SURGERY FOR ACQUIRED HEART DISEASE

PROTECTION OF RAT SPINAL CORD FROM ISCHEMIA WITH DEXTROPHAN AND CYCLOHEXIMIDE: EFFECTS ON NECROSIS AND APOPTOSIS

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Objective: We examined the characteristics of neuronal cell death after transient spinal cord ischemia in the rat and the effects of an *N*-methyl-D-aspartate antagonist, dextrorphan, and a protein synthesis inhibitor, cycloheximide. **Methods:** Spinal cord ischemia was induced for 15 minutes in Long-Evans rats with use of a 2F Fogarty catheter, which was passed through the left carotid artery and occluded the descending aorta, combined with a blood volume reduction distal to the occlusion. The rats were killed after 1, 2, and 7 days. Other groups of rats were pretreated with dextrorphan (30 mg/kg, intraperitoneally, *n* = 7), cycloheximide (30 mg, intrathecally, *n* = 7), or vehicle (saline solution, *n* = 12) and killed after 2 days. **Results:** This model reliably produced paraplegia and histopathologically distinct morphologic changes consistent with necrosis or apoptosis by light and electron microscopic criteria in different neuronal populations in the lumbar cord. Scattered necrotic neurons were seen in the intermediate gray matter (laminae 3 to 7) after 1, 2, and 7 days, whereas apoptotic neurons were seen in the dorsal horn laminae 1 to 3 after 1 and 2 days. Deoxyribonucleic acid extracted from lumbar cord showed internucleosomal fragmentation (laddering) on gel electrophoresis indicative of apoptosis. The severity of paraplegia in the rats treated with dextrorphan and cycloheximide was attenuated 1 day and 2 days after ischemia. The numbers of both necrotic and apoptotic neurons were markedly reduced in both dextrorphan- and cycloheximide-treated rats. **Conclusions:** The results suggest that both *N*-methyl-D-aspartate receptor-mediated excitotoxicity and apoptosis contribute to spinal cord neuronal death after ischemia and that pharmacologic treatments directed at blocking both of these processes may have therapeutic utility in reducing spinal cord ischemic injury. (J Thorac Cardiovasc Surg 1997;114:609-18)

Spinal cord ischemia is a devastating complication after operations on the descending thoracic and thoracoabdominal aorta.¹ Despite improvements in surgical technique, this complication remains unpredictable and unpreventable. Little is known about the mechanisms of neuronal death after spinal cord ischemia, particularly in patients who undergo operations on the descending thoracic and thoracoab-

dominal aorta.² Such knowledge would be valuable in facilitating the development of strategies aimed at minimizing spinal cord ischemic injury after aortic operations.

One likely contributing factor to ischemia-induced spinal cord injury is excitotoxic neuronal death. Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system. Extracellular con-

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centrations of glutamate increase strikingly during ischemia in the brain³ and the spinal cord.⁴ Substantial evidence suggests that glutamate neurotoxicity contributes to neuronal death after brain ischemia.⁵ The *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors in particular can play a prominent role in excitotoxic neuronal death, because they are linked to Ca^{2+} -permeable channels.⁵ An increase in intracellular Ca^{2+} concentrations is believed to trigger a chain of reactions leading to neuronal death. NMDA antagonist drugs reduce brain⁶ and spinal cord⁷ damage after ischemic insults.

Classically, ischemia has been considered to induce necrosis of brain cells, in which excitotoxicity may play a major role. However, recent studies have shown that apoptosis also occurs.⁸ Apoptosis is a form of cell death that occurs normally during embryonic development and is characterized by coarse chromatin condensation, loss of cell volume, and endonuclease cleavage of deoxyribonucleic acid (DNA) into internucleosomal fragments.⁹ Apoptosis can be inhibited by protein synthesis inhibitors.¹⁰ Brain damage after ischemic insults and spinal cord damage after traumatic insults can be reduced by the administration of protein synthesis inhibitors.^{8,11}

