Glutamate excitotoxicity: A mechanism of neurologic injury associated with hypothermic circulatory arrest

Glutamate, the major central nervous system neurotransmitter, may have potent neurotoxic activity under conditions of metabolic stress. By receptor autoradiography, we have demonstrated that brain regions most vulnerable to injury during prolonged hypothermic circulatory arrest have the highest density of glutamate receptors. To test the hypothesis that such injury could be mediated by glutamate excitotoxicity, we used dizocilpine (MK-801), a selective *N*-methyl-D-aspartate-glutamate receptor antagonist in a canine survival model of hypothermic circulatory arrest. Eighteen male dogs (20 to 25 kg) were supported by closed-chest cardiopulmonary bypass, subjected to 2 hours of hypothermic circulatory arrest at 18° C, and rewarmed on cardiopulmonary bypass. All were mechanically ventilated and monitored for 20 hours before extubation and survived for 3 days. Group A dogs (*n* = 9) received a prearrest intravenous bolus of dizocilpine (0.75 mg/kg) followed by continuous infusion (75 µg/kg per hour), resulting in electroencephalographic silence. Dizocilpine was weaned before extubation. Group B dogs received vehicle only. According to a species-specific behavior scale that yielded a neurologic deficit score ranging from 0 (normal) to 500 (brain dead), all animals were neurologically assessed every 12 hours. After the dogs were killed at 72 hours, brains were examined by receptor autoradiography and histologically for patterns of selective neuronal necrosis; they were scored blindly from 0 (normal) to 100 (severe injury). Group A dogs had better neurologic function than group B (neurologic deficit score 21 ± 15 versus 192 ± 40 , $p < 0.001$) and had less neuronal injury (7.3 ± 3 versus 48.3 ± 9 , $p < 0.0001$). Densitometric receptor autoradiography revealed preservation of neuronal *N*-methyl-D-aspartate-glutamate receptor expression in group A only. These results represent the first direct evidence of a role for glutamate excitotoxicity in the development of hypothermic circulatory arrest-induced brain injury and suggest that selective glutamate receptor antagonists may have a neuroprotective capacity in prolonged periods of hypothermic circulatory arrest. (*J THORAC CARDIOVASC SURG* 1994;107:776-87)

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Supported by National Institutes of Health grant 1 RO1 NS31238-01.

Read at the Seventy-third Annual Meeting of The American Association for Thoracic Surgery, Chicago, Ill., April 25-28, 1993.

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0022-5223/94 \$3.00 + 0 12/6/50928

The technique of profound hypothermia and circulatory arrest (HCA) was introduced into pediatric cardiac surgery in 1952 to facilitate repair of complex congenital heart lesions.¹ Because it provides a bloodless operative field unobstructed by vascular clamps and cannulas, HCA has been widely adopted as an adjunct for an expanding array of cardiac and noncardiac procedures. Characteristic delayed neurologic sequelae after HCA include choreoathetosis, learning and memory deficits, and impaired intellectual development.^{2,3} The morbidity and mortality increase substantially when the period of arrest extends beyond 45 minutes.²

Evidence is gathering that excitatory amino acid (EAA) neurotransmission may contribute to neuronal ischemic injury during conditions of metabolic stress.^{4,5} Excessive synaptic accumulation of glutamate can cause neuronal overactivation, precipitating a cascade of cellular events that lead ultimately to cell death, a phenomenon termed glutamate excitotoxicity. Glutamate receptors, including the *N*-methyl-D-aspartate (NMDA) subtype and several non-NMDA subtypes, are transiently overexpressed in neonates and infants, inasmuch as EAAs play a critical role in the development of the central nervous system.⁶ This would render the pediatric population particularly vulnerable to glutamate excitotoxicity.

We⁷ have previously shown that those brain regions most susceptible to injury after HCA have the highest concentration of glutamate receptors.⁷ Hypothesizing that glutamate excitotoxicity could underlie the distinctive neurologic injury caused by prolonged periods of HCA, we used dizocilpine (MK-801), a selective NMDA-glutamate receptor antagonist, in a canine survival model of HCA. The potential neuroprotective properties of dizocilpine and its effects on glutamate receptor expression after HCA were explored.

