White Matter Injury in Spinal Cord Ischemia
Protection by AMPA/Kainate Glutamate Receptor Antagonism

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Background and Purpose—Spinal cord ischemia is a serious complication of surgery of the aorta. NMDA receptor activation secondary to ischemia-induced release of glutamate is a major mechanism of neuronal death in gray matter. White matter injury after ischemia results in long-tract dysfunction and disability. The AMPA/kainate receptor mechanism has recently been implicated in white matter injury.

Methods—We studied the effects of AMPA/kainate receptor blockade on ischemic white matter injury in a rat model of spinal cord ischemia.

Results—Intrathecal administration of an AMPA/kainate antagonist, 6-nitro-7-sulfamoyl-(f)-quinoxaline-2,3-dione (NBQX), 1 hour before ischemia reduced locomotor deficit, based on the Basso-Beattie-Bresnahan scale (0=total paralysis; 21=normal) (sham: 21±0, n=3; saline: 3.7±4.5, n=7; NBQX: 12.7±7.0, n=7, P<0.05) 6 weeks after ischemia. Gray matter damage and neuronal loss in the ventral horn were evident after ischemia, but no difference was noted between the saline and NBQX groups. The extent of white matter injury was quantitatively assessed, based on axonal counts, and was significantly less in the NBQX as compared with the saline group in the ventral (sham: 1063±44/200×200 μm, n=3; saline: 556±104, n=7; NBQX: 883±103, n=7), ventrolateral (sham: 1060±135, n=3; saline: 411±66, n=7; NBQX: 676±122, n=7), and corticospinal tract (sham: 3391±219, n=3; saline: 318±23, n=7; NBQX: 588±103, n=7) in the white matter on day 42.

Conclusions—Results indicate severe white matter injury in the spinal cord after transient ischemia. NBQX, an AMPA/kainate receptor antagonist, reduced ischemia-induced white matter injury and improved locomotor function.

(Stroke. 2000;31:1945-1952.)

Key Words: aorta ■ axons ■ excitotoxins ■ myelin ■ paraplegia ■ spinal cord ■ rats

Ischemic injury to the spinal cord leading to paraplegia continues to represent a serious complication of surgery on the descending thoracic and thoracoabdominal aorta. Numerous surgical techniques have been proposed and are being used to attenuate the severity of the ischemic insult to the spinal cord during these extensive surgical procedures. No techniques have been found to reliably prevent intraoperative spinal cord injury. A different approach to prevent ischemic injury of the spinal cord is pharmacological prophylaxis directed at enhancing tolerance of the spinal cord to ischemia.

Glutamate is the major excitatory neurotransmitter in the central nervous system of vertebrates. Under normal conditions, neurons are exposed to physiological concentrations of glutamate in the course of excitatory neurotransmission. Such exposure is not injurious. During ischemia, a massive release of glutamate into the extracellular space, coupled with a decreased capacity of metabolically impaired glia to transport glutamate, augments injury and facilitates neuronal death.

See Editorial Comment, page 1952

Disability after spinal cord injury (SCI) is primarily caused by axonal injuries or dysfunction in the white matter. Neurological deficit, to a large extent, is determined by the lesion size in the white matter. A gray matter lesion in the cord sparing most white matter (eg, central cord syndrome) results in segmental motor or sensory dysfunction and usually does not cause deficit below the affected level. The most severe disability after SCI generally stems from loss of communication between the brain and spinal cord secondary to dysfunction of the axons that constitute the long tracts in the white matter. White matter injury in the spinal cord, even segmental, may disrupt axonal conduction in long tracts leading to paralysis below the lesion.

Studies of central nervous system ischemia have mainly focused on gray matter injury. Degeneration of the white matter after spinal cord ischemia has not been systematically

Received January 12, 2000; final revision received April 20, 2000; accepted May 16, 2000.