The purpose of the present study was to test the hypothesis that spinal cord damage after ischemia is mediated by both excitotoxicity and apoptosis. We examined the effects of treatment with an NMDA antagonist, dextrorphan, and a protein synthesis inhibitor, cycloheximide. Dextrorphan is a well-characterized noncompetitive NMDA antagonist,¹² which attenuates hypoxic neuronal injury in cortical culture, decreases infarct volume in models of focal cerebral ischemia, and reduces paraplegia after spinal cord injury.^{13,14}

Material and methods

Characterization of a spinal cord ischemia model

Induction of ischemia. We used male adult Long-Evans rats (Harlan Sprague Dawley Inc., Indianapolis) weighing 290 to 375 gm. Housing and anesthetic treatment concurred with guidelines established by the institutional Animal Studies Committee and were in accordance with the Public Health Service "Guide for the Care and Use of Laboratory Animals," U.S. Department of Agriculture regulations, and the American Veterinary Medical Association Panel on Euthanasia guidelines. The rats were allowed free access to food and water before and after the operation. Anesthesia was induced with 2% to 3% halothane in oxygen and maintained with 0.5% to 1.5% halothane delivered through a face mask. Spinal cord ischemia was induced by occlusion of the descending aorta with a 2F Fogarty balloon catheter (modification of the method of Coston and associates¹⁵),

combined with blood volume reduction. The left femoral artery was cannulated with PE50 polyethylene tubing for monitoring of arterial blood pressure distal to the occlusion and for obtaining blood samples for blood gas analysis and blood glucose measurements. The left carotid artery was exposed, and the catheter was passed into the thoracic aorta. The balloon was then inflated with a small amount of water (0.02 ml) and was withdrawn until it reached the origin of the left carotid artery just above the origin of the left subclavian artery. The balloon was then fully inflated with 0.1 ml of water, occluding the descending aorta together with the left carotid artery and the left subclavian artery. Blood was then withdrawn through the femoral arterial cannula to maintain the distal blood pressure at less than 10 mm Hg. The distal blood pressure dropped rapidly after balloon inflation from 80 to 90 mm Hg to 10 to 20 mm Hg. Five to eight milliliters of blood was withdrawn over several minutes to maintain the distal blood pressure at less than 10 mm Hg. At the end of the occlusion period, the catheter was deflated, and the shed blood was infused. The blood pressure returned to preischemic levels within 10 minutes. Ischemia was induced at normothermia, and the postischemic rectal temperature was monitored. To determine the time course of histopathologic processes, groups of rats were subjected to ischemia for 15 minutes and were killed after 1 day ($n = 5$), 2 days ($n = 6$), and 7 days ($n = 11$) of survival. Five sham-operated rats were also included in the study.

Hind-limb motor function. An observer who was blinded to the experimental conditions carefully examined all the animals for neurologic deficits, including paraplegia, at 1 hour, 2 hours, 4 hours, 6 hours, 1 day, 2 days, and 7 days after ischemia. The hind-limb motor function of the animals was graded according to a 0 to 4 scoring system with 0 being normal walking; 1, mildly impaired walking with toes flat under the body but with weakness or spasticity present; 2, knuckle walking; 3, dragging legs with movement in the knees; and 4, dragging lower extremities without any movement, including both spastic paraplegia and flaccid paraplegia.⁴

Histopathology and TUNEL staining. The rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally) at the time points previously indicated, and the spinal cords were perfusion fixed with buffered formalin after a brief saline solution flush. The lumbar spinal cords were removed, postfixed overnight in the same fixative, and embedded in paraffin. Transverse sections were cut to a thickness of 5 μm at the level of L2-5. The sections were stained with hematoxylin and eosin for histopathologic observations. The sections were also stained with the terminal deoxynucleotidyl-transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) technique,¹⁶ with use of the ApopTag kit (Oncor, Gaithersburg, Md.) for the localization of DNA fragmentation. The sections were counterstained with hematoxylin. The Rexed cytoarchitectonic scheme, dividing the gray matter into 10 laminae, was used to describe the locations of damaged neurons (Fig. 1).