Materials and methods

Preparation. Eighteen conditioned heartworm-negative 10-month-old hound dogs (20 to 25 kg) were used. Animals were of a similar age and from an in-bred strain, to preclude potential variation in microscopic brain anatomy and glutamate receptor subtype expression. Dogs were sedated with fentanyl (20 μ g/kg intravenously) and anesthesia was induced with thiamylal sodium (17.5 mg/kg intravenously). After endotracheal intubation, dogs were maintained on fluothane (halothane) inhalational anesthesia (0.5% to 2.0%) and 100% oxygen.

Bilateral tympanic membrane probes and nasopharyngeal and rectal temperature probes were placed. Tympanic membrane temperature correlates very closely with brain temperature. Animals were prepared and draped in a sterile fashion. Swan-Ganz (Baxter Healthcare Corp., Irvine, Calif.) and arterial catheters were placed percutaneously. Electroencephalograms (EEGs) were recorded with a Grass M 8-16 EEG (Grass Co., Quincy, Mass.). Nine needle electrodes (Grass) were inserted in the dog scalp and the head was insulated with trans-

parent adhesive material and aluminum foil to reduce artifact during cardiopulmonary bypass (CPB). EEG recording parameters included a time constant of 0.3 to 1 Hz, a high-frequency filter of 35 Hz, a paper speed of 30 mm/sec, and initial sensitivity of 70 μ V. Electrode impedance was measured below 3 kOhm. Eight channels were used in bipolar montage and a variable number of channels in referential montage. The EEG baseline was recorded with the dog under anesthesia before CPB and cooling. Electrocerebral silence was defined as absence of EEG activity at a gain of 2 μ V.

CPB and HCA. The CPB circuit included a Bentley-10 Plus bubble oxygenator (Baxter), a 40 μ m in-line arterial filter, and a Sarns roller pump (Sarns/3M, Inc., Ann Arbor, Mich.). The circuit prime consisted of 1 L of lactated Ringer's solution with 20 mEq of sodium bicarbonate and 10 mEq of potassium chloride added. Heparin was administered (300 U/kg intravenously) and animals were cannulated for closed-chest CPB; venous cannulas (16F to 18F) were advanced to the level of the right atrium through the right femoral and right external jugular veins; the arterial cannula (12F to 14F) was placed in the descending thoracic aorta via the right femoral artery.

CPB was initiated and animals were cooled to a tympanic membrane temperature of 18° C with surface (ice bags around head and cooling blanket) and core (CPB) cooling. During CPB, mean blood pressure was maintained at 50 to 55 mm Hg with pump flows of 80 to 100 ml/kg and was reduced to 50 to 60 ml/kg when the tympanic membrane temperature was less than 32° C. Arterial blood gases were controlled by means of the alpha-stat strategy. The arterial pump was turned off and the venous blood was allowed to drain by gravity, thereby exsanguinating the animal. Circulatory arrest was maintained for 2 hours at 18° C.

CPB with rewarming was then reinstituted. Dogs received bicarbonate to maintain the base deficit less than 7 mEq. At normothermia (37° C), the dogs were weaned from CPB and decannulated. The right femoral artery and vein were ligated. Protamine was administered and the wounds were irrigated and closed.

After the procedure, animals continued to have ventilatory support and underwent 20 hours of intensive care monitoring. Anesthesia was maintained with nitrous oxide (2.5 L/min) and intravenous fentanyl. Cardiac rhythm, arterial blood pressure, Swan-Ganz catheter parameters, and urine output were monitored, along with arterial blood gases and hemoglobin and glucose levels; appropriate adjustments were made as needed. Animals were weaned from ventilatory support at the end of 20 hours and were extubated according to standard protocol.

Animals were followed up for 72 hours from the cessation of CPB. Neurologic assessment was performed every 12 hours. At the conclusion of the 3-day period, after the final neurologic examination, animals were reanesthetized, intubated, and their lungs were ventilated. Through a median sternotomy, the ascending aorta was cannulated and the descending aorta was clamped. Dogs were killed by perfusion-fixation of the brain with 3 L of paraformaldehyde (3%, pH 7.4) via the aortic cannula. After 24 hours, the brains were removed and further fixed for 14 days before being sliced into sections for subsequent microscopic examination.

