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Short communication

# Lamotrigine inhibits $Ca^{2+}$ currents in cortical neurons: functional implications

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**Abstract**

In pyramidal cortical cells, high-voltage-activated $Ca^{2+}$ currents affect seizure propagation and the release of excitatory amino acids at the corticostriatal axon terminals. The new antiepileptic drug lamotrigine (Lamictal) produced a large and dose-dependent inhibition of high-voltage-activated $Ca^{2+}$ currents ($IC_{50}$ = 12.3 $\mu$M) in rat cortical neurons. This action was not blocked by the dihydropyridine receptor antagonist nifedipine; instead, the response was blocked by the concomitant application of the N-type $Ca^{2+}$ channel blocker, $\omega$-conotoxin GVIA (1–3 $\mu$M) and the P-type $Ca^{2+}$ channel blocker, $\omega$-agatoxin-IVA (20–100 nM). These findings demonstrate that lamotrigine, at therapeutic doses, is capable of modulating the $Ca^{2+}$ conductances involved in excitatory amino acid release in the corticostriatal pathway, partially explaining lamotrigine usefulness in the therapy of epilepsy as well as in the treatment of excitatory amino acid-induced neurotoxicity.

*Keywords:* Anti-epileptic drug; Cortex; $Ca^{2+}$ current; Neuroprotective agent

## 1. Introduction

The new antiepileptic drug lamotrigine (Lamictal) has emerged as a broad-spectrum anticonvulsant with clinical efficacy in partial and generalized seizures (Pellock, 1994). Interference with voltage-gated $Na^+$ channels is indicated as its prominent mechanism of action (Cheung et al., 1992). The well established efficacy of lamotrigine to block high-frequency firing activity is comparable to that of previously studied antiepileptics (Lang et al., 1993). Lamotrigine was also indicated to affect the glutamate release from human brain preparation (During and Spencer, 1993). Potent activation of excitatory amino acid receptors is involved in the development of infarction after ischemic insults and plays a major role in the long-lasting events correlated to neurodegeneration (Rothman, 1994). In this regard, the possible use of lamotrigine congeners as neuroprotective agents has been proven effective (Smith and Meldrum, 1995). The electrophysiological mechanisms underlying this action are, however, poorly known. Mechanisms other than the use-dependent reduction of $Na^+$-driven action potentials might be involved: a direct interference of lamotrigine with those $Ca^{2+}$ conductances, which are suggested to be responsible for excitatory amino acid transmission at many central synapses (Takahashi and Momiyama, 1993), could be important. Oxcarbazepine, for instance, has been shown to decrease the glutamatergic synaptic potentials through the modulation of $Ca^{2+}$ signals (Stefani et al., 1995). Therefore, we have studied the effects of lamotrigine on high-voltage-activated $Ca^{2+}$ currents recorded from the soma of isolated pyramidal cortical neurons.

## 2. Materials and methods

The preparation of acutely isolated neurons has been described previously in detail (Stefani et al., 1994). We obtained cortical neurons from coronal slices, 450 $\mu$m thick, of adult rats. Small portions of sensorimotor cortex were first dissected under stereomicroscopy, then incubated in a Hepes-buffered Hanks' balanced salt solution (HBSS), bubbled with 100% $O_2$ and warmed at 35°C.

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From 30 to 60 min later, one slice was transferred in HBSS containing 1.5 mg/ml protease XIV (Sigma). After 30–45 min of proteolytic treatment (pronase E, Sigma), the tissue was rinsed in HBSS and mechanically triturated. Finally, the cell suspension was placed in a Petri dish mounted on an inverted microscope (Nikon). Cells were allowed to settle for 10 min. Cortical neurons were chosen for recordings when considered to be pyramidal cells based on their morphology (major axis 15–25 μm, 1–3 fine apical processes spared by the dissociation, Fig. 1A). Whole-cell recordings were performed using pipettes (3–8 MΩ in impedance) pulled with a Flaming-Brown electrode puller, fire-polished just before use and filled with the internal solution consisting of (in mM): *N*-methyl-*d*-glucamine 160, Hepes 40, EGTA 10, Mg 4, phosphocreatine 20, ATP 2–4, GTP 0–0.2, leupeptin 0.2; pH = 7.3 with phosphoric acid, osmolarity = 265–275 mosmol/l. The neurons were bathed in a medium composed of (in mM): NaCl 140 (or tetraethylammonium · Cl 160), $BaCl_2$ 5, $CsCl_2$ 5, Hepes 10, tetrodotoxin 0.001, glucose up to 300–305 mOsm. Control and drug solutions were applied with a linear array of gravity-fed capillaries positioned within 500 μm of the patched neuron. Recordings were made with an Axopatch 1D at room temperature (21°C). Series resistance compensation (70–80%) was routinely used. Barium ($Ba^{2+}$) currents were studied with voltage steps and ramps. Nifedipine, pronase E, tetraethylammonium chloride and tetrodotoxin were obtained from Sigma. ω-Conotoxin was purchased from Latoxan (Rosiens, France). ω-Agatoxin was a kind gift of Dr. N.