Electron microscopy. Five other rats were used for electron microscopic study. The lumbar spinal cords of three rats subjected to 15 minutes of ischemia and those of two rats subjected to sham operation followed by 24 hours of survival were perfusion fixed with 2% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L phosphate buffer

(pH 7.4). The cords were postfixed in 1% osmium tetroxide in phosphate buffer for 1 hour and embedded in Epon fixative. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined with a JEM-100CX II transmission electron microscope (JEOL Ltd., Tokyo, Japan).

DNA fragmentation analysis by agarose gel electrophoresis. Six other rats were used for DNA fragmentation analysis. Rats subjected to 15 minutes of spinal cord ischemia ($n = 4$) or sham operation ($n = 2$) were killed after 2 days. The lumbar spinal cords were quickly removed and frozen in liquid nitrogen. DNA was extracted from the tissue and was electrophoresed on a 1.5% agarose gel, as previously described.⁸

Effects of treatment with dextrorphan or cycloheximide.

Other groups of rats were randomly assigned to receive one of the drugs (dextrorphan or cycloheximide) or saline solution. The doses were chosen on the basis of studies using brain ischemia models.^{13, 17} Dextrorphan (30 mg/kg, $n = 7$; Hoffmann-La Roche Inc., Basel, Switzerland) or the vehicle (saline solution, 2 ml/kg, $n = 6$) was injected intraperitoneally 15 minutes before ischemia. Cycloheximide (30 μ g, $n = 7$; Sigma Chemical Co., St. Louis, Mo.) or the vehicle (saline solution, 10 μ l, $n = 6$) was injected intrathecally 30 minutes before ischemia through the L6-S1 intervertebral space by the technique of Sloane-Stanley and Chase¹⁸ with modifications. The vertebral arches of L6 and S1 were exposed and an injecting needle was inserted rostrally 2 cm into the vertebral canal through the gap between the vertebral arches. In a preliminary experiment, we confirmed that dye injected by this method was present around the cauda equina and the lumbosacral cord. Administration of the cycloheximide by the intrathecal route was used because systemic treatment of the ischemic rats with cycloheximide (0.5 to 1.5 mg/kg, intraperitoneally) was associated with an unacceptably high mortality rate in a preliminary study.

Neurologic deficits in the rats were assessed periodically using the hind-limb motor function score as described. The rats were killed after 2 days and the spinal cords were processed for hematoxylin and eosin and TUNEL staining. The numbers of necrotic neurons and apoptotic neurons in each microscopic section examined were counted in a blinded fashion. The definitions of necrosis and apoptosis were the same as those used in the model characterization study (see Results section). The animals were killed at 48 hours because, according to the model characterization study (1) both necrosis and apoptosis could be seen in the spinal cord, (2) most of the reversal of paraplegia was seen before 48 hours, and (3) no animals died before this time.

Statistical analysis of the hind-limb motor function was performed by Kruskal-Wallis nonparametric analysis of variance and the Mann-Whitney U test. Statistical analysis of the numbers of necrotic and apoptotic cells was performed by analysis of variance and the Dunnett multiple comparison test. In both analyses, the two groups treated with saline solution were not statistically different, and results from them were pooled as one control group for further analysis.

Fig. 1. Outline of the rat spinal cord at the lumbar level. The gray matter is divided into 10 cytoarchitectonic regions according to Rexed: laminae 1 to 9 and an area around the central canal (area 10).

Results

Model characterization

Hind-limb motor function. We observed no neurologic deficits in sham-operated rats. All the rats subjected to 15 minutes of ischemia exhibited severe flaccid paraplegia soon after recovery from anesthesia. The rats began to exhibit spastic paraplegia within several hours. The majority of the rats had spastic paraplegia after 2 days, but a few rats had further recovery. Further recovery from paraparesis between 2 days and 7 days was unusual. Among 11 rats in the 7-day survival group, one rat died after 3 days and one after 4 days. These exhibited paraplegia and hematuria suggesting renal damage. In the 1-day and 2-day survival groups, no rats died.

