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An Electrophysiological Analysis of the Protective Effects of Felbamate, Lamotrigine, and Lidocaine on the Functional Recovery From In Vitro Ischemia in Rat Neocortical Slices

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ABSTRACT We used field potential recording techniques to examine whether felbamate (FBM), lamotrigine (LTG), and lidocaine (LID) protect against the irreversible functional damage induced by transient ischemia. Five minutes of ischemia caused a depression of the field potential in rat cortical slices, which did not recover even after more than 1 h of washout. The N-methyl-D-aspartate (NMDA) antagonist ketamine (50 μM) protected against depression of the field caused by ischemia. On the other hand, the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2.3-dione (CNQX) (10 μM) had protective effects only if co-applied with ketamine. We found that either FBM (30–300 μM), which did not modify the amplitude of the field EPSP, or LTG (10–300 μM), which reversibly depressed the excitatory synaptic transmission, had a marked protective effect when superfused before and during the ischemic insult. After FBM (100 μM) and LTG (100 μM), the field EPSP recovered by 84 ± 1% and 73 ± 2.7% of control, respectively. Furthermore, LID (30–300 μM) was less effective than FBM and LTG in inducing a functional recovery from the damage caused by ischemia (58 ± 1.8%). The rank order of potency, based on the maximal protection caused by the three drugs, was FBM > LTG > LID. Our results suggest that a noticeable neuroprotection can be obtained during glucose and O_2 deprivation by preventive therapeutic regimens which use the two recently marketed anticonvulsant drugs, FBM and LTG. **Synapse 30:371–379, 1998.**

INTRODUCTION

Several factors might contribute to the damage of neurons during ischemic disease. It is well known that ischemic injury in stroke triggers a massive release of excitatory amino acids (EAAs) (Benveniste et al., 1984; Drejer et al., 1985; Hagberg et al., 1985; Silverstein et al., 1986). The consequent excessive activation of EAA receptors is an important determinant of the neuropathological changes induced by ischemia (Rothman and Onley, 1986; Meldrum, 1990; Choi and Rothman, 1990). In fact, the death of neurons is reduced either by EAA antagonists (Germano et al., 1987; Park et al., 1988; Roman et al., 1989; Meldrum, 1990; Simon and Shirashi, 1990; Buchan et al., 1991; Gill and Lodge, 1992; Smith and Meldrum, 1992) or by drugs which inhibit the release of glutamate (Meldrum et al., 1992; Graham et al., 1993). Another contributor to the cellular vulnerability during stroke is an increased uptake of sodium and calcium into the neurons caused by a spreading membrane depolarization and excitation (Kass and Lipton, 1982; Balestrino and Somjen, 1986; Lobner and Lipton, 1993; Mies et al., 1993). The consequent deranged distribution of ions across the membrane would lead to an activation of proteolytic enzymes and cell swelling (Kass and Lipton, 1982; Rothman and Onley, 1987; Siesjo and Bengtsson, 1989).

Any treatment of the functional deficits caused by ischemia on neurons should consider the physiopathological premises delineated above. There is also increasing evidence that EAA-mediated processes contribute significantly to neuronal loss in conditions such as

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epilepsy. Thus, several agents have been proposed to offer protection against the cellular failure caused by ischemia and epilepsy (for reviews, see Taylor and Meldrum, 1995; Dorman et al., 1996). Among these agents felbamate (FBM), lamotrigine (LTG), and lidocaine (LID) have recently been reported to reduce the damage caused by O_2 and glucose deprivation (Wasterlain et al., 1993; Weber and Taylor, 1994; Fried et al., 1995; Smith and Meldrum, 1995). In spite of this, no studies have yet examined and compared the neuroprotective effects of these compounds in the same ischemic model. Considering that the neurons of the cerebral cortex are often injured by a stroke, we used a field potential analysis to investigate the protective effects of FBM, LTG, and LID on the irreversible synaptic failure evoked by an ischemic insult in rat prefrontal and frontal cortical slices.

MATERIALS AND METHODS

Preparation of the cortical slices

Adult male Wistar rats, 150–200 g body weight, (Morini, Reggio Emilia) were anesthetized with halothane. After brain removal, coronal slices (300 μm) of prefrontal and frontal poles were prepared with the use of a vibratome (Siniscalchi et al., 1997). A single slice was then transferred to a recording chamber, submerged, and continuously perfused with an artificial cerebrospinal fluid (ACSF). The ionic composition of the ACSF was (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.4, glucose 10, and $NaHCO_3$ 20; the solution was gassed with 95% O_2 and 5% CO_2. The temperature was maintained in the bath chamber at 36°C and the perfusion rate was 2.5 ml/min.

Electrophysiological recordings

Extracellular recording electrodes were filled with 2 M NaCl and had a resistance of 3–10 MΩ. For synaptic stimulation, tungsten bipolar electrodes were positioned 0.5–3 mm distant from the recording electrode. The field potentials were obtained following orthodromic electrical stimulation (10–40 V, 0.03–0.05 ms) of superficial (II–III) as well as deep (IV–V) cortical layers at 0.05 Hz. The field potential amplitude was defined as the average of the amplitude from the peak of the early positivity to the peak of the negativity and the amplitude from the negativity to the peak of the late positivity. An Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) was used in Bridge mode for recordings. Traces were displayed on an oscilloscope and stored in a digital system (Pclamp 5.5, Axon Instruments). Four responses were averaged. Data were presented as a mean ±SE of the mean. The statistical significance of the experiments was evaluated with the use of Student's *t*-test. The program for statistical calculations was Primer.

Ischemia and drug application

The ischemic stimulus was produced by switching to a solution saturated with 95% N_2 and 5% CO_2 in which glucose was removed and substituted with sucrose (Whittingham et al., 1984). In order to exclude factors such as age, temperature, flow rate, and duration of ischemia, which could play a part in the ischemic outcome, the experiments were conducted under controlled conditions. O_2 and glucose deprivation were maintained for a period ranging between 1 and 5 min, then switched to the normal ACSF. During ischemia, the amplitude of the field EPSP was depressed. No functional recovery was observed for severe ischemic stimulus lasting 5 min. The postsynaptic population spikes were then observed for 60–180 min during reoxygenation. The drugs were applied 10–20 min before and during the ischemic stimulus. They were washed out when the system was reequilibrated with 95% CO_2 and 5% O_2 and glucose 10 mM. The recovery of the field was calculated by dividing its amplitude at 1 h post-ischemia with the amplitude of the pre-ischemic field. Drugs were bath-applied by switching the superfusing solution to one containing known concentrations of substances. The drugs studied were ketamine and 2-amino-5-phosphonovalerate (APV) (Sigma, St. Louis, MO). LTG was kindly given by Glaxo Wellcome (Beckenham, England); CNQX and FBM were from Tocris Cookson, and LID from RBI (Natick, MA).

RESULTS

Effects of ischemia on the cortical field potential

In control conditions the amplitude of the cortical field ranged between 0.5 and 2.3 mV, mean 1.5 ± 0.05 mV (n = 198), and varied by less than 10% over the 180-min period. During ischemia the amplitude of the field EPSP rapidly decreased, and in 5 min the field completely disappeared. The recovery of the field was gradual and depended on the duration of the ischemic period. For ischemia lasting 1 and 3 min the field recovered at 60 min of washout to 82 ± 1.5% (n = 9) (control 1.2 ± 0.2 mV) and 55 ± 2% (n= 13) (control 1.6 ± 0.1 mV) of its pre-ischemic amplitude, respectively (Fig. 1). However, no recovery was observed for a period of ischemia lasting 5 min even after 180 min of washout (n = 20) (Fig. 1). During the recovery phase the amplitude of the field potential did not change between 30 and 180 min.

Protective effects of EAA antagonists on the depression of the field caused by ischemia

The participation of glutamate receptors in the irreversible changes of the field caused by ischemia was studied by using EAA antagonists. In spite of the fact that the preexposure of the slices with CNQX (10 μM) blocked the intracortical field potential (Calabresi et al., 1996) (Fig. 2), this AMPA/kainate antagonist did not

