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## Lamotrigine Inhibits Extracellular Glutamate Accumulation during Transient Global Cerebral Ischemia in Rabbits

Andreas Bacher, M.D.,\* Mark H. Zornow, M.D.†

**Background:** During cerebral ischemia, an influx of  $\text{Na}^+$  may be partially responsible for the release of the excitatory amino acid glutamate. When glutamate is released in excessive concentrations during ischemia, it may become neurotoxic. The ability of the  $\text{Na}^+$  channel blocker lamotrigine to inhibit glutamate release during episodes of transient global cerebral ischemia was investigated.

**Methods:** After approval was given by the animal care and use committee, 24 New Zealand white rabbits were randomly assigned to one of four groups each containing six animals (control, L20, L50, and a hypothermic group). After anesthesia (1% halothane) was induced, the tracheas were intubated and the lungs mechanically ventilated before microdialysis probes were placed in the hippocampus. Ninety minutes before the onset of ischemia, either 20 or 50 mg/kg lamotrigine was administered intravenously (in the L20 and L50 groups). Esophageal temperature was maintained at 38°C in the control, L20, and L50 groups, whereas the animals in the hypothermic group were cooled to 30°C. Two 10-min periods of cerebral ischemia, separated by a 90-min interval, were generated by inflating a neck tourniquet. High-performance liquid chromatography was used to determine the glutamate concentration in the microdialysate. Analysis of variance and Dunnett's test were used for statistical analysis. Data are presented as means  $\pm$  SD.

**Results:** During the first ischemic period, glutamate concentration increased only slightly from baseline. A significant increase was observed during the second ischemic period for the control (sixfold) and the L20 (threefold) groups. Glutamate

concentrations in the L50 and the hypothermic groups were significantly lower than in the other two groups and remained at the baseline level during the entire experiment.

**Conclusions:** This study shows that the  $\text{Na}^+$  channel blocker lamotrigine is effective in inhibiting extracellular glutamate accumulation during transient global cerebral ischemia. This attenuation of ischemia-induced glutamate release may explain the previously reported neuroprotective properties of  $\text{Na}^+$  channel blockers. (Key words: amino acid: excitatory; glutamate: ischemia: cerebral; lamotrigine; rabbit.)

VARIOUS studies have shown that the excitatory amino acid glutamate plays a pivotal role in ischemic neuronal cell death.<sup>1</sup> Various models of ischemia have shown an increase in glutamate concentration in the cerebral extracellular space.<sup>2,3</sup> There is considerable evidence that glutamate released during ischemia originates in the synaptosomes. Both an impaired reuptake into presynaptic neurons and an excessive release probably are involved.<sup>4-6</sup> A breakdown of intra- and extracellular ion balance caused by a failure of cellular energy supply can contribute to ischemic glutamate accumulation.<sup>7</sup> Furthermore, glutamate reuptake depends at least in part on the maintenance of the transmembrane  $\text{Na}^+$  gradient.<sup>8</sup> Various ion channel blockers have been studied for their potency to inhibit increases in extracellular glutamate concentrations during ischemia. The ability of neuronal  $\text{Ca}^{++}$  channel blockers to inhibit glutamate release recently was shown for the conopeptide MVIIC.<sup>9</sup> Certain  $\text{Na}^+$  channel blockers also may be effective in attenuating glutamate release. Lamotrigine [3,5-diamino-6(2,3-dichlorophenyl)-1,2,4-triazine] is a  $\text{Na}^+$  channel blocker used as an anticonvulsant and recently was released for clinical use.<sup>10-12</sup> It has few side effects and can also be administered orally. Based on lamotrigine's interaction with  $\text{Na}^+$  channels and its ability to penetrate the blood-brain barrier, interest has focused on its neuroprotective properties. The ability of lamotrigine to reduce cerebral glutamate accumulation has been proved *in vitro*,<sup>13</sup> and several animal trials of focal or transient global cerebral ischemia showed that lamo-

\* Research Fellow, Department of Anesthesiology and General Intensive Care, University of Vienna, and Department of Anesthesiology, University of Texas Medical Branch.

† Clara T. Phillips Professor, Department of Anesthesiology, University of Texas Medical Branch.

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Address reprint requests to Dr. Zornow: Department of Anesthesiology, The University of Texas Medical Branch, Galveston, Texas 77555-0830. Address electronic mail to: abacher@marlin.utmb.edu.

trigine had beneficial effects on histologic cerebral changes, neurological outcome, or both.<sup>14,15</sup>

The present study was designed to examine the effect of two different doses of lamotrigine on the perischemic extracellular cerebral concentrations of glutamate.