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1945
explored. The conventional view that the white matter is less vulnerable to ischemic injury as compared with the gray matter is now being questioned. Increasing in vivo evidence indicates that ischemia may primarily damage white matter in the spinal cord and the brain. Whereas excitotoxins play a major role in the pathogenesis of ischemic gray matter injury, the role of glutamate receptor action in ischemic white matter lesion is less clear. Glial elements and axons have traditionally been considered resistant to injury caused by excitotoxins exposure. However, activation of the AMPA/kainate glutamate receptor has recently been shown to cause oligodendrocyte death in vitro and in vivo. In addition, the AMPA/kainate receptor was found to mediate oligodendrocyte death after oxygen-glucose deprivation in vitro. Interestingly, AMPA/kainate receptor antagonism has also been found effective in salvaging white matter after traumatic SCI in the rat.

The present study aimed to investigate (a) the pathological effects of spinal cord ischemia induced by aortic occlusion on the white matter and (b) the efficacy of 6-nitro-7-sulfonyl-2,3-dione (NBQX), a competitive and highly potent antagonist of the AMPA/kainate glutamate receptor, in reducing the white matter injury and neurological deficit after spinal cord ischemia.

Materials and Methods

Surgery

Forty-eight Long-Evans outbred male rats (body weight 360±30 g) were used in the study. All animals were allowed free access to laboratory chow and tap water in a day-night regulated quarters at 24°C. Animal care complied with the “Principles of Laboratory Animal Care” and the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 85-23, 1985) and was approved by the Animal Studies Committee, Washington University School of Medicine. The model of spinal cord ischemia used in this study has been described in detail previously. In brief, the surgery was performed under inhalation anesthesia with halothane. The tail artery and the left internal carotid artery were cannulated for monitoring the distal aortic blood pressure and the temperature probe inserted 3 cm into the rectum. During the surgical preparation, the temperature was maintained at 37.0±0.5°C with the use of a heating lamp connected to a temperature monitor and a thermostat. Heparin (100 U/kg) was administered intra-arterially before aortic occlusion. Arterial blood gases and blood glucose were determined just before aortic occlusion. A 2F Fogarty catheter was inserted through the left common carotid artery and subsequently advanced into the thoracic aorta. Inflation of the catheter balloon with 0.10 mL water was performed and maintained for 11 minutes, occluding the aortic arch and the origins of the left common carotid and left subclavian arteries. The left femoral artery was partially incised transversely immediately after inflation of the catheter balloon to equilibrate the arterial pressure below occlusion to the atmospheric pressure. Blood was collected in a 10-mL syringe containing 25 U of heparin during the period of ischemia. The recovered blood was administered to the animals through the internal carotid artery cannula, if needed during the later stage of aortic occlusion, to maintain the mean distal pressure in this artery at 50 mm Hg. The remaining blood was administered to the animal within a period of 2 minutes after deflation of the balloon. The rectal temperature was recorded for ≥3 hours after the onset of reperfusion.

Drug Administration

The rats were randomized into 3 groups. The control group (n=15) received a single intrathecal injection of 20 μL 0.9% NaCl 1 hour before aortic occlusion. The intrathecal injection was conducted as described by Sloane-Stanley and Chase, with modifications. The vertebral arches of L6 and S1 were exposed through a 1-cm vertical incision; a 27-gauge needle was inserted into the vertebral canal through the L6-S1 intervertebral space and was advanced in the rostral direction for 2 cm. In a series of preliminary experiments in 5 rats, we confirmed that injection of 20 μL of methylene blue solution consistently resulted in the distribution of the dye in the subarachnoid space from the caudal cord to thoracic segments 30 minutes to 3 hours after injection. The treatment group (n=15) received 425 μg NBQX disodium diluted in 20 μL 0.9% NaCl in a similar manner as in the saline group. We administered NBQX through the intrathecal route to avoid the nphrotoxic effects of the drug at higher doses that are required in systemic administration. The therapeutic strategies for reducing ischemic injury are directed at a specific patient population undergoing invasive surgery. Intrathecal administration of a neuroprotective agent may not pose much technical difficulty in this group of patients. The NBQX dose chosen for the present study was based on a series of preliminary experiments in which NBQX in doses ranging from 375 to 600 μg was intrathecally administered under halothane anesthesia in another set of animals (n=6). Animals that received up to 425 μg NBQX disodium did not exhibit sedation or respiratory disturbance, and none of these animals died. However, these animals exhibited severe but transient flaccid paraplegia lasting for ~2 hours after NBQX delivery. This was followed by gradual and complete recovery of the motor function in the lower limbs within 6 hours after injection. The sham-operated group (n=7) received intrathecal injection of 20 μL 0.9% NaCl and underwent Fogarty catheter insertion in the aorta without the aortic arch occlusion. The animals were killed at either 48 hours (acute series: 8 in the saline group, 8 in the NBQX group, and 4 in the sham group) or 42 days (chronic series: 7 in the saline group, 7 in the NBQX group, and 3 in the sham group). Crede’s maneuver was used for evacuation of the urinary bladder when necessary.

Evaluation of Hindlimb Function

The hindlimb function was scored by means of the Basso, Beattie, and Bresnahan (BBB) open-field locomotion scale developed for traumatic SCI. The BBB scale ranges from 0 (no detectable movement in the hind limbs) to 21 (normal locomotion). BBB scores were recorded at 1, 6, 12, 24, and 48 hours in the acute series and on days 7, 14, 21, 28, 35, and 42 in the chronic series by experienced investigators who did not conduct the surgery and were blinded to the treatment codes.

Light Microscopy

In both the acute and chronic series, the animals were anesthetized with an overdose of pentobarbitial (150 mg/kg IP) and transcardially perfused with 0.9% NaCl for 1 minute followed by 400 mL 10% buffered formalin. The cadavers were kept at 4°C for 4 hours. The spinal cord was harvested and postfixed in the same fixative at 4°C overnight. The 3rd and 4th lumbar spinal cord segments were isolated and embedded in paraffin. Consecutive 8-μm sections were cut serially and were mounted and stained with hematoxylin and eosin and Nissl. In the acute series, spinal cord damage was assessed by means of a semiquantitative scoring system in a blinded fashion as previously described. A score was given according to the extent and severity of histopathological changes in 3 sets of hematoxylin and eosin–stained and Nissl-stained specimens in the mid-segment of the 4th lumbar cord. The grading of the acute gray matter injury was based on percent abnormal or dead neurons in the vertebral horns: 0, no neuronal injury or death; 1, mild damage (<10%); 2, moderate damage (10% to 50%); and 3, severe damage (>50%). Three regions of the spinal cord gray matter were scored: the ventral horn with the large motoneurons (Rexed’s laminae 8 and 9), the intermediate gray matter (laminae 7 and 10), and the dorsal horn (laminae 1 to 6).
(Figure 1). The acute white matter damage in the ventral and ventrolateral funiculi was assessed on the basis of the extent of vacuolation: 0, normal (no vacuolations seen); 1, mild damage (<10% area affected); 2, moderate damage (10% to 50%); and 3, severe damage (>50%) (Figure 1). The score for the gray or white matter damage in each animal was the average of the right and left hemisegments in 3 consecutive sets of specimens from each animal. In the chronic series, the number of neuronal cell bodies per micros-copy field was counted in the ventral horn (laminae 8 and 9, the area with the large motoneurons and most of the adjacent part of lamina 7) with a ×400 magnification (Figure 1). The numbers obtained from the left and right hemisegments were averaged for each animal. To avoid sampling errors, similar neuronal counts were also obtained from specimens derived from the 3rd lumbar segments in the same fashion. Investigators without knowledge of the injury modes (sham or ischemia) or treatment codes (saline or NBQX) performed the morphological assessment of the extent of gray and white matter injury.

Immunohistochemistry

In the chronic series, the 4th lumbar spinal cord segments were obtained after perfusion fixation with buffered formalin (see above). The spinal cord segments were transferred to 30% sucrose solution obtained after perfusion fixation with buffered formalin (see above). The spinal cord segments were transferred to 30% sucrose solution. On the second day, the sections were rinsed in 0.01 mol/L PBS for 3 periods of 10 minutes each and subsequently processed with biotinylated horse serum at a concentration of 1:10 000. The primary antibody was a mouse antibody specifically against the phosphorylated component-H of neurofilaments in axons (SMI-31; Sternberger-Meyer Immunochemicals Inc). On the second day, the sections were rinsed in 0.01 mol/L PBS for 3 periods of 10 minutes each and subsequently processed with biotinylated horse anti-mouse IgG (1:100) and Vector Avidin-biotin-peroxidase complex (Vector Laboratories, Inc). The final peroxidase conjugate was reacted with H2O2 in the presence of 0.05% 3,3-diaminobenzidine (DAB; Sigma). The DAB reaction was enhanced with nickel ammonium sulfate. The sections were mounted, dehydrated, and coverslipped. Areas of interest were specified in the ventral (200 ×200 μm) and ventrolateral (200 ×200 μm) white matter. The ventralmost portion of the dorsal funiculus (100 ×100 μm) (Figure 1) corresponds to the corticospinal tract in the rat.20 The ventral and ventrolateral areas of the spinal cord white matter contain 2 major brain stem–spinal pathways in the rat, namely the vestibulospinal and reticulospinal tracts, respectively.21 These 2 tracts, along with the rubrospinal tract, form the major descending brain stem–spinal pathways that regulate the reflexive posture and locomotor function.22 SMI-31–labeled axons were counted by a blinded investigator using an Olympus BX60 upright microscope equipped with a ×100 oil immersion lens and a 20×20 grid eyepiece. SMI-31 reacts with a phosphorylated epitope in extensively phosphorylated neurofilament H and also with neurofilament M in most mammalian species including the rat. Since phosphorylation, and, to a lesser extent, dephosphorylation, are required for the maintenance of axonal function, SMI-31 can react with almost all axons of variable diameters. SMI-31 immunostaining for axonal counts has been previously correlated with conventional toluidine blue stain in previous studies.23

Statistical Analyses

Data are expressed as mean±SD. A Student’s t test with Dunn-Sidak adjustment as a protection for multiple testing was used for the analysis of the differences in the physiological parameters. Differences in the hindlimb function based on the BBB scores (acute and chronic series) and the histopathological scores (acute series) were assessed by Kruskal-Wallis nonparametric ANOVA and the Mann-Whitney U test. Differences in the axonal and neuronal cell body counts were analyzed by 1-way ANOVA followed by a post hoc Tukey’s test. A probability value <0.05 was considered significant.

Results

Physiological variables are presented in the Table. No difference between the control and treatment groups was noted in any parameter.

Hindlimb Function

The hindlimb function based on the BBB score is summarized in Figure 2. The sham group had very little deficit, even at the acute stage (24 hours: 20.1±0.7, n=7), and showed normal function on day 42 (21±0, n=3). Animals with ischemia in the saline and NBQX groups exhibited severe flaccid paraplegia after recovery from anesthesia. The rats in the saline group developed spastic paraplegia within the first few hours that persisted beyond the first 24 hours in most animals. The transition from flaccid to spastic paraplegia is characteristic of this model and has been described previously.15,19 The rats in the NBQX group demonstrated less pronounced spasticity in the hind limbs during the first few
Figure 2. Effect of NBOX on locomotor function after spinal cord ischemia. Locomotor function was rated based on the BBB scale, which returned to full score*6 hours after surgery. In the ischemic groups, no hindlimb movement was noted at 1 hour and showed little recovery for up to 42 days in the saline-treated (control) animals. Rats with ischemic insult treated with NBOX had significantly better functional recovery than those treated with saline (control). The BBB scale is expressed as mean±SD. *Difference between NBOX- and saline-treated (control) groups is significant. Number below each time point denotes total number of animals (1, 6, and 12 hours, n=20; sham=4, control=8, NBOX=8; d 1, 2, n=37: sham=7, control=15, NBOX=15; d 7, 14, 21, 28, 35, 42, n=17: sham=3, control=7, NBOX=7). Note overlap between the acute and chronic groups on d 1, 2 (20 acute+17 chronic animals=37).

Histopathology in the Acute Series
On day 2, there were no evident histopathological changes in the 4th lumbar spinal cord segment in the sham group. In contrast, significant ischemic injury was noted in the saline group. In the gray matter, many neurons showed features characteristic of ischemic cell death, including cytoplasmic eosinophilia with disintegration of cytoarchitecture and nuclear pyknosis. Shrinkage of cell bodies with occasional budding were noted in some of these ischemic neurons. In addition, vacuolation was noted in the neuropil. In the white matter, vacuolation was widespread and was prominent in the ventral and ventrolateral funiculi (Figure 3). The dorsolateral funiculus was also affected (see below). These histopathological abnormalities were similar to those described in this model previously. Animals in the NBOX group showed similar histopathological changes. There was no difference in the degree of histopathological damage in the spinal cord gray matter between the saline and the NBOX groups on day 2 (Figure 4). However, the grading of the acute white matter damage in the ventral and ventrolateral white matter of animals treated with NBOX (0.8±0.5, n=8) was significantly less than the saline group (2.4±0.9, n=8 P<0.05) (Figure 4).