Histopathology and TUNEL staining. There were no histopathologic abnormalities in sham-operated rats. Generally, histopathologic cell damage was restricted to neurons. Two distinct forms of neuronal damage were observed (Fig. 2). The first form was characteristic of necrosis¹⁹ and included pyknotic nuclei and eosinophilic, structureless cytoplasm (red neurons) or loss of nuclear hematoxylin staining (ghost neurons). The second form was characteristic of apoptosis⁹ and included nuclear and cytoplasmic condensation, nuclear budding, and fragmentation into membrane-bound small bodies (apoptotic bodies). Necrosis was seen predominantly in small- to medium-sized neurons in the intermediate areas of the gray matter (laminae 3 to

Fig. 2. Hematoxylin and eosin staining (**a** through **c**, **e**, **f**, **i**, **j**) and TUNEL staining (**d**, **h**) of the lumbar spinal cord of a rat subjected to 15 minutes of ischemia. **a** through **d**, Laminae 3 to 7; **e** through **h**, lamina 2; **i** and **j**, anterolateral portion of the white matter. **a**, Sham operation. Normal appearance of neurons in laminae 3 to 7. **b**, After 2 days, a necrotic neuron (*arrow*) exhibits eosinophilic, structureless cytoplasm and loss of nuclear hematoxylin stainability. **c**, After 7 days, a ghost neuron still can be observed (*arrow*) and proliferation of macrophages and glial cells is seen. **d**, After 2 days, the nuclei of necrotic neurons are TUNEL positive (*arrows*). **e**, Sham operation. Normal appearance of small neurons in lamina 2. **f**, After 2 days, apoptotic neurons exhibiting shrinkage, chromatin condensation, nuclear budding, and fragmentation are seen (*arrows*). **g**, After 7 days, neuronal loss is obvious. **h**, After 2 days, the nuclei of apoptotic neurons are TUNEL positive (*arrows*). **i**, Sham operation. Normal appearance of white matter. **j**, After 2 days, prominent vacuolation is apparent. Bar in **a** applies to **a** through **d**, **i**, and **j** and indicates 20 μ m; bar in **e** applies to **e** through **h** and indicates 10 μ m.

7) between 1 and 7 days. The nuclei of the necrotic neurons were uniformly TUNEL positive, even when nuclear hematoxylin staining disappeared (Fig. 2). The nuclei of the necrotic neurons were round, oval, or triangular and did not appear fragmented. Large motoneurons in the ventral horn laminae 8 and 9 were relatively resistant to ischemia. When they were damaged, they showed necrotic morphologic features. Apoptosis was seen only between 1 and 2 days in small neurons located in the most dorsal part of the dorsal horn (laminae 1 to 3, especially lamina 2). The nuclei of these apoptotic neurons were also TUNEL positive (Fig. 2). Apoptosis in other laminae was rare. After 7 days, ghost neurons were still present in laminae 3 to 7, and the

areas with neuronal damage showed astroglial proliferation. In severe cases, foci of infarction had developed with macrophage infiltration. In laminae 1 to 3, apoptotic neurons could no longer be seen. In the white matter, many vacuolations were seen predominantly in the anterior and lateral portions (Fig. 2).

Electron microscopy. Electron microscopy provided ultrastructural evidence of necrosis in the damaged neurons in laminae 3 to 7 (Fig. 3),⁹ including small, loosely textured aggregations of chromatin, dilated endoplasmic reticulum, dissolution of ribosomes, and mitochondrial swelling. In advanced stages of necrosis, karyolysis, disintegration of cytoplasmic organelles, and disruption of

Fig. 3. Ultrastructural appearance of normal (a) and necrotic (b through d) neurons in intermediate gray matter (laminae 3 to 7) 24 hours after 15 minutes of ischemia. Necrotic neurons show nuclear and cytoplasmic disintegration with breakdown of membranes (b and c; c shows advanced stage). d, Higher magnification of the identified area in c shows no nuclear membrane between the nucleus (N) and cytoplasm (C) and disrupted mitochondria (M). nc, Nucleolus; bars, 3 μ m in a through c and 0.5 μ m in d.