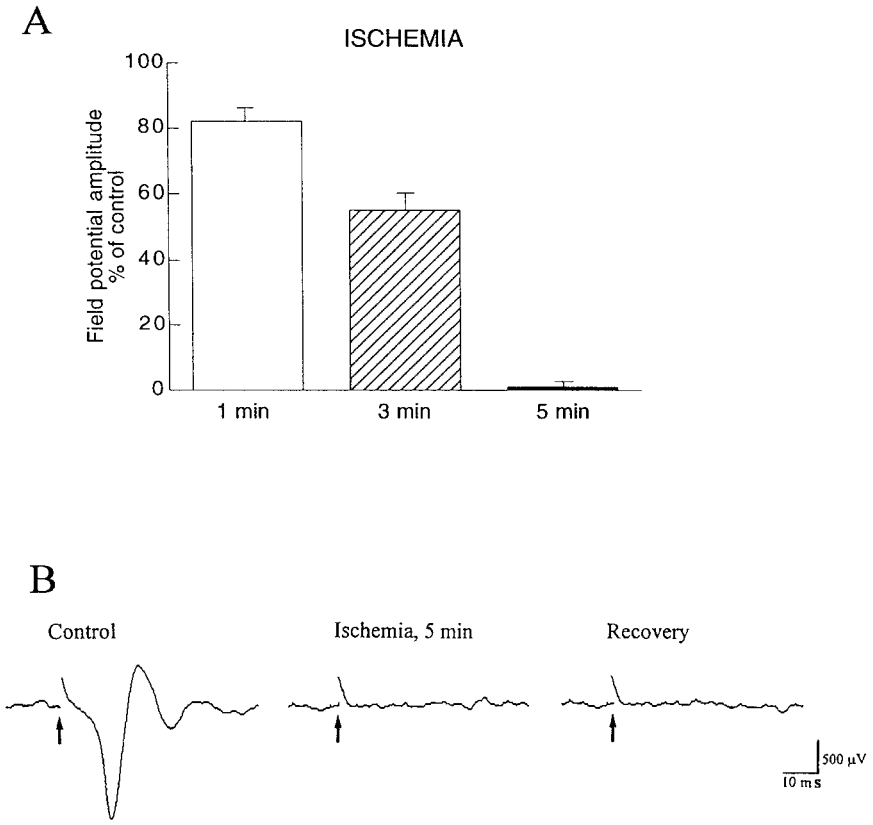

Fig. 1. Effects of ischemia on field EPSP. (**A**) Exposure of the cortical slices to ischemia depresses the field excitatory potential. A partial recovery of the field is observed after 1 and 3 min, while the synaptic response was completely suppressed after 5 min ischemia. In this and the followings histograms the vertical bars indicate ±SEM. (**B**) Extracellular recordings of the persistent depression of the field caused by ischemia even after 180 min of washout (Recovery). In this and the followings figures the arrows indicate synaptic stimulation

influence the recovery of the field when applied 20 min prior and during 5 min of ischemia (n = 9) (Fig. 2). On the other hand, no significant change in amplitude of the field was caused in control conditions by the NMDA antagonist ketamine (50 µM) (Fig. 2) and APV (50 µM; not shown). In the presence of ketamine or APV, 5 min ischemia completely depressed the field EPSP. However, the superfusion of ketamine (50 µM), applied before (more than 10 min) and during ischemia enabled the recovery of the field to the 78 ± 3.9% (n = 19 $P <$ 0.005) of the initial amplitude (1.6 ± 0.2 mV). Almost the same neuroprotective effect (78.4 ± 3%) (n = 4, $P <$ 0.005) of control (1.7 ± 0.4 mV) was caused by preexposure of APV (50 µM) (data not shown). When both CNQX and ketamine were perfused before and during ischemia there was a small but significant increase (n = 12, $P < 0.005$) of the field recovery to 90 ± 3.2% of control (1.7 ± 0.2 mV), (Fig. 2).

Effects of FBM, LTG, and LID

Felbamate

In control medium, FBM (10–300 µM) neither affected the amplitude of the field potential (Calabresi et al., 1996) (Fig. 3) nor significantly influenced the ischemia-induced depression of the synaptic potential. However, FBM applied before (20 min) and during ischemia protected from the derangement of the cortical field by increasing the degree of functional recovery upon reoxygenation. The minimal effective concentration of FBM was 30 µM. The amplitude of the field during reoxygenation with FBM (30 µM) was 30 ± 1.3% (n = 5, $P <$ 0.005) of control (1.4 ± 0.2 mV). A saturating concentration of FBM (100 µM) caused a recovery of the amplitude of the field potential to 84 ± 1% (n = 15, $P < 0.001$) of its initial level (1.6 ± 0.2 mV).

Lamotrigine

In normal ACSF, bath application of LTG depressed in a dose-dependent manner the cortico-cortical field potential (Fig. 4). This effect could be reversed in 8–10 min of washout (Calabresi et al., 1996). The exposure of the slices to LTG (20 min before and during the ischemic period) protected from the ischemia-induced depression of the field. As shown in Figure 4, the minimal effective concentration of LTG was 10 µM (residual field amplitude 16 ± 0.9%, n = 9, $P < 0.005$) (control 1.6 ± 0.2 mV n = 9). Higher concentration of LTG (100 µM) produced a 73 ± 2.7% recovery of the

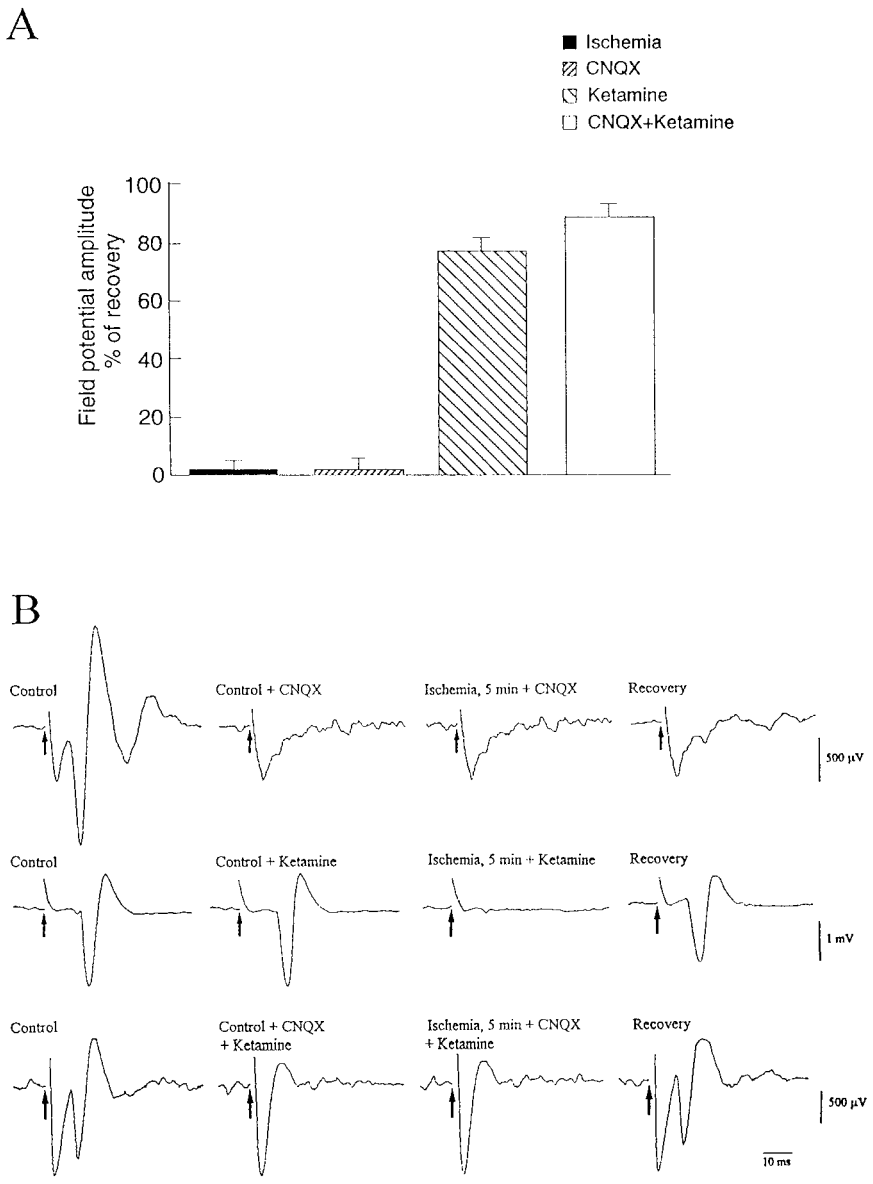

Fig. 2. Protective effects of EAA antagonists. (**A**) The graph shows the recovery of the cortical field after the ischemic stimulus in the presence of ketamine (50 µM), CNQX (10 µM), and CNQX plus ketamine. Note the synergistic protective effects of ketamine (50 µM) plus CNQX (10 µM). (**B**) Indicative traces of the field potential which were included (Recovery) in the different columns of the graph.

initial amplitude of the field (1.3 ± 0.2 mV) (n = 13, $P <$ 0.001).