Dizocilpine protocol. Experimental dogs (group A, *n* = 9) received dizocilpine in an investigator-blinded manner. A pre-arrest intravenous bolus (0.75 mg/kg) was administered before the animals were cannulated for CPB. This dose produced EEG silence without altering brain temperature. Animals then received a similar postarrest bolus followed by a continuous

Fig. 1. Tympanic membrane temperatures during cooling, HCA, and rewarming.

infusion (0.75 $\mu\text{g/kg}$ per hour). The animal was gradually weaned from the drug over the 20-hour recovery period before extubation. Control animals (group B, $n = 9$) received vehicle only.

Neurologic injury evaluation. All animals were neurologically assessed according to a species-specific behavioral scale developed and validated for dogs at the International Resuscitation Research Center, University of Pittsburgh.⁸ Neurologic deficit scoring consisted of five major components, including level of consciousness, cranial nerve function, breathing pattern, motor and sensory function, and behavior. A score of 100 was assigned to each category; 0 was normal function, and 500 was brain death. Final neurologic deficits were agreed on by at least two members of the team.

Histopathology. Histopathologic analysis was performed on 12 dogs, six from each group. After fixation, each brain was sliced into 3 mm coronal sections; 18 to 20 sections were embedded in paraffin and 10 μm sections of each block were stained with hematoxylin and eosin, cresyl violet (for cell bodies), and luxol-fast blue (for white matter and myelin). Sections of 25 anatomic areas were examined blindly by a neuropathologist for evidence of neuronal injury. Each population of neurons was scored as follows: normal = 0; mild changes of neuronal injury (shrunken and angular) = 1; moderate neuronal injury (hypereosinophilic) = 2; frank neuronal necrosis with loss of some neurons = 3; and neuronal and vessel necrosis = 4. The neuronal populations were then divided into nine anatomically related regions for comparison between experimental groups of dogs. The total histopathologic score for each brain was the sum of all scores for each of the 25 anatomic areas; the worst possible score is 100.

Receptor autoradiography. To study glutamate receptor subtype expression, we performed densitometric receptor autoradiograms on the remaining three dogs in each group. After the animals were killed, 20 μm sections of fresh brain were processed for autoradiographic imaging by labeling with ^3H -tritiated glutamate (to image NMDA receptors) or with ^3H -tritiated β -D-aspartylaminomethylphosphonic acid (AMPA) (non-NMDA receptors), by a method described previously.⁹ Density of recep-

tors were calculated as disintegrations per minute per microgram of protein and compared with three normal (negative control) dog brains (group C).

Statistical analysis. All values are expressed as mean $\pm$ standard deviation. Comparisons between groups were made by analysis of variance for repeated measures or Student's t test where appropriate. Receptor autoradiographic density values were compared between groups by means of a nested analysis of variance.

Animal care. Animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Results

All animals survived the 2 hours of HCA and the 3 days of postarrest observation. The mean cooling times on CPB were similar for both groups (25.8 ± 3 minutes in group A versus 25.1 ± 4 minutes in group B). There were no significant differences in tympanic membrane temperatures between groups throughout the cooling, arrest, and rewarming phases (Fig. 1). Esophageal and rectal temperatures were similar for the two groups throughout all phases of the procedure.

During the 20-hour recovery period, there were no differences in mean arterial pressure (Fig. 2); cardiac output ranged from 2.1 to 2.8 L/min for all dogs. Acidosis associated with HCA was rapidly corrected for both groups with no significant differences in arterial carbon dioxide tensions or bicarbonate requirements during recovery (Fig. 3). Mild hyperglycemia and hemodilution were similar in the two groups (Fig. 4). There were no significant differences between groups in the quantities of anesthetic agents required during the procedure or the 20-hour recovery period.

Fig. 2. Mean arterial pressure (MAP) versus time, before and after CPB.

EEG. After the first dizocilpine bolus in group A dogs, suppression of EEG activity was noted within 5 ± 1 minutes, while EEG silence was obtained within 10 ± 2 minutes (Fig. 5). EEG activity remained depressed during administration of dizocilpine but returned gradually to baseline as the dizocilpine was weaned during the 20-hour recovery period.

