic. **D:** Fluorescent image of the neuron labelled with biocytin. A sample from its physiological records is shown in **B** (bottom)

Discussion

It appears likely that Lamotrigine exerts a complex effect on nociceptive synaptic transmission in the spinal dorsal horn. First, the substantial decrease in the amplitudes of the evoked EPSPs and the consecutive increase in the failure rate of synaptic transmission are likely due to the interaction of Lamotrigine with pre-synaptic transmitter release mechanisms [6, 13]. The modulation of N-type Ca^{2+} channels could be a component of this interaction [5]. Second, the decline in the amplitude and frequency of spontaneous EPSPs and EPSCs indicates that the Lamotrigine-evoked blockade of voltage-sensitive Na^+ channels, depressing neural activities in the superficial spinal dorsal horn. However, the occasional appearance of small-amplitude spontaneous EPSCs during Lamotrigine application suggests that accidental transmitter release from axon terminals resulting in miniature post-synaptic currents is not affected by Lamotrigine. Third, the Lamotrigine-evoked prominent changes in Na^+ conductance alter the intrinsic firing pattern of neurons, making them incapable of producing repetitive action potentials [14]. Fourth, the strong alteration of voltage-sensitive Na^+ and Ca^{2+} channels may also have an indirect effect on some K^+ channel-mediated ion currents. Such a consecutive increase in K^+ conductance may explain the finding that Lamotrigine application causes a gradual hyperpolarization of post-synaptic neuronal membranes.

It is also likely that the effectiveness of Lamotrigine is dependent on the activity of neural circuits. It exerts its action more rapidly in slices in which more neurons are active simultaneously, indicating that Lamotrigine blocks Na^+ channels in a use-dependent manner. Previous studies suggested that, because of its use-dependent character, Lamotrigine can effectively block synaptic transmission in nociceptive pathways only in chronic pain conditions [15, 16]. Our present results question this idea. Here, we have shown that Lamotrigine effectively blocks synaptic transmission between primary afferents and secondary sensory neurons even in an acute *in vitro* slice preparation obtained from naive animals, indicating that Lamotrigine might be active not only in chronic but also in acute pain conditions. The finding of Blackburn-Munro et al. [15] showing that mexiletine, another use-dependent Na^+ channel blocker, increases the nociceptive response latency of animals to acute thermal stimuli strongly reinforces this notion.

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