Lidocaine

LID (30 µM and 100 µM) caused by itself a small reduction of the field 10 ± 3% (n = 5) and 30 ± 1.5% (n = 12), respectively (Fig. 5B). When applied 20 min before and during ischemia (n = 17), LID was able to facilitate recovery of the field potential after an ischemic insult. The minimal effective concentration for a significant protection of LID was 30 µM (recovery 32 ± 0.8%, n = 6, $P < 0.005$) (control amplitude of the field, 1.6 ± 0.16 mV). The maximal protective effect was obtained with 100 µM LID (58 ± 1.8%) (n = 16, $P <$ 0.005) (control amplitude 1.3 ± 0.12 mV).

No significant recovery of the field potential was observed when FBM (100 µM), LTG (100 µM), or LID (100 µM) was applied during the ischemic period without preexposing the slices to these anticonvulsants (four experiments for each compound).

DISCUSSION

It is generally accepted that the loss of the population spike after O_2 and glucose deprivation in a slice preparation is a reliable index of ischemic damage (Kass and Lipton, 1982; Whittingham et al., 1984).

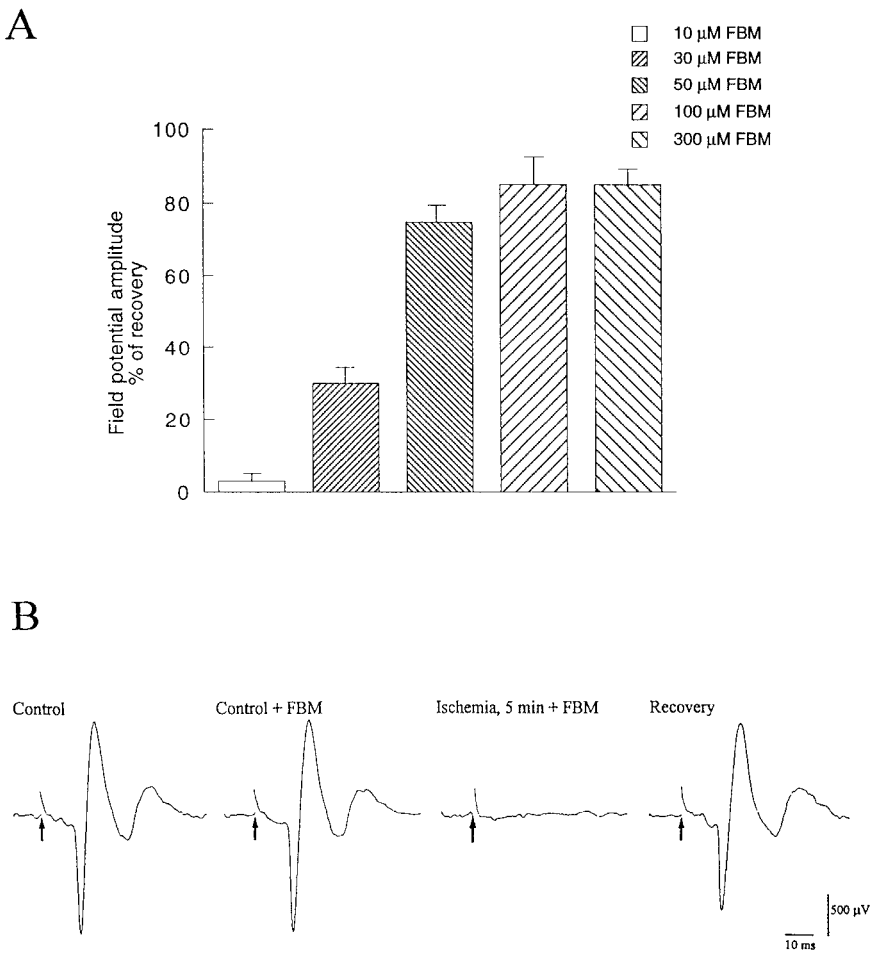

Fig. 3. Felbamate causes a concentration-dependent protection against the ischemic damage. (**A**) The graph shows the rescue of the field after ischemia in the presence of different concentrations of FBM. (**B**) Indicative traces of the field potential before and after the ischemic stimulus in the presence of FBM (100 μM). Note that this antiepileptic drug had no effect by itself on the amplitude of the field potential.

Thus, this in vitro study presents an analysis of the protective effects of FBM, LTG, and LID on the cortico-cortical excitatory transmission after an ischemic episode which produces complete and irreversible depression of the cortical EPSP. In addition, it shows clear evidence for a protective effect against the ischemic damage by pretreatment of the slices with NMDA antagonists.

Effects of EAA antagonists

Despite the fact that NMDA receptors are not implicated in the normal excitatory transmission in the prefrontal and frontal lobe (Calabresi et al., 1996; Siniscalchi et al., 1997), NMDA-mediated events play an important role in the progression of neuronal damage during and after ischemia (Simon et al., 1984; Rothman and Onley, 1986; Gill et al., 1987; Choi, 1988; Swan et al., 1988; Meldrum, 1990; Graham et al., 1993; Szatkowski and Attwell, 1994). Certainly the pronounced activation of the NMDA receptor in the ischemic brain could promote the death of selectively vulnerable neurons by favoring membrane depolarization and influx of calcium ions (Gill et al., 1987; Lobner and Lipton, 1993). In line with a crucial role of NMDA receptors in ischemic injury (Hansen, 1985; Rothman et al., 1987; Swan and Meldrum, 1990), we found that ketamine and APV protected from the derangement of the field potential caused by ischemia. Interestingly, while the blockade of non-NMDA receptors did not increase the resistance of the slices to ischemia, the combination of CNQX (AMPA/kainate antagonist) with ketamine (NMDA antagonist) increased the recovery of the field after ischemia. A similar result was obtained by Lobner and Lipton (1993) in the CA1 region of hippocampus. It is also worth mentioning that the blockade of both the AMPA and the NMDA receptors increased the period of time required for oxygen and glucose deprivation to produce widespread neuronal death in the cortex (Kaku et al., 1991; Lynch et al., 1995).