Axonal and Neuronal Counts in the Chronic Series
In sham-operated animals, axons with variable diameters were clearly defined by SMI-31 immunoreactivity, as shown in the selected areas of the ventral and ventrolateral white matter (Figure 5, A and B) and the corticospinal tract (data not shown) at the 4th lumbar spinal cord segment on day 42. In the ventral white matter, the density of labeled axons in the sham group (1063±44/200 μm, n=3) was substantially greater than those in the ischemic animals treated with saline (559±104/200 ×200 μm, n=7) or NBOX (883±103/200 ×200 μm, n=7). The difference in axonal density was also significant between the saline and NBOX groups (P<0.05). Similar findings were noted in the ventrolateral white matter (sham: 1060±135/200 ×200 μm; saline: 883±103/200 ×200 μm; NBOX: 783±110/200 ×200 μm; NBOX: 563±135/200 ×200 μm; NBOX: 483±103/200 ×200 μm; NBOX: 383±110/200 ×200 μm; NBOX: 363±103/200 ×200 μm; NBOX: 343±99/200 ×200 μm; NBOX: 323±96/200 ×200 μm; NBOX: 303±93/200 ×200 μm; NBOX: 283±90/200 ×200 μm; NBOX: 263±87/200 ×200 μm; NBOX: 243±84/200 ×200 μm; NBOX: 223±81/200 ×200 μm; NBOX: 203±78/200 ×200 μm; NBOX: 183±75/200 ×200 μm; NBOX: 163±72/200 ×200 μm; NBOX: 143±69/200 ×200 μm; NBOX: 123±66/200 ×200 μm; NBOX: 103±63/200 ×200 μm; NBOX: 83±60/200 ×200 μm; NBOX: 63±57/200 ×200 μm; NBOX: 43±54/200 ×200 μm; NBOX: 23±51/200 ×200 μm; NBOX: 0.49, n=8 P<0.05) (Figure 4).

Figure 3. Vacuolation reflecting acute white matter injury 2 days after ischemia. Animals underwent sham operation or SCI with saline (SCI + Vehicle) or NBOX (SCI + NBOX) treatment. No vacuolation was noted in representative sham-operated animal. Extensive vacuolation was noted in another rat with ischemia pretreated with saline (SCI + Vehicle). Vacuolation was substantially less in representative ischemic animal pretreated with NBOX (SCI + NBOX).
Eleven minutes of ischemia resulted in a loss of 47%, 58%, and 91% of SMI-31–labeled axons, respectively, in the white matter of the rat spinal cord 1 to 2 months after transient ischemia have been previously reported. In one of these studies, degenerating axons containing aggregates of microtubules and dense bodies, disintegrating myelin sheaths, and scavenger cells were seen in the corticospinal tract of the lumbar cord 32 days after injury. The postischemic loss of axons in the white matter of the 4th lumbar spinal cord segment may have resulted from local axonal injury or from a lesion in the more proximal axonal segments up to their cell bodies. 

Discussion

In this study, impairment of locomotor function was evident after ischemia, consistent with extensive axonal degeneration, based on qualitative analysis. NBQX pretreatment improved locomotor function and attenuated axonal loss up to 6 weeks after ischemia. However, the gray matter damage was not affected by NBQX pretreatment.

White Matter Damage After Spinal Cord Ischemia

Eleven minutes of ischemia resulted in a loss of 47%, 58%, and 91% of SMI-31–labeled axons, respectively, in the ventral and ventrolateral white matter areas and the corticospinal tract of the 4th lumbar spinal cord segment at 6 weeks after the insult. We performed axonal counts in the lumbar region because spinal cord damage in this model is mostly seen distal to the lower thoracic spinal cord. We evaluated the numbers of axons at 6 weeks after transient ischemia, avoiding intermediate morphological changes that might compound quantitative axonal counts. It is unclear whether ischemia may induce changes in the structure of axonal neurofilaments, resulting in the loss of phosphorylated epitopes and therefore reduced SMI-31–labeled axonal counts in the posts ischemic white matter. Descriptions of morphological changes in the white matter of the rat spinal cord 1 to 2 months after transient ischemia have been previously reported. 

Figure 5. Photomicrographs demonstrating SMI-31–labeled axons in the ventral (left column) and ventrolateral (right column) white matter of the lumbar spinal cord segment in sham-operated (Sham, A and B), ischemia with saline treatment (SCI+Vehicle, C and D) and ischemia with NBQX treatment (SCI+NBQX, E and F) groups. Ischemic animals with or without NBQX treatment underwent 11 minutes of aortic arch occlusion and were killed on day 42. In sham-operated animals, numerous large (arrows) or small (seen in clusters within circles) axons labeled with SMI-31 were seen (A, B). In saline-treated group (SCI+Vehicle), the number of labeled axons was substantially reduced in both ventral and ventrolateral white matter (C and D). Treatment with NBQX before ischemic insult led to better preservation of large (arrowheads) and small (clusters within circles) axons after ischemia-reperfusion (E and F). Horizontal bar in A equals 10 μm. All photomicrographs apply to all other panels.

411 ± 66/200 × 200 μm; NBQX: 676 ± 122/200 × 200 μm, n = 7) and the corticospinal tract (sham: 3391 ± 219/200 × 100 μm, n = 3; saline: 318 ± 23/200 × 100 μm, n = 7; NBQX: 588.2 ± 103/200 × 100 μm, n = 7) (Figure 6). On day 42, neuronal counts in the ventral horn of the 4th lumbar spinal cord segment in both the control and NBQX groups were significantly lower than those in the sham group. However, the difference in the neuronal count between the saline and NBQX groups was not statistically significant (sham: 29 ± 4, n = 3; saline: 16 ± 4, n = 7; NBQX: 17 ± 3, n = 7). To avoid any sampling bias, neuronal counts were repeated in another set of sections in the adjacent 3rd lumbar segment. Similar results were noted (sham: 27 ± 3, n = 3; saline: 17 ± 5, n = 7; NBQX: 17 ± 4, n = 7).

Figure 6. Axonal counts in the 3 regions of white matter in the 4th lumbar spinal cord. Significant difference between *sham and vehicle control (ischemia with saline treatment) groups and between **vehicle control and NBQX (ischemia with NBQX treatment) groups.
that underlie vacuolation in the spinal cord white matter early after ischemia is not known. However, segmental swelling of axons and astrocyte processes as well as formation of spaces between myelin sheaths and axolemma were responsible for the production of vacuoles in the brain white matter 12 to 24 hours after permanent ligation of the middle cerebral artery in rats.9

NBQX and White Matter Damage After Spinal Cord Ischemia

In the present study, pretreatment with NBQX reduced the loss of SMI-31–labeled axons in the ventral white matter from 47% to 17% in the saline group and in the ventrolateral area from 61% to 36% in the saline group. Similarly, NBQX administration reduced the loss of labeled axons in the corticospinal tract from 91% to 83% in control. The effect of NBQX on ischemic axonal loss in the white matter has not been previously reported. Although there are important differences in the pathophysiology between spinal cord ischemia and trauma, it is interesting to note that NBQX significantly increased the serotonin immunoreactivity caudal to the injury site 4 weeks after injury in a rat model of spinal cord compression trauma.14 This observation suggests that the AMPA/kainate receptor mediates the damage of descending axonal pathways caused by mechanical injury and that NBQX is effective in attenuating damage to these long tracts. In addition to the preservation of white matter axons, NBQX administration decreased the severity of histopathological changes in the ventral and ventrolateral funiculi of the white matter 2 days after the onset of reperfusion. This finding is in accord with findings from a rabbit model of SCI induced by combining ischemia with administration of exogenous glutamate, in which NBQX appeared to reduce the white matter damage at 48 hours after insult.26

AMPA/Kainate Glutamate Receptor and Pathogenesis of Axonal Degeneration After Spinal Cord Ischemia

Our findings support the contention that AMPA/kainate receptor activation contributes to axonal loss and white matter damage after ischemia and reperfusion. It has been demonstrated from studies with the in vitro rat optic nerve that anoxia may directly injure axons by disrupting ion homeostasis,7,28 leading to gradual Ca2+ accumulation in axons and activation of deleterious cascades. There is no evidence indicating that neuronal excitatory amino acid receptors could modulate ischemia-induced ionic disturbances in axons. It therefore appears unlikely that NBQX preserved axons in this study by attenuating direct ischemic injury to axons. This view is further supported by the time-honored observation that exposure of neurons to excitotoxins produces morphological changes that spare the axons.4 Loss of myelinated axons in the white matter may also be secondary to injury or death of oligodendrocytes, which myelinate axons in the central nervous system. There is increasing evidence that oligodendrocytes may be highly vulnerable to ischemic injury. In a rat model of permanent middle cerebral artery occlusion, pathological changes in oligodendrocytes appeared early after the onset of ischemia and appeared to be concomitant with but independent of neuronal perikaryal injury.9 In a different rat model of sustained moderate brain ischemia, the earliest and perhaps primary change in the white matter was disturbed metabolism and synthesis of myelin.29 Furthermore, there is accumulating evidence that the marked elevations in extracellular glutamate concentration, which accompany ischemic injury of the brain2 and the spinal cord,3,30 might mediate the oligodendrocyte ischemic injury. In the case of white matter, nonsynaptic mechanisms for extracellular release of excitatory neurotransmitters are important. It has been suggested that glutamate could leak out of axons during ischemia through the Na+-K+-glutamate transporter, creating high neurotransmitter concentrations in the restricted submyelinic and interlamellar spaces.28 Ischemia-reperfusion injury also may cause glutamate efflux from energy-depleted astrocytes31 through multiple mechanisms, including reversed glutamate transport32 and swelling-evoked33 or Ca2+-dependent34,35 release. Glutamate also might spill into the white matter from the neighboring ischemic gray matter.21 Ischemia-induced increases in extracellular glutamate concentration could result in toxic activation of functional AMPA/kainate glutamate receptors on oligodendrocytes and astrocytes.11–13,36 Differentiated rat oligodendrocytes have been recently found to be highly vulnerable to AMPA/kainate receptor–mediated excitotoxic death in vitro, whether induced by exposure to agonists12,13 or by deprivation of oxygen and glucose.12 Injection of AMPA/kainate receptor agonists into the rat thalamus52 or external capsule13 caused marked oligodendrocyte death. In the present study, ischemic destruction of axons in the spinal cord might result from soluble mediators, such as oxidative products or free radicals, produced by glial cells after toxic exposure to glutamate. In addition, axons might degenerate after ischemia-induced excitotoxic death of the oligodendrocytes. Myelin-forming glial cells are capable of modifying axonal morphology and axonal transport.37,38 Findings from a recent study in vivo indicate that degeneration of axons in the central nervous system may occur when crucial local support from oligodendrocytes becomes inadequate.39 Antagonism of AMPA/kainate receptors on glial cells11 may have preserved white matter axons in the present study by attenuating ischemia-induced excitotoxic injury or death of oligodendrocytes.

NBQX and Gray Matter Degeneration After Spinal Cord Ischemia

NMDA receptor blockade has been shown to improve spinal cord tolerance to ischemia.40 In view of the prominent role of NMDA receptor mechanism in gray matter injury, the neuroprotective effects of NMDA antagonists, such as MK-801,43 CGS-19755, and LY233053,46 in spinal cord ischemia might be on gray matter. In the present study, pretreatment with NBQX failed to attenuate the severity of histopathological changes in the lumbar cord gray matter 2 days after ischemia in this model. Furthermore, administration of NBQX was not associated with preservation of neurons in the ventral horns of the lumbar cord 42 days after ischemia, suggesting that NBQX is not effective against neuronal degeneration in the postischemic gray matter. In a previous
study. NBQX reduced the length of the gray matter lesion but failed to increase the cross-sectional area of the remaining gray matter at the epicenter compared with control 3 weeks after laser-induced photochemical thrombosis in the rat spinal cord. In another study, administration of NBQX attenuated gray matter degeneration in an acute rabbit model of spinal cord injury caused by a combination of ischemia and administration of exogenous glutamate. The significant differences in the experimental conditions between the previous two studies and the present one may be responsible for the discrepancies in the results of NBQX administration against ischemic gray matter degeneration. It is interesting to note that AMPA/kainate receptor antagonism alone fails to protect cultured cortical neurons from simulated ischemic damage. The devastating effects of NMDA glutamate receptor activation after ischemic release of glutamate may mask any neuronal protection by AMPA/kainate receptor antagonism.

**NBQX and Locomotor Function After Spinal Cord Ischemia**

In the present study, intrathecal administration of NBQX before ischemia resulted in significant improvement of the locomotor function as compared with vehicle-treated controls. The improved locomotor outcome was apparent by 24 hours after the insult and was maintained throughout the 6-week follow-up period. Our findings are in agreement with observations in previous studies showing functional improvement after NBQX treatment in rat and rabbit. In addition, administration of a different AMPA antagonist, LY293558, given 5 minutes after the onset of reperfusion, significantly increased the duration of ischemia required to produce paraplegia in an acute rabbit model of spinal cord ischemia. The spinal cord was not examined specifically for white matter histopathology in that study. In both the acute and the chronic phases of the present study, the improved locomotor function in animals pretreated with NBQX was associated with decreased severity of degeneration in the white but not in the gray matter of the lumbar spinal cord.

**Timing of NBQX Treatment**

Blockade of glutamate receptors to reduce ischemia-induced spinal cord damage may have a very limited therapeutic window. In the present study, NBQX was tested in a pretreatment regimen. The effectiveness of NBQX in a posttreatment dosing was not examined. Because the present study aimed to explore preventive measures that may protect spinal cord from ischemic insult sustained in elective surgery of the aorta, an effective pretreatment regimen is clinically relevant. Under the circumstances, pretreatment to prevent the injury is preferred to posttreatment.

**Concluding Remarks**

Spinal cord ischemia with resultant neurological deficit continues to be a serious complication after surgery on the descending thoracic and thoracoabdominal aorta of patients. Our data support the notion that white matter degeneration is an important mechanism of ischemia-induced paralysis. NBQX treatment attenuated the postischemic white matter degeneration, possibly by favorably interfering with axonal-glial interactions and improved locomotor function. Administration of agents that modify the function of AMPA/kainate glutamate receptors before surgery may be an efficacious measure in attempts to prevent white matter degeneration caused by perispinal cord ischemia.

**Acknowledgments**

This study was supported in part by National Institutes of Health grants NS25545, 28995, and 37230. We thank Dr G.S. Fan for technical assistance and Dr Shah-Hinan Ahmed for editorial assistance.

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The neuroprotection of NBQX, an AMPA/kainate glutamate receptor antagonist, in cerebral ischemia was first reported in 1990. This generated tremendous interest and offered hope that cerebral ischemia could be ameliorated by the AMPA receptor antagonist. Enthusiasm for the therapeutic potential was significantly dampened later by the fact that NBQX is toxic to the liver. Nevertheless, several second-generation AMPA receptor antagonists have been developed. These drugs primarily target the ischemic neurons. Recent studies have demonstrated that inhibition of the AMPA/kainate receptor can provide neuroprotection against excitotoxicity in white matter oligodendrocytes and a reduction in functional impairment after spinal cord trauma.

In this well-written and carefully performed study, Kanellopoulos and colleagues have furthered the preclinical utility of NBQX by showing amelioration of white matter injury in a rat model of spinal cord ischemia. In view of the failure of current neuroprotective agents that mainly target neurons and endothelial cells, this study provides further consideration for alternative therapeutic approaches that target white matter injury in ischemic stroke.

References
White Matter Injury in Spinal Cord Ischemia: Protection by AMPA/Kainate Glutamate Receptor Antagonism
Georgios K. Kanellopoulos, Xiao Ming Xu, Chung Y. Hsu, Xiaobin Lu, Thoralf M. Sundt and Nicholas T. Kouchoukos

Stroke. 2000;31:1945-1952
doi: 10.1161/01.STR.31.8.1945

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/31/8/1945

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