nuclear and cell membranes were seen. Damaged neurons in laminae 1 to 3 exhibited ultrastructural features of apoptosis (Fig. 4),⁹ including the aggregation of chromatin into sharply delineated masses, with intact cytoplasmic organelles and nuclear and cell membranes. Cells in advanced apoptosis exhibited nuclear and cytoplasmic condensation, and the fragmentation of the cells into membrane-bound

Fig. 4. Ultrastructural appearances of apoptosis in lamina 2 after 15 minutes of spinal cord ischemia and 24 hours of survival. a, Next to a neuron with minimal changes (to the left) there is an apoptotic cell showing nuclear and cytoplasmic condensation and shrinkage. b, Early apoptosis. Aggregation of chromatin into dense, sharply delineated masses is seen but cytoplasmic organelles and membranes are structurally intact. c, Sharply demarcated, condensed chromatin (asterisk) within a convoluted nucleus with intact nuclear membrane (arrowheads) and condensed cytoplasm with vacuolations. d, Two nuclear fragments containing chromatin are seen at the periphery of a cell. e, Apoptotic bodies (arrows); the largest one contains part of a nucleus and the smaller ones contain only cytoplasm. f, An apoptotic body containing a small fragment of chromatin (arrow) whereas cytoplasmic organelles and membranes are structurally intact. N, Nucleus; C, cytoplasm; bars, 2 μ m except for in d, where it represents 5 μ m.

fragments (apoptotic bodies) containing aggregated chromatin and cytoplasmic organelles was observed.

Agarose gel electrophoresis. DNA extracted from animals subjected to ischemia showed a characteristic internucleosomal breakdown ("laddering") on the gel, superimposed on light smearlike staining

Fig. 5. Gel electrophoresis of the DNA extracted from lumbar spinal cord of the rats subjected to sham operation (A) and 15 minutes of ischemia followed by 48 hours of reperfusion (B). Bands with a laddering pattern are seen in B, indicating the presence of nucleosome-sized DNA fragmentation, superimposed on smearlike staining, which is indicative of necrosis. DNA markers in kbp are shown on the left.

(Fig. 5). This was not observed in the sham-operated animals.

Treatment with dextrorphan or cycloheximide

Physiologic parameters. Physiologic parameters of the animals treated with saline solution, dextrorphan, and cycloheximide are shown in Table I. The mean arterial blood pressure decreased significantly in dextrorphan-treated rats, but not in cycloheximide-treated animals. Blood gas values were not different among the three groups. Blood glucose levels decreased in cycloheximide-treated rats but not in dextrorphan-treated animals. The animals were normothermic when ischemia was induced. The rectal temperature decreased by 2° to 3° C for several hours after ischemia, probably because of decreased motor activity as a result of severe paraplegia, and returned to normal within 1 day. There were no statistical differences in the rectal temperatures of the animals of the three groups.

Hind-limb motor function. The time course of the hind-limb motor function recovery after ischemia is shown in Fig. 6. All rats subjected to ischemia had severe flaccid paraplegia soon after recovery from anesthesia. The rats demonstrated spastic paraplegia within a few hours, and the rats of all three groups continued to have spastic paraplegia within the first 6-hour period. After 1 day and 2 days, saline-treated rats continued to exhibit spastic paraplegia, but most of the dextrorphan- and cycloheximide-treated rats showed significant recovery of the hind-limb motor function and were able to walk, although not normally. The neurologic scores after 1 day and 2 days for dextrorphan-treated rats (1.4 ± 0.79 [mean plus or minus standard deviation] for both 1 and 2 days) and cycloheximide-treated rats (1.6 ± 1.13 for both) were significantly lower than those for saline-treated rats (3.6 ± 1.00 for both) (Fig. 6).

Histopathology and TUNEL staining. Both necrosis and apoptosis were seen in the lumbar spinal cord of the rats treated with dextrorphan or cycloheximide, but the numbers of damaged neurons were strikingly reduced compared with those in saline-treated rats. The numbers of necrotic and apoptotic neurons per section in dextrorphan-treated rats (13 ± 11.1 and 10 ± 7.8 , respectively) and cycloheximide-treated rats (27 ± 16.0 and 14 ± 10.8 , respectively) were significantly less than those in saline-treated rats (56 ± 30.2 and 35 ± 25.9 , respectively) (Fig. 7).