## Materials and Methods

### Animals

The study protocol was approved by our institution's animal care and use committee. Twenty-four New Zealand white rabbits, ages 8–10 weeks and weighing  $2.8 \pm 0.2$  kg (mean  $\pm$  SD), were randomly assigned to one of four groups (control, L20, L50, and hypothermic), each containing six animals. The animals were fasted for 24 h before the start of the experiment and housed one per cage at the institutional Animal Resource Center, where they received veterinary care.

### Surgical Procedure

The rabbits were anesthetized with 4% halothane in oxygen in a clear plastic box. After loss of consciousness, a 22-gauge ear vein catheter was inserted and 0.9% saline was infused at  $6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ . The trachea was intubated with a 3-mm endotracheal tube and mechanical ventilation was started to maintain end-tidal carbon dioxide between 35 and 40 mmHg (Capnomac Ultima; Datex, Helsinki, Finland). Halothane concentration was reduced to 1% in oxygen as soon as mechanical ventilation was established. The animals were positioned in a stereotactic frame, with the interauricular line approximately 12 cm above the mid-chest. After infiltration with 0.25% bupivacaine, femoral arterial and central venous catheters were inserted and mean arterial pressure was recorded continuously. Serial arterial blood samples were withdrawn to measure arterial pH, carbon dioxide tension ( $P_{\text{CO}_2}$ ), oxygen tension ( $P_{\text{O}_2}$ ), and base excess (BE). Mechanical ventilation was adjusted to maintain  $P_{\text{CO}_2}$  between 35 and 40 mmHg. Body temperature was monitored by an esophageal temperature probe and servocontrolled by an infrared heating lamp and a warming mattress for maintenance at 38.0°C. In one study group (the hypothermic group), body temperature was actively reduced to 30°C by putting ice on the dorsal region of the animal. Hypothermia was maintained throughout the entire experiment in the hypothermic group.

After infiltration with 0.25% bupivacaine, the scalp

was incised in the midline and reflected laterally to expose the skull. Bilateral burr holes, 2 mm in diameter, were drilled 4 mm posterior and 4 mm lateral to the bregma to place microdialysis catheters into the dorsal hippocampus at a depth of 8 mm using stereotactic manipulators. The microdialysis catheters were secured to the skull using dental acrylic before they were released from the carriers. Needle electrodes were placed in the scalp for continuous recording of the frontoparietal electroencephalogram. Finally, an inflatable neck tourniquet (6 cm wide) was secured loosely around the rabbit's neck. At the end of the experiment the animals were killed by increasing halothane concentration to 5% and by injecting 10 mmol potassium chloride.

### Drug Administration

Lamotrigine (Burroughs Wellcome, Research Triangle Park, NC), at a dose of 20 mg/kg (L20 group) or 50 mg/kg (L50 group), was diluted in 60 ml 0.5% methyl cellulose. The drug was administered intravenously in these two study groups for 20 min starting 1.5 h before the first ischemic period was induced. A third study group not receiving lamotrigine served as a control group. Finally, the hypothermic group was treated in the same way as the control group, except for the reduction in body temperature.

### Microdialysis

The microdialysis probes (BAS, West Lafayette, IN) were 500  $\mu\text{m}$  in diameter and had a dialysis membrane that was 4 mm long. Before insertion, the probes were perfused with artificial cerebrospinal fluid (147 mM NaCl, 2.3 mM  $\text{CaCl}_2$ , 0.9 mM  $\text{MgCl}_2$ , 4 mM KCl) at a rate of 2  $\mu\text{l}/\text{min}$ . The perfusion rate of 2  $\mu\text{l}/\text{min}$  was maintained throughout the entire study. No dialysate samples were collected and no manipulation was performed on the animals for 60 min after insertion. Thereafter, dialysate was collected every 10 to 15 min for later analysis.

### Induction of Ischemia

After collection of baseline samples of microdialysate, mean arterial pressure was decreased to 25–50 mmHg with a bolus of 5 mg trimethaphan given intravenously. The neck tourniquet was inflated to a pressure of 80 mmHg within 0.5 s using a regulated tank source of compressed air. Ischemia was considered effective if an isoelectric electroencephalogram was achieved within 30 s after the tourniquet was inflated. After 10 min of ischemia, the neck tourniquet was deflated and mean

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arterial pressure was restored to 80–100 mmHg with a bolus of phenylephrine (5–10  $\mu$ g given intravenously). The electroencephalogram was examined for spontaneous activity in the postischemic period. This sequence was repeated after 90 min to produce a second episode of ischemia.

#### Determination of Glutamate Concentration

High-performance liquid chromatography with fluorescence detection was used to analyze the dialysate samples. The concentrations were quantified based on linear calibration with known amino acid standards.

#### Statistical Analysis

All data were analyzed using a commercially available computer program (Stat View 4.01; Abacus Concepts, Berkeley, CA). Factorial analysis of variances and Dunnett's test were used to determine differences in peak glutamate concentrations among the groups. To test for statistical significance of changes within one group between baseline values and peak levels of glutamate concentration, a paired *t* test was used. Changes in body temperature, pH,  $P_{O_2}$ ,  $P_{CO_2}$ , and BE were compared with repeated-measures analysis of variance and Scheffé's test.

## Results

#### Vital Parameters

The induction of ischemia resulted in an isoelectric result of electroencephalogram within 30 s after the neck tourniquet was inflated in all animals. Partial spontaneous activity was observed after 25 min of reperfusion, and full spontaneous recovery occurred within 45 min after ischemia was terminated in all animals, after both ischemic episodes. There were no significant differences in pH,  $P_{CO_2}$ ,  $P_{O_2}$ , or BE among the groups, and no significant changes over time were observed, except for a decrease in pH and BE, which occurred in all groups. Figure 1 shows the course of body temperature for all study groups. Body temperature was well maintained at approximately 38°C in the control, L20, and L50 groups. In the hypothermic group, body temperature was already lower than in the other groups ( $P < 0.05$ ) at the initial measuring point (*i.e.*, 15 min before the onset of the first ischemic period). During the rest of the study, body temperature remained stable at approximately 30°C in the hypothermic group.

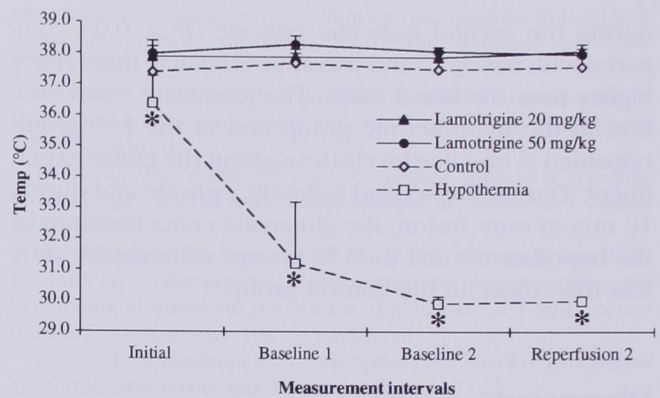


Fig. 1. Changes in body temperature (Temp) during the experiment in all study groups. Initial = 15 min before ischemia 1, baseline 1 = immediately before ischemia 1, baseline 2 = immediately before ischemia 2, reperfusion 2 = 25 min after ischemia 2. \* $P < 0.05$  compared with other study groups (analysis of variance, Scheffé's test).

#### Glutamate Concentration

Figure 2 shows the glutamate concentrations for each of the groups during the experiment. Glutamate increased slightly during the first ischemic period in the control and the L20 groups. During the second ischemic episode, glutamate concentration in the control group peaked at levels approximately six times higher than the initial values. Twenty-five minutes later, glutamate concentrations had returned to baseline values. A similar pattern of changes in glutamate concentrations was observed in the L20 group. The peak value occurred

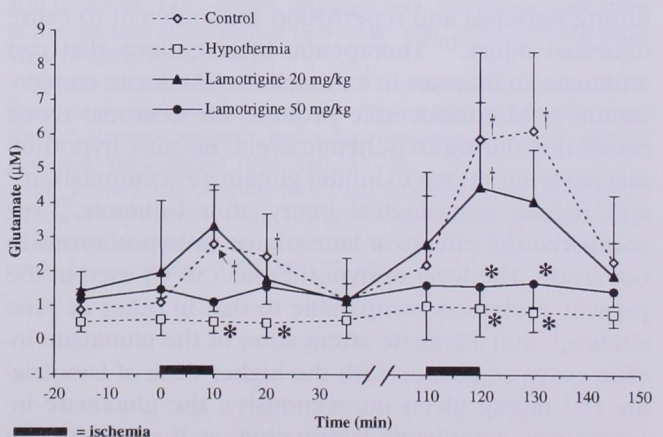


Fig. 2. Changes in extracellular glutamate concentration during the experiment in all study groups. \* $P < 0.05$  compared with the control group (analysis of variance, Dunnett's test). † $P < 0.05$  compared with the preceding baseline value (dialysate collection between 15 min before and onset of ischemia).

during the second ischemic episode ( $P < 0.05$  compared with baseline) and was approximately three times higher than the initial value. The glutamate concentration in the hypothermic group and in the L50 group remained at baseline levels throughout the entire experiment. During the second ischemic episode and during 10 min of reperfusion, the glutamate concentrations of the hypothermic and the L50 groups were significantly less than those in the control group.

## Discussion

The present study was performed to investigate the effects of the  $\text{Na}^+$  channel antagonist lamotrigine on *in vivo* glutamate accumulation in an animal model of transient global cerebral ischemia. Extracellular glutamate concentration increased six times in the untreated, normothermic control group during a second 10-min period of ischemia and reperfusion. Hypothermia ( $30^\circ\text{C}$ ) prevented any increase in glutamate concentration. If 20 mg/kg of lamotrigine was administered intravenously 90 min before the onset of ischemia, glutamate accumulation was only partially inhibited. However, with 50 mg/kg the values during ischemia and reperfusion remained at the baseline level, comparable to the hypothermic animals.

Glutamate accumulation in the hippocampus occurring during transient ischemia is a well-known phenomenon, and there is evidence that glutamate plays an important role in inducing neuronal cell death during ischemia.<sup>2,3,16,17</sup> The glutamate concentrations obtained during ischemia and reperfusion are sufficient to cause neuronal injury.<sup>18</sup> Therapeutic interventions that can attenuate an increase in extracellular glutamate concentration hold considerable promise for neuronal tissue protection during an ischemic event. Because hypothermia has been shown to inhibit glutamate accumulation<sup>19</sup> and reduce neurological injury after ischemia,<sup>20</sup> we compared the effects of lamotrigine to hypothermia in our study. The level of hypothermia ( $30^\circ\text{C}$ ) used in the present study was comparable to that in other *in vivo* studies,<sup>19</sup> and adequate attenuation of the glutamate increase was achieved. With the higher dose of lamotrigine (50 mg/kg given intravenously), the glutamate increase was completely suppressed, as it was with the application of hypothermia.

Although blocking postsynaptic receptors would represent another possible approach to reduce neuronal injury caused by excitatory neurotransmitters, there are

at least three different postsynaptic receptors for excitatory amino acids.<sup>21</sup> Glutamate activates not only N-methyl-D-aspartate receptors but also other postsynaptic receptors such as the S- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate and the kainate receptor.<sup>22</sup> Therefore, it might appear preferable to prevent the release of glutamate rather than to block its postsynaptic binding sites.

Various other studies have shown neuroprotective effects of lamotrigine. *In vitro*, lamotrigine inhibited glutamate release in a preparation of rat brain slices after electrical stimulation as well as after veratrine application.<sup>13</sup> *In vivo*, administration of lamotrigine (20 mg/kg intravenously) to rats immediately after middle cerebral artery occlusion resulted in smaller cerebral infarct volumes and in less neurological deficit assessed by a neurological scoring system.<sup>14</sup> At a higher dose (50 mg/kg given intravenously), lamotrigine caused a decrease in mean arterial pressure and was not neuroprotective in rats. In the present study, 50 mg/kg of lamotrigine given intravenously did not induce hypotension but did prevent glutamate accumulation during 10 min of global cerebral ischemia. The neuroprotective potency of lamotrigine has also been investigated in a gerbil model of transient global cerebral ischemia. Hippocampal neuronal injury was assessed histologically, and changes in spatial memory were tested in a water maze after common carotid artery occlusion.<sup>15</sup> Lamotrigine prevented histologic cell damage after ischemia of 5, 10, and 15 min. The impairment of spatial learning that was observed after 15 min of ischemia in control animals was attenuated by 50 mg/kg when given 2 h before and again immediately after common carotid artery occlusion.

An inhibition of extracellular glutamate accumulation seems to be a reasonable explanation for at least some of the neuroprotective properties of lamotrigine in these models of cerebral ischemia. However, it is still unclear by which mechanism glutamate release during ischemia is inhibited. A detailed electrophysiologic analysis it showed that lamotrigine inhibits  $\text{Na}^+$  currents in a concentration-dependent and voltage-dependent manner.<sup>23</sup> Lamotrigine mainly acts on the slow inactivation of  $\text{Na}^+$  channels and can inhibit rapid repetitive firing in a use-dependent manner. Glutamate release is enforced by the inward movement of  $\text{Na}^+$ , and N-methyl-D-aspartate receptor channels are opened by removing  $\text{Mg}^{++}$ .<sup>7</sup>

In our study, the glutamate concentration returned to baseline levels quickly after reperfusion in the control group. It is noteworthy that the glutamate concentra-

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tion during the second period of ischemia in our control group was more than twice as high as during the first period of ischemia. This observation was made in previous studies with a similar design.<sup>24</sup> Repetitive ischemia appears to enhance the excitatory response of neurons. Further investigations are required to identify any effects of lamotrigine on elevated glutamate concentrations during a longer period of ischemia.

The Na<sup>+</sup> channel blocker lamotrigine is a potent agent for attenuating extracellular glutamate accumulation during transient global cerebral ischemia. The efficacy of lamotrigine with regard to the inhibition of ischemic extracellular glutamate increase is comparable to that of moderate (30°C) hypothermia.

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