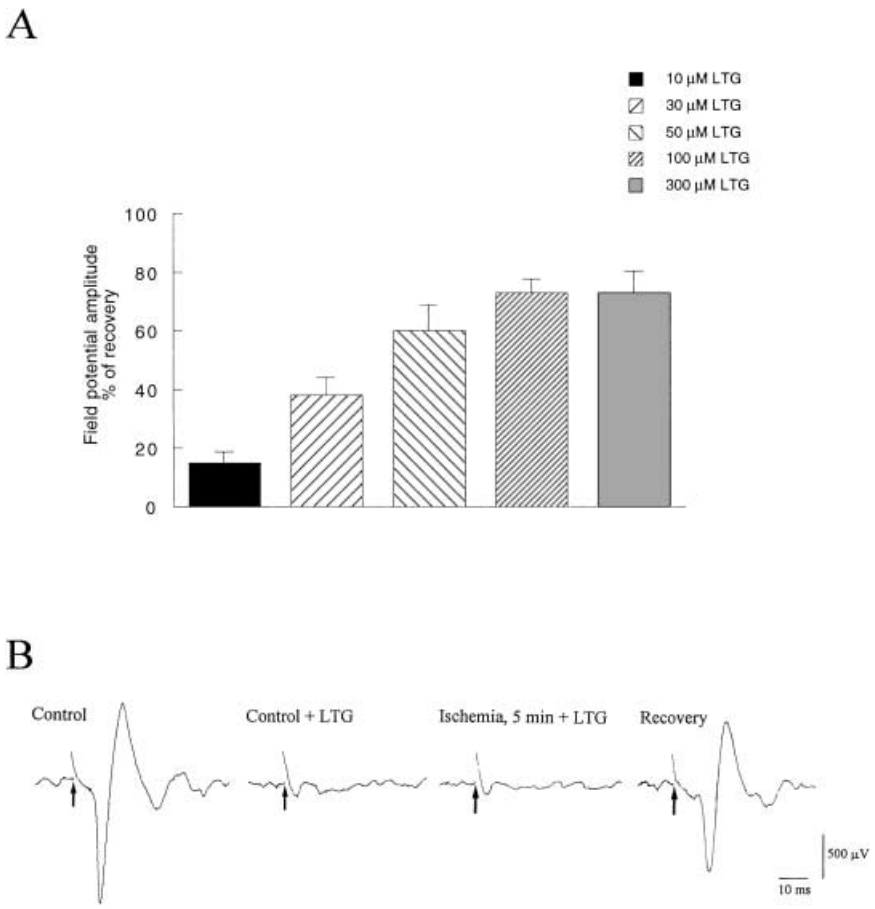

Fig. 4. Lamotrigine exposure facilitates the recovery of the field potential after ischemia. (**A**) The protective influences of lamotrigine on the degree of field recovery are shown. (**B**) Indicative traces of the recovery of the field after ischemia in the presence of LTG (100 μM). Note that LTG depressed the field EPSP.

The simple reduction of the cortico-cortical excitatory synaptic transmission before and during the ischemic episode is not sufficient for neuroprotection. This is confirmed by the use of CNQX, which was able to fully depress the field EPSP but did not influence functional recovery after ischemia.

Effects of anticonvulsants

The neuroprotective effects of FBM, LTG, and LID can be accounted for by different mechanisms: 1) an antagonism of EAA receptors, 2) a reduction in the release of EAAs, and 3) direct interference with the excessive fluxes of ions inside (sodium and calcium) and outside the neurons (potassium).

In view of the fact that a significant functional recovery of the field was not obtained when the anticonvulsants were applied only during the ischemic stimulus, the preexposure of the cells with these compounds is a prerequisite for recovery of electrophysiological parameters.

It is generally accepted that FBM has NMDA antagonistic properties. In fact, it reduces NMDA-induced cellular responses (Rho et al., 1994) by interacting with strychnine-insensitive binding sites (McCabe et al., 1993). In addition, FBM reduces the NMDA-mediated component of the cortical excitatory synaptic potential, which is elicited in low-magnesium (Calabresi et al., 1996). However, FBM not only antagonizes the effects of EAAs on NMDA-type receptors, it also reduces voltage-activated sodium and calcium currents in cortical and striatal cells (White et al., 1992; Pisani et al., 1995; Stefani et al., 1996a). Thus, the antagonistic effects of FBM on the NMDA-induced responses, together with the depression of the transmembrane ion fluxes during ischemia can account for the marked protective effect of this compound. Accordingly, FBM has recently been shown to have rescuing properties in the hypoxic hippocampus in vitro (Wallis and Panizzon, 1993) and in vivo (Wasterlain et al., 1996).

LTG is a novel anticonvulsant which has various effects—it reduces the evoked release of glutamate (Leach et al., 1986), and inhibits the glutamate-induced burst firing and the excitatory synaptic transmission in the cortex by blocking sodium conductances (Cheung et