For group B animals, EEG silence occurred 21.6 ± 3 minutes after the initiation of CPB and cooling, at a tympanic membrane temperature of $21.3^\circ \pm 1^\circ \text{C}$; continuous EEG activity returned after 20.4 ± 3 minutes of rewarming on CPB corresponding to a tympanic membrane temperature of $22.4^\circ \pm 1^\circ \text{C}$. The predominant frequency 2 hours after arrest was nonrhythmic, diffuse with a frequency of 18 to 25 Hz, which we¹⁰ have previously reported as a predictor of poor neurologic outcome after prolonged HCA.

Neurologic outcome. At each postarrest neurologic evaluation, the neurologic deficit score was significantly higher in group B than in group A ($p < 0.001$ by analysis of variance, Fig. 6). Apart from mild abnormalities of gait in two dogs, animals receiving dizocilpine were neurologically normal before being killed on postarrest day 3; all could maintain posture and feed normally.

Group B dogs, however, had severe injury; although all dogs were conscious, four had features of choreoathetosis and two dogs had recurrent epileptic seizures. None of these animals could stand normally, walk, or feed itself.

The final neurologic deficit score was 192 ± 40 for group B compared to 21 ± 15 for group A ($p < 0.001$).

Histopathology. The neuronal injury observed on microscopy was that of selective neuronal necrosis (Fig.

Fig. 3. Top, Arterial pH versus time, before and after CPB. Bottom, Arterial carbon dioxide tension (PaCO_2) versus time, before and after CPB.

7 and Table I). The most severely affected regions were the pyramidal cells of the CA-1 hippocampus, the molecular layer of the dentate nucleus and entorhinal cortex; the Purkinje cells of the cerebellum, lamina 3 and 5 of the neocortex, and the basal ganglia (particularly the globus pallidus) also were severely injured. No injury was observed in the white matter or brain stem in any animal.

In all affected brain regions there was significantly less neuronal injury in dizocilpine-treated dogs. The total his-

Fig. 4. Top, Blood hemoglobin (Hb) concentration versus time. Bottom, Blood glucose concentration versus time.

topathologic score was 11.3 ± 3 in group A compared with 48.3 ± 9 in group B ($p < 0.0001$).

Receptor autoradiography. Densitometric receptor autoradiography in group B revealed a significant decline in NMDA-sensitive ^3H -glutamate binding after HCA, particularly in brain regions with high NMDA receptor density (Figs. 8 to 10). Receptor autoradiography in the hippocampus showed that in the CA-1, zone 2 (representing the Stratum Oriens and Pyramidal cell layer), and CA-3, zone 2, there were significant reductions in NMDA receptor densities of 41% and 31%, respectively, in group B (untreated dogs) compared with group C (negative control) dogs. In the molecular layer of dentate nucleus and the molecular layer of the cerebellum, NMDA-sensitive ^3H -glutamate binding in group B diminished significantly by 48% and 40%, respectively, compared with group C values. In the corresponding regions in group A, dizocilpine-treated dogs, however, there was no significant decline in NMDA-sensitive ^3H -glutamate binding, indicating preservation of glutamate receptor expression of these neuronal populations by dizocilpine.

In CA-1, zone 1, and CA-3, zone 1, of the hippocampus, where NMDA receptor density is low, there was no significant reduction of NMDA binding in group B or group A dogs compared with group C dogs.

AMPA binding (non-NMDA) was significantly diminished in group B dogs after HCA. Unexpectedly, in several brain regions, β -D-aspartylaminomethylphosphonic acid receptor density was preserved by dizocilpine, a selective NMDA receptor antagonist. Such protection

BASELINE EEG

EEG 5 MINS AFTER MK-801 BOLUS

EEG 10 MINS AFTER MK-801 BOLUS

Fig. 5. Tracings of the suppressive effect of dizocilpine on EEG activity in group A dogs.

was observed, however, only in brain regions where NMDA receptor concentrations were high (Fig. 11).