Discussion

The present study demonstrates that different neuronal populations in the rat spinal cord exhibit two distinct forms of death after ischemia, one characteristic of necrosis and the other characteristic of apoptosis. This distinction is supported by both light and electron microscopic criteria. The determination of apoptosis is additionally supported by the demonstration of internucleosomal fragmentation in DNA extracted from ischemic spinal cords. Of note, TUNEL positivity, which has been used in some studies as a sole criterion for apoptosis, developed in the nuclei of both necrotic and apoptotic neurons. However, this is not unexpected because nonspecific DNA breakdown will increase the number of available 3'-OH ends detected by the TUNEL method. Several recent studies have demonstrated that necrotic neurons can be TUNEL positive.^{20, 21}

Necrosis was seen predominantly in the central gray matter (laminae 3 to 7), whereas apoptosis was

Fig. 6. Time course (1 hour to 48 hours) of the hind-limb motor function after 15 minutes of spinal cord ischemia. The severity of paraplegia (mean plus or minus the standard deviation) was graded according to a scoring system from 0 (normal) to 4 (paraplegia). Hind-limb function in rats treated with dextrorphan ($n = 7$) and cycloheximide ($n = 7$) was significantly better than that in saline-treated rats ($n = 12$). p Values determined by Mann-Whitney U test.

Table I. Physiologic parameters of the rats treated with saline solution, dextrorphan, and cycloheximide

	Blood gases				Glucose (mg/dl)	Rectal temperature ($^{\circ}$ C)			
	MABP (mm Hg)	PaO_2 (mm Hg)	$PaCO_2$ (mm Hg)	pH		Before	1 hr	2 hr	4 hr
Saline solution ($n = 12$)	84 ± 5.5	298 ± 45.4	44 ± 7.7	7.40 ± 0.027	145 ± 14.9	37.4 ± 0.19	34.6 ± 0.41	33.9 ± 0.61	33.4 ± 1.11
Dextrorphan ($n = 7$)	$70 \pm 3.8^*$	337 ± 37.8	46 ± 5.6	7.40 ± 0.036	129 ± 20.6	37.4 ± 0.09	34.5 ± 0.47	33.5 ± 1.07	33.4 ± 0.91
Cycloheximide ($n = 7$)	86 ± 7.1	295 ± 19.6	45 ± 3.2	7.38 ± 0.019	$106 \pm 8.5^*$	37.5 ± 0.19	34.8 ± 0.31	34.7 ± 0.54	34.4 ± 0.83

The values are expressed as mean plus or minus the standard deviation. Mean arterial blood pressure (MABP), blood gases, and blood glucose were measured just before ischemia. Rectal temperature was measured before and 1 hour, 2 hours, and 4 hours after ischemia. PaO_2 , Arterial oxygen tension; $PaCO_2$, arterial carbon dioxide tension.

predominantly seen in the most dorsal part of the dorsal horn (laminae 1 to 3, especially lamina 2). One possible explanation for the different distribution of the necrotic and apoptotic neurons is the severity of the ischemic insult. Because apoptosis occurred in the peripheral portion of the spinal cord, where better collateral blood supply is available, less severe ischemia may have triggered apoptosis rather than necrosis. However, arguing against a predominant role for the severity of the insult in determining the development of neuronal necrosis or apoptosis, we observed the same distribution of necrosis and apoptosis even in animals sustaining relatively less histologic and neurologic

damage after shorter durations of ischemia (unpublished observations). Thus we suspect that systematic differences in the environment or in the intrinsic cellular properties of dorsal horn versus central gray neurons account for most of the observed difference in type of death after ischemia.