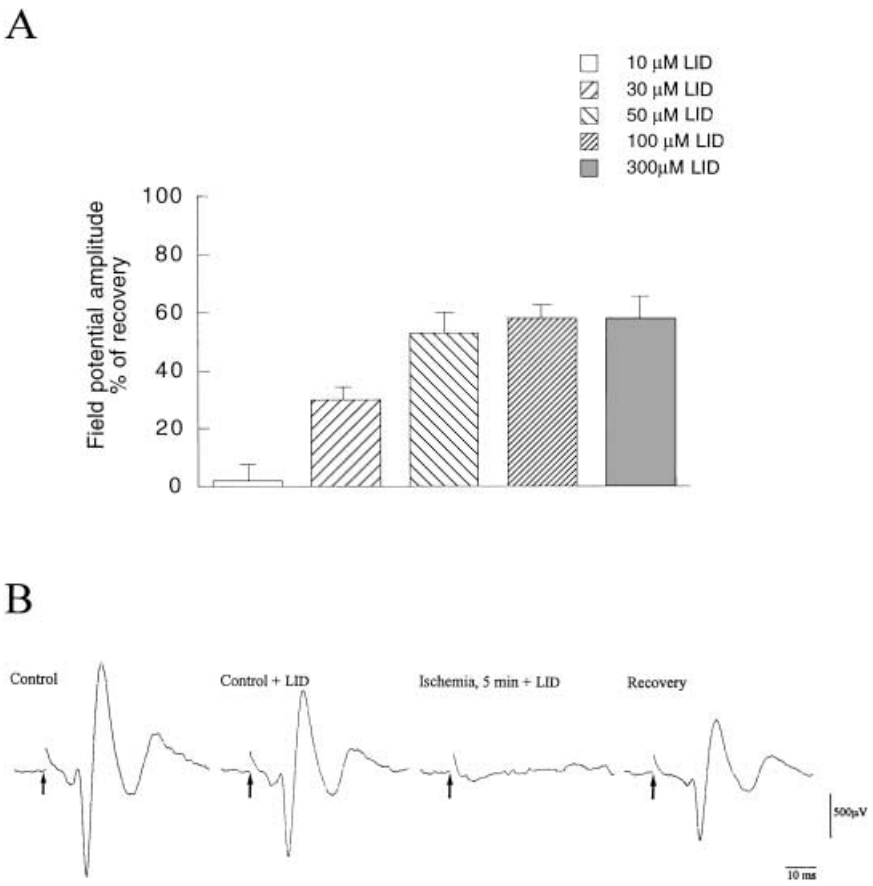

Fig. 5. Protective effects of lidocaine. (**A**) The graph shows the recovery of the field after ischemia in the presence of different concentrations of LID. (**B**) Indicative traces of the protective effects of LID (100 μM).

al., 1992; Lees and Leach, 1993; Xie et al., 1995; Calabresi et al., 1996). An interaction of LTG with voltage-activated calcium currents has also been reported in neurons of the cortex and amygdala (Stefani et al., 1996b; Wang et al., 1996). These multiple actions of LTG can account for the functional recovery observed after an ischemic insult in our preparation. In accordance, it has been demonstrated that LTG has a cerebroprotective effect after focal ischemia (Smith and Meldrum, 1995).

LID is a local anesthetic that reduces the conductance of Na^+ channels (Kaneda et al., 1989). It has been reported that LID depresses the release of aspartate, glutamate, and the efflux of potassium during ischemia (Astrup et al., 1981; Shokunbi et al., 1990). These various actions of LID might underlay its protective effects during ischemia. In agreement with our study, LID protects the neurons from anoxia in vitro (Fried et al., 1995) and from ischemia in vivo (Ayad et al., 1994) conditions by blocking Na^+ channels.

Comparison of the effects of FBM, LTG, and LID

Although all three antiepileptic drugs tested in the present study protect against the functional derangement caused by ischemia, there is a different neuroprotective potency between them. In spite of the fact that the least effective dose of LTG (10 μM) is more effective than an equivalent concentration of FBM, when the absolute level of neuroprotection was examined FBM was more potent than LTG and LID.

It is possible that the antagonistic effect of FBM on the NMDA responses is mainly responsible for the virtually complete protection after ischemia with this anticonvulsant. Thus, both the NMDA antagonism and the block of voltage-dependent sodium and calcium currents plays a synergistic role in the neuroprotection induced by FBM.

A potential important target of LTG, which is also common to FBM, is inhibition of voltage-dependent sodium and calcium currents in neurons during ischemia. A block of inactivated sodium channels during the ischemic depolarization by FBM, LTG, and LID can account, at least in part, for the anticonvulsant-induced neuronal survival after energy deprivation (Taylor and Meldrum, 1995).

In order to see the protective effects on neuronal function, either EAA antagonists or anticonvulsants had to be applied before and during ischemia in our in

vitro model. A similar phenomenon was observed for LID (Fried et al., 1995), mannitol, and fructose (Huang et al., 1996) in hippocampal slices. This might depend on the time necessary for complete diffusion of the drugs into the tissue. The combined glucose and O_2 deprivation might also change the conformation of the sites activated by the protective agents. Thus, insufficient binding may occur if the drugs are perfused only during 5 min of ischemia. In addition, pretreatment of the tissue with anticonvulsants and glutamate antagonists may cause a reduction in neuronal activity, which can in turn interfere with the production and diffusion of toxic agents (e.g., free radicals, nitric oxide, and arachidonic acid) (Pellegrini-Giampietro et al., 1990; Globus et al., 1995).

CONCLUSION

Our results demonstrate that it is possible to prevent most of the irreversible functional changes caused by ischemia by treating the slices with the anticonvulsants FBM and LTG. This may render both drugs of interest in clinical practice for the treatment of stroke in order to prevent and reduce the extension of cerebral ischemia by halting those physiopathological processes that ultimately result in cell death.

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