In Figs 12 and 13 quantitative autoradiograms depict diminished NMDA and AMPA receptor binding observed in group B dogs and the preservation of receptor density in group A dogs in the hippocampus.

Discussion

The prevalence of neurologic sequelae after HCA becomes prohibitive when the period of arrest extends beyond 1 hour. In fact, despite curtailed arrest times, clinically apparent sequelae of HCA-induced brain damage can occur in up to 12% of children¹¹ and 15% of adults.¹² Clearly, improved methods of cerebral protection are required to reduce the attendant neurologic complications during standard periods of HCA, particularly if the safe time limit for HCA is to be extended to allow surgeons a greater latitude in the approach to complex cardiac and noncardiac defects.

Pharmacologic means to protect the central nervous

Fig. 6. Neurologic deficit score versus time. The neurologic recovery was significantly different between groups ($p < 0.001$ by analysis of variance).

system during HCA have included steroids and barbiturates. Although often used empirically,^{12, 13} there is no conclusive evidence of their efficacy in HCA. Selective antegrade cerebral perfusion through the carotid vessels^{14, 15} and retrograde cerebral perfusion via the superior vena cava^{16, 17} have been used to ameliorate the cerebral injury associated with circulatory arrest. These techniques, however, can be cumbersome and complicated and for technical reasons cannot be used in all patients. The feasibility of intermittent antegrade hypothermic cerebral perfusion with either asanguineous solutions¹⁸ or cold blood¹⁹ as cerebroprotective strategies has also been demonstrated. However, apart from providing effective and possibly more evenly distributed cerebral hypothermia, the techniques of cerebroprotection have not been proved to have other unique beneficial properties. Furthermore, none of these techniques has been shown to consistently prevent selective neuronal necrosis, the hallmark of the global anoxic neurologic injury attributable to HCA.

Following the work of Choi and Olney, evidence is accumulating that the EAAs, in particular glutamate, the major neurotransmitter mediating synaptic excitation in the mammalian central nervous system, have potent neurotoxic activity during conditions of depleted cellular energy such as hypoxia or ischemia, when the synaptic reuptake of EAAs, a highly energy-dependent process, becomes compromised.^{4, 5} The resultant overaccumulation of glutamate leads to excessive excitation of the NMDA and non-NMDA glutamate receptors, leading to a rise in intracellular calcium that triggers protease, lipase, protein kinase C, altered transcription, and release of free oxygen radicals, eventually producing neuronal injury and death. This process is a delayed phenomenon extending over several hours after the inciting insult.

Table I. Histopathologic injury score for each region or related regions

	Group A	Group B	p Value
Neocortex	1.2 ± 1	16 ± 4	<0.001
Hippocampus	1	4	<0.001
Dentate gyrus	0.3	4	<0.001
Entorhinal cortex	0.3	3	<0.001
Amygdala	0	2.5 ± 2	<0.001
Deep central white matter	0	0	
Basal ganglia	2.4 ± 1	7.7 ± 1	<0.001
Brain stem	0	0	
Cerebellum	2.1 ± 1	11.1 ± 1	<0.001
Total histopathology score	7.3 ± 3	48.3 ± 9	<0.0001

Neocortex includes frontal, parietal, temporal, occipital, insular, and cingulate regions; therefore the most severe cumulative injury score is 24. Basal ganglia includes globus pallidus, putamen, caudate nucleus, and thalamus; the worst score is 16. Brain stem includes midbrain, substantia nigra, periaqueductal gray matter, pons, and medulla; the worst score is 20. Cerebellum includes Purkinje cells and granular and molecular layers; the worst score is 12. Deep central white matter includes the anterior commissure and corpus callosum; the worst score is 12. Total injury score for all 25 brain regions is 100. There were marked differences in scores between groups for each region or related regions, and the total histopathology scores were significantly different ($p < 0.0001$).

Much attention has recently been focused on selective NMDA and non-NMDA glutamate receptor antagonists. These compounds have been shown to be neuroprotective in a variety of ischemic/hypoxic paradigms. This was first demonstrated in vitro by Rothman.²⁰ Hypoxic neuronal injury in cortical and hippocampal culture can be reduced by several NMDA antagonists,^{21, 22} and systemically administered NMDA antagