Experimental evidence suggests that excitatory amino acids potentiate neuronal ischemic damage. Excessive stimulation of excitatory neurotransmitter receptors is damaging to neurons, and this neurotoxicity can be blocked by specific antagonists. Similarly, antagonists of excitatory neurotransmitters have been shown to blunt cell injury in a variety of in vitro and in vivo models of neuronal ischemia. Such drugs are now in clinical trial for treatment of stroke, and early analysis suggests benefit. However, side effects can be considerable and may limit clinical usefulness. Conversely, agonists of inhibitory neurotransmitters are commonly used clinically (with acceptable side-effect profiles) yet have not been extensively tested as potential neuroprotective agents.

A variety of evidence suggests that increased inhibitory neurotransmitter activity may protect ischemic neurons. At the cellular level, inhibitory neurotransmitters such as γ-aminobutyric acid (GABA) increase chloride conductance, effectively blunting depolarization and therefore the opening of voltage-dependent calcium channels. Such inhibition may also reduce the metabolic requirements of the normally perfused and the ischemic neuron. Neurons producing GABA are relatively resistant to both N-methyl-D-aspartate (NMDA)-induced neurotoxicity and ischemia. Neuronal death related to excitotoxin exposure can be ameliorated by local application of GABA agonists.

Background and Purpose Antagonists of excitatory neurotransmitters are effective in limiting ischemic damage to the brain and spinal cord, but use in clinical stroke may be limited by side effects. Agonists of inhibitory neurotransmitters, such as γ-aminobutyric acid (GABA), may provide similar neuroprotection with less severe side effects. This study examines the effect of an agonist and antagonist of the GABA-A receptor on neuronal ischemic damage.

Methods Either muscimol (a GABA-A agonist) or bicuculline (a GABA-A antagonist) was administered intravenously to groups of rabbits exposed to reversible spinal cord ischemia induced by temporary occlusion of the infrarenal aorta. The duration of occlusion for individual animals was varied, providing a range of ischemia for each experimental group. The group P50 represents the duration (in minutes) associated with 50% probability of resultant permanent paraplegia. Neuroprotection was demonstrated if a drug prolonged the P50 compared with the control group.

Results The P50 of the control group was 26.3±2.0 minutes. Treatment with intravenous muscimol at 5 mg/kg significantly prolonged the P50 (32.4±1.3; P=.01). Treatment with intravenous bicuculline at 0.1 mg/kg significantly shortened the P50 (20.6±2.1; P=.03). The physiological and apparent behavioral effects of the drugs at these doses did not appear substantial.

Conclusions Pharmacological manipulation of the GABA-A receptor may offer another avenue of therapy for central nervous system ischemia, possibly with less severe associated physiological side effects than other effective drugs. (Stroke. 1994;25:2271-2275.)

Key Words • excitotoxins • GABA • neuroprotection • rabbits

Effect of γ-Aminobutyric Acid Modulation on Neuronal Ischemia in Rabbits

Kenneth P. Madden, MD, PhD

See Editorial Comment, page 2274

Regional vulnerability to ischemic injury can also be correlated with the concentration of excitatory neurotransmitters relative to that of GABA. The turtle brain, which is remarkably resistant to anoxic injury, produces markedly elevated GABA levels during anoxic exposures. In addition to metabolic effects, GABA may also elicit a local vasodilatory response. Previous experimental work has correlated pharmacologically increased levels of GABA activity with a lessened degree of neuronal ischemic injury in both brain and spinal cord. Other investigators, however, have not been able to demonstrate the benefit of GABAergic drugs in ischemia, particularly those agents relatively specific for the GABA-B receptor. In a rabbit model and a rat model of multiple cerebral emboli, Lyden and colleagues recently demonstrated neuroprotection by the GABA-A agonist muscimol, both alone (Lyden and Hedges) and in synergy with the NMDA antagonist MK-801 (Lyden and Lonzo). In this study we further explore the therapeutic effect of pharmacological modulation of the GABA-A receptor, using both the agonist muscimol and the specific antagonist bicuculline in a model of spinal cord ischemia.

Materials and Methods

This experimental protocol was approved by the Animal Use Committee of the Marshfield Medical Research Foundation. New Zealand White rabbits (weight, 2.5 to 3.5 kg) were anesthetized with halothane before invasive procedures. Physiological studies were initially performed to determine the effects of muscimol (Sigma; freshly dissolved, concentration 5 mg/mL) and bicuculline (Sigma; freshly dissolved in 0.1N HCl, pH corrected with NaOH, concentration 2 mg/mL) on rectal temperature, blood pressure, pulse rate, and behavior in...
rabbits. A dose-finding study was also performed to approximate the threshold dose of bicuculline for production of seizures in the rabbit. Based on preliminary work showing little therapeutic benefit of muscimol in this model at 2.5 mg/kg, a dose of 5 mg/kg was selected for this study.

For the spinal cord ischemia study, a 10-cm ventral midline incision was made to place a snare ligature about the infrarenal aorta. The snare device consisted of a large-bore Tygon tubing housing a loop of thin Tygon tubing that encircled the vessel. One end of the large tubing was exteriorized, allowing easy manipulation of the thin tubing within, and animals were then allowed to recover from anesthesia. Temporary spinal cord ischemia was accomplished in the awake rabbit by tightening the thin tubing for a predetermined duration, then releasing the snare to allow reperfusion. This ischaemic duration was varied among the animals in each experimental group to provide a range of durations for the group. Eighteen hours after ischemia, the animals were examined for paraplegia. A binary scoring system was used, with animals rated in a blinded fashion as either paraplegic or not paraplegic (includes animals with minimal motor function). Within each experimental group, the duration of ischemia producing a 50% probability of paraplegia in animals was termed the \( P_{50} \) (similar to the \( LD_{50} \) of pharmacological studies).

One group of animals was treated with a bolus of 5 mg/kg IV muscimol 5 minutes after the onset of spinal cord ischemia. A second group received 0.1 mg/kg IV bicuculline 5 minutes before spinal cord ischemia. An untreated group of animals was used as a control. The effect of either drug on neuronal ischemia was assessed by shift of the \( P_{50} \) of treated animals compared with that of the control group. A rightward shift (greater \( P_{50} \)) indicates that animals are able to tolerate longer durations of ischemia without resultant paraplegia; a leftward shift (shorter \( P_{50} \)) indicates less tolerance of ischemia.

The statistical significance of shifts in \( P_{50} \) was assessed by group \( t \) test and adjusted for multiple comparisons with the Bonferroni correction (\( P<0.05 \)).

### Results

#### Bicuculline Dose-Finding Study

Doses of bicuculline of 0.5 mg/kg or greater consistently resulted in rapid onset of generalized seizure accompanied by what appeared to be rapid cardiac arrest. Rabbits receiving 0.2 to 0.3 mg/kg consistently survived but had rapid onset of generalized seizure. Of 24 rabbits receiving the study dose of 0.1 mg/kg, 7 displayed very brief (<5 seconds) apparent seizure activity consisting of rhythmic eye blinking only, without generalized motor phenomena. The remainder exhibited no behavioral change to suggest seizure occurrence.

#### Physiologic Effects of Muscimol and Bicuculline

Other than the very mild convulsive effects described above, bicuculline at 0.1 mg/kg caused no apparent change in behavior, nor were any significant physiological effects noted on body temperature, blood pressure, or heart rate.

Animals receiving 5.0 mg/kg muscimol displayed nystagmus and moderate ataxia, with minimal actual apparent sedation. Behavioral effects seemed to dissipate by 4 hours. The drug caused a relative bradycardia (approximately 20% to 30% decrease) without blood pressure change. Heart rate normalized by 4 hours. Rectal temperatures were unchanged during the first 2 hours, usually followed by a slight decline during the next 2 hours (0.2°C to 1.8°C) before returning to baseline.

#### Spinal Cord Ischemia

The Table lists duration of ischemia and functional outcome for three groups of animals. Fifteen untreated control animals received varying durations of spinal ischemia; a leftward shift (shorter duration of ischemia) versus functional outcome, were used to graphically display therapeutic efficacy. More detailed explanations of this data analysis method have been published.

### Table: Duration of Ischemia and Subsequent Outcomes for the Three Experimental Groups

<table>
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<th>Group</th>
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cord ischemia, with a resultant $P_{50}$ ($\pm$SE) of 26.3±2.0 minutes. Eleven animals received 5 mg/kg muscimol 5 minutes after initiation of ischemia, resulting in significant prolongation of the $P_{50}$ to 32.4±1.3 minutes ($P=0.01$). Ten animals received 0.1 mg/kg bicuculline 5 minutes before initiation of spinal cord ischemia. This resulted in a significant decrease of the $P_{50}$ to 20.6±1.5 minutes ($P=0.03$). The Figure graphically depicts the probability of resultant paraplegia after spinal cord ischemia for these groups. The rightward displacement of the muscimol curve demonstrates greater tolerance to ischemia relative to the control group; the leftward shift of the bicuculline curve demonstrates the converse.

Discussion

As might be intuitively expected, these data suggest that the modulatory effect of inhibitory neurotransmitters on ischemic outcome is directly contrary to that of excitatory neurotransmitters. The direct application of the excitatory transmitter NMDA is toxic to cerebral neurons, and NMDA antagonists attenuate ischemic damage in both brain and spinal cord. In contrast, an agonist of the inhibitory transmitter GABA improves functional outcome after spinal cord ischemia in this study, and a GABA antagonist worsens outcome.

The apparent neuroprotection demonstrated by muscimol in this study is consistent with protective effects noted in cerebral ischemic models. Muscimol, as well as other agents with GABAergic properties, limits delayed hippocampal cell loss after ischemia in a gerbil model. Lyden and Hedges demonstrated that muscimol improves survival and limits infarct volume in models of multiple cerebral emboli. Behavioral learning was also less impaired by ischemia after muscimol treatment. These effects were similar to the protective benefit of MK-801. The current data extend the apparent neuroprotection of muscimol to spinal cord ischemia and demonstrate therapeutic efficacy in a functional rather than histological model.

The mechanism of neuroprotection by modulation of these neurotransmitters remains unclear. Given the diametric effects of inhibitory and excitatory neurotransmitters during ischemia, it appears unlikely that independent toxic mechanisms are involved. The two types of transmitters more likely have opposing effects on similar cellular processes (such as membrane potential) affecting the ischemic neuron. Alternatively, inhibitory transmitters may directly antagonize the action of excitatory transmitters, which are known to become substantially elevated during ischemic conditions, although the demonstration of synergy between MK-801 and muscimol argues against this. The subtype of GABA receptor pharmacologically activated may also be important. Although the limited data involving relatively specific GABA-A agonists (such as muscimol) have shown consistent neuroprotective benefit, prior investigations involving GABA-B agonists have been conflicting. The prominent effect on membrane polarization via chloride channels exerted by GABA-A receptor activation may be more critical during ischemic conditions than the presumed presynaptic mechanism of inhibition mediated by GABA-B receptors. Alternatively, the disparate results may reflect differences in concentration of the two receptor types, particularly in varying species.

The physiological effects of the drugs do not appear to be responsible for the neurological outcomes demonstrated in this study. There was a tendency in some rabbits to develop a slight drop in rectal temperature 2 to 4 hours after muscimol injection. While more substantial degrees of hypothermia have been shown to have a potentially confounding neuroprotective effect during ischemia, this was a minimal decline that did not fall below 37°C. Although rectal temperature should closely approximate spinal cord temperature, it should be noted that this study did not directly measure central nervous system temperature in these alert animals. The drug also caused a relative bradycardia, although blood pressure was unaffected. Since it is difficult to postulate how bradycardia could be protective in spinal cord ischemia, such an effect does not appear significant for these purposes. The epileptogenic potential of bicuculline can also potentially contaminate these data. Not only could the occurrence of a seizure possibly worsen functional recovery from spinal cord ischemia, but the seizure itself might initiate a cascade of other neuro-
transmitter releases and biochemical events that could affect neurological function. For this reason, the dose of bicuculline was selected in this study to be just below the threshold for seizure production in this species. The few apparent seizures that were noted with these doses were very brief and consisted of eye blinking only.

In summary, these data support the potential therapeutic benefit of the GABA-A agonist muscimol in this rabbit model of spinal cord ischemia. Muscimol had a relatively mild effect on behavior in this investigation, which is encouraging in terms of its potential use in humans. The magnitude and type of side effects in humans, of course, may differ substantially from these relatively crude animal behavioral observations. However, there is no reason to suspect that this drug will have the psychotomimetic effects that may limit human use of NMDA antagonists, and in fact GABA-B agonists are already used clinically for spastic disorders. While extrapolation of animal studies to human use is always fraught with uncertainties, the modest behavioral effects noted in this study and the clinical familiarity with similar agents currently in use should encourage further research with these drugs in ischemic models and possibly in future clinical stroke trials.

Acknowledgment
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References

Editorial Comment

The role of excitatory amino acid neurotransmitters in the pathophysiology of ischemic neuronal injury has been addressed by numerous experimental investigations. Indeed, glutamate antagonists protect neurons against injury in vitro, can reduce ischemic neuronal injury in animal models, and are in clinical stroke trials in the United States and elsewhere in the world. On the contrary, the role that inhibitory neurotransmitters might play in limiting ischemic neuronal injury has, by comparison, been studied much less. Receptors for the inhibitory amino acid neurotransmitter y-aminobutyric acid (GABA) serve to counterbalance the excitatory influences of glutamate in the central nervous system and are the sites of action of clinically used anticonvulsants, eg, gamma-vinyl GABA and benzodiazepines. Agonists selective for GABA-A receptors have been described and are reported to be anticonvulsant, but few studies have addressed their neuroprotective potential. In cortical cell culture, while...
Effect of gamma-aminobutyric acid modulation on neuronal ischemia in rabbits.

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