All animals showed flaccid paraplegia after recovery from the anesthesia, which may have been a result of acute suppression of the spinal cord function (spinal shock) caused by ischemia or ischemic injury to other peripheral tissues. Within a few hours, the animals started to exhibit spastic paraplegia, which is indicative of spinal cord injury. One of the major findings of this study is that pretreatment

Fig. 7. The numbers (per section; mean plus or minus the standard deviation) of necrotic neurons in laminae 3 to 10 and apoptotic neurons in laminae 1 to 3 of the spinal cord gray matter 48 hours after 15 minutes of spinal cord ischemia. The numbers of necrotic and apoptotic neurons in rats treated with dextrorphan ($n = 7$) and cycloheximide ($n = 7$) were significantly less than those in saline-treated rats ($n = 12$). p Values determined by Dunnett multiple comparison test.

with either dextrorphan or cycloheximide attenuated the severity of paraplegia and reduced the numbers of neurons that died by both necrosis and apoptosis. These observations suggest that NMDA receptor activation and protein synthesis are required for the neuron-damaging effects of spinal cord ischemia. NMDA antagonists MK-801 and Mg^{2+} attenuate paraplegia after spinal cord ischemia in the rat.⁷ To our knowledge, there have been no reports on the effects of protein synthesis inhibitors on spinal cord ischemia, but cycloheximide reduces brain damage after ischemia⁸ and attenuates spinal cord damage after traumatic injury.¹¹

The finding that either dextrorphan or cycloheximide administration reduced both neuronal necrosis and neuronal apoptosis was unexpected. Recent in vitro studies have suggested that excitotoxic neuronal death still represents necrosis even when triggered slowly with submaximal insults and is insensitive to cycloheximide.²² We hypothesized that cycloheximide administration would reduce only apoptotic death. Further studies will be necessary to unravel the mechanisms underlying this apparent

crossover protection observed with dextrorphan and cycloheximide. One possible explanation is that our morphologically based classification of cell death may be flawed and that the pharmacologic response to dextrorphan versus cycloheximide is a more accurate indicator of type of death. Nevertheless, we currently favor the alternative explanation that cell death in vivo induces widespread perturbations, such as changes in circuit excitability, loss of glutamate uptake, loss of trophic factor support, and oxidative stress, that could reduce the threshold of insult required for the death of other cells, whether necrosis or apoptosis. In support of this concept, injection of excitotoxins in vivo has been found to produce both necrosis and apoptosis.^{20, 23} The finding in this study that dextrorphan prevented apoptosis to a greater degree than cycloheximide prevented apoptosis might indicate that excitotoxicity is a relative prerequisite for the apoptotic death. It therefore appears that the two mechanisms of neuronal death may be interrelated in a way yet to be defined.²⁴

Some of the neuroprotective effects of the

NMDA antagonist MK-801 against ischemic damage to the CA1 pyramidal neurons after global cerebral ischemia may be explained by drug-induced hypothermia.²⁵ Cycloheximide is also known to induce hypothermia, which protects against ischemic neuronal damage to the CA1 area of the hippocampus.²⁶ In this study, ischemia was induced at normothermia in both dextrorphan- and cycloheximide-treated rats (Table I). After ischemia, however, the body temperature decreased by 2° to 3° C for several hours, but this moderate postischemic hypothermia was observed in both saline- and drug-treated rats. Probably, this hypothermia was spontaneous because of the reduced motor activities caused by severe paraplegia. Therefore hypothermia cannot account for the protective effects seen in this study. Dextrorphan decreased preischemic arterial blood pressure (Table I), but it is unlikely that this mild hypotension worked protectively against ischemic spinal cord damage. Blood glucose levels in cycloheximide-treated animals were mildly lower than those in saline-treated animals, although they were still in the physiologic range. We doubt that this mild degree of blood glucose reduction in cycloheximide-treated animals was sufficient to account for the protective effects seen. Although pretreatment with insulin attenuates motor deficits after aortic occlusion in the rat and the rabbit,^{27,28} glucose levels in these studies were in the hypoglycemic range. Furthermore, cycloheximide reduces ischemic brain damage in rats even when the drug-induced glucose reduction is corrected.²⁹

In conclusion, the results of our study support the hypothesis that ischemia can simultaneously trigger excitotoxic and apoptotic neuronal cell death in the spinal cord. This suggests that specific pharmacologic treatments directed at each of these components, alone or in combination, may provide neuroprotection from spinal cord ischemia subsequent to aortic occlusion in human beings and such treatments deserve further exploration.

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