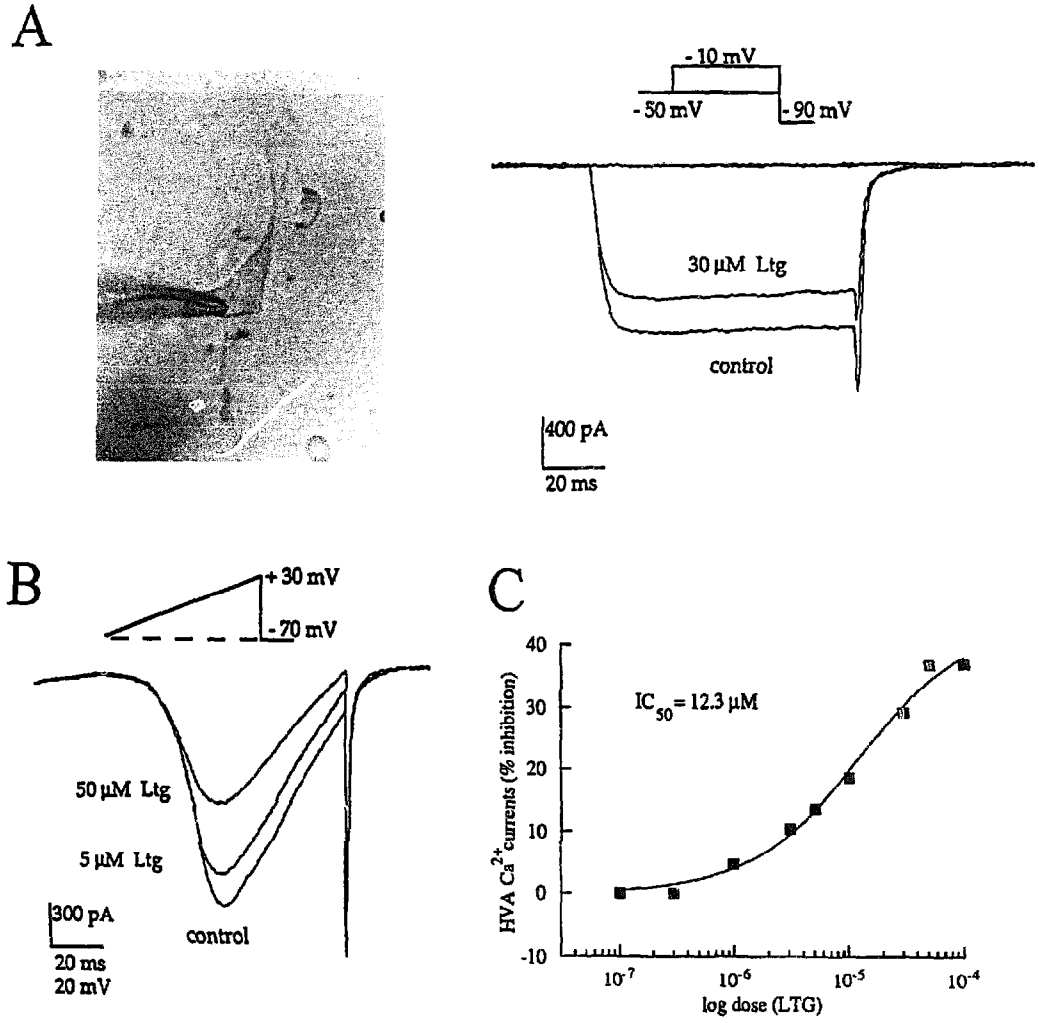


Fig. 1. Effect of lamotrigine on high-voltage-activated $Ca^{2+}$ currents of cortical neurons. A: In a typical pyramidal cortical cell (left), the step pulse to −10 mV activated a non-inactivating $Ba^{2+}$ conductance (control trace). 30 μM lamotrigine caused a 22% decrease in conductance. B: Ramp-activated (0.8 mV/ms) $Ba^{2+}$ currents under control conditions, lamotrigine 5 μM and lamotrigine 50 μM. Note the absence of substantial change in the current/voltage relation. C: Dose-response curve of lamotrigine-mediated reduction of high-voltage-activated $Ca^{2+}$ currents. Each point represents the average of three experiments. $IC_{50}$ is $m_2$ in the following equation:

$$y = m_3 \frac{m_0^{m_1}}{m_0^{m_1} + m_2^{m_1}}$$

where $m_1$ is the slope (0.89).

Saccomano (Pfizer, CT, USA). Lamotrigine was given by the Wellcome Foundation (R. Di Virgilio, Wellcome Italia).

## 3. Results

Experiments were performed on 60 adult patched cells, in which large $Ba^{2+}$ currents were activated by step pulses (Fig. 1A) or ramps (Fig. 1B) from holding potentials ranging from −70 to −40 mV. Under these conditions, $Ba^{2+}$ currents were dominated by high-voltage-activated components. The current shown in Fig. 1A was activated by a step pulse to −10 mV, manifested negligible inactivation in the 100 ms time frame and was suppressed by 25 μM cadmium (not shown), confirming that it was driven by the opening of $Ca^{2+}$ channels. A representative response to 30 μM lamotrigine is shown in Fig. 1A: conductance at −10 mV was decreased by 22%. Lamotrigine (0.3–100 μM) was effective to reduce $Ba^{2+}$ currents in all the neurons tested ($n = 48$). The maximal inhibition observed was −36.7% (±4.1, $n = 10$). Lamotrigine did not produce a significant shift in the voltage dependence of the ramp. The threshold concentration was close to 1 μM. Other responses to 5 and 50 μM lamotrigine are shown by individual traces in Fig. 1B. The dose-response curve of Fig. 1C, made from pooled observations (at least two experiments for each concentration), revealed saturating doses at 50 μM and $IC_{50}$ of 12.3 μM. Since the slope was close to 0.9, a single binding site was postulated. The lamotrigine-mediated inhibition of $Ba^{2+}$ currents was fully reversible.

In order to investigate which type of $Ca^{2+}$ channel was modulated by lamotrigine, we studied the lamotrigine effects in the presence of 10 μM nifedipine, a dihydropyridine receptor antagonist at L-type $Ca^{2+}$ channels, 2–3 μM ω-conotoxin GVIA, which is known to inhibit N-type $Ca^{2+}$ channels and 20–100 nM ω-agatoxin, one of the selective blockers of P-type $Ca^{2+}$ channels.

In eight out of ten nifedipine-treated neurons, supramaximal concentrations of lamotrigine (50–100 μM) were still effective and the observed modulation ($-32 \pm 4.6$, $n = 8$) was not significantly different from that observed under control conditions. In the two remaining neurons, however, the lamotrigine response was reduced by nifedipine (−16.4 and −19%). Since these results were not wholly consistent, we also applied the dihydropyridine receptor agonist, Bay K 8644, unmasking a long-lasting tail (Fig. 2A). As shown in Fig. 2A, the lamotrigine-mediated inhibition of the current was not paralleled by a corresponding modulation of the L-channel tail, implying that dihydropyridine-sensitive channels do not play an important role in the lamotrigine responses of cortical neurons.

More consistent findings were obtained with the use of N- and P-type $Ca^{2+}$ channel blockers. ω-Conotoxin GVIA per se reduced the ramp-activated conductances by about

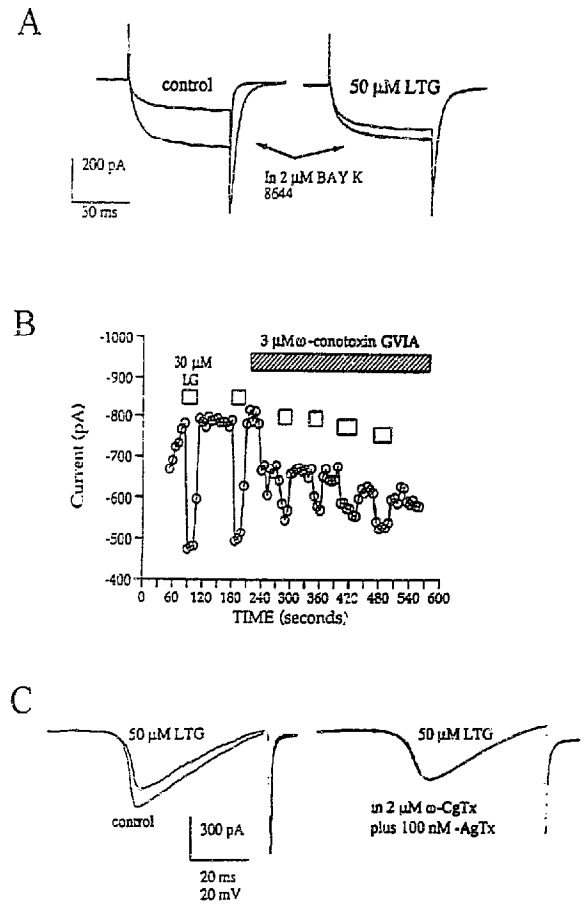


Fig. 2. Pharmacology of the lamotrigine-mediated response. A: Under 2 μM Bay K 8644, the test-activated current was increased and the long-lasting tail was unmasked (left trace, arrow); 50 μM lamotrigine reduced the current but did not affect the dihydropyridine-sensitive tail (right trace). B: Time course of the effect of 50 μM lamotrigine before and after the application of 2.5 mM ω-conotoxin; the lamotrigine-mediated inhibition of $Ca^{2+}$ currents was reduced to 10.8% by the N-type $Ca^{2+}$ channel blocker. C: The lamotrigine-mediated inhibition in control (left trace) was fully blocked by the concomitant application of 100 nM ω-agatoxin and 2 μM ω-conotoxin (right trace).

20% ($-20.51 \pm 4\%$, $n = 9$) and partially blocked the lamotrigine-mediated responses. The lamotrigine inhibition, in the presence of ω-conotoxin GVIA, averaged 11.5% (±3.2%, $n = 6$) whilst it was > 30% in the control. An example of this partial block of the lamotrigine response by ω-conotoxin is illustrated in Fig. 2B. In this case, the −36% inhibition observed in the control was reduced to −10.8% by ω-conotoxin GVIA (if ω-conotoxin GVIA-sensitive channels were not affected by lamotrigine, the current reduction should approximate 25%). With 20–100 nM ω-agatoxin, we also observed a reduction of $Ca^{2+}$ conductances by 19.6% (±5.1, $n = 6$) and a strong decrease of lamotrigine modulation. The lamotrigine-mediated reduction of $Ca^{2+}$ currents averaged 4.7% ($n = 5$) and, in addition, was obscured in one cell. Significantly, complete block of the lamotrigine modulation of $Ca^{2+}$ currents was obtained with the concomitant applica-

tion of 2 μM ω-conotoxin GVIA and 100 nM ω-agatoxin ($n = 4$, Fig. 2C).

## 4. Discussion

We have described a novel action of lamotrigine on cortical pyramidal neurons: a reversible inhibition of high-voltage-activated $Ca^{2+}$ currents. Since ω-conotoxin GVIA and ω-agatoxin antagonised the lamotrigine-mediated inhibition of $Ca^{2+}$ currents, both N- and P-type $Ca^{2+}$ channels are involved in the lamotrigine responses. In addition to their presence on somatic membranes, these channels mediate synaptic transmission in many central synapses (Takahashi and Momiyama, 1993). Therefore, it might be suggested that the lamotrigine action, apart from decreasing the $Ca^{2+}$-dependent excitability of cortical neurons, will also be reflected by a substantial decrease of excitatory amino acid release in target structures.

The lamotrigine effect on cortical high-voltage-activated $Ca^{2+}$ currents was detected in response to low, micromolar doses of lamotrigine. Moreover, the $IC_{50}$ for the described modulation was very similar to the lamotrigine concentrations required to block the sustained sodium-dependent firing discharge. It is conceivable that both mechanisms are active at the same time and concentration range. In this regard, it should be kept in mind that the mean plasma concentration of this AED, at therapeutic doses (Peck, 1990; Yuen and Peck, 1988), is comparable to the $IC_{50}$ in this study.

A surprising discrepancy remains between our results and those of other recent work which have not shown a clear modulation of $Ca^{2+}$ conductances by lamotrigine at the same concentration range (Lees and Leach, 1993). It is possible that this modulation is mainly observed in adult cortical neurons (used in our experiments) rather than in cultured young neurons. In a recent study on hippocampal neurons, Xie et al. (1995) showed that only in those epileptiform bursts which caused cumulative inactivation of $Na^+$ spikes lamotrigine did produce potent inhibition. To what extent the depression of the $Ca^{2+}$ signals had contributed to the observed inhibition was not investigated.

There is strong evidence regarding the inhibition of low-voltage-activated $Ca^{2+}$ currents by different antiepileptic drugs; for instance, ethosuximides produce a large block of T channels in thalamic neurons (Coulter et al., 1989). Whether the modulation of high-voltage-activated $Ca^{2+}$ currents represents a mechanism of action shared by old and new antiepileptic drugs is still debated (Dichter and Zona, 1989). Similarly to the findings with lamotrigine, we have recently described the nifedipine-insensitive, oxcarbazepine-induced inhibition of the same conductances (Stefani et al., 1995). Nevertheless, the lamotrigine responses were large when compared to those induced by the carbamazepine derivative. On the other hand, felbamate was shown to interact with dihydropyridine-sensitive $Ca^{2+}$ channels (Stefani et al., 1996).

In conclusion, the strong action of lamotrigine on high-voltage-activated $Ca^{2+}$ currents seems to represent an interesting mechanism of action which might contribute to explain its efficacy to elevate the threshold for seizure propagation as well as prevent the calcium overload which is associated with neuronal $Ca^{2+}$-dependent damage. The latter can occur either acutely as in the sequelae of stroke, or in the long-lasting progression of neurodegenerative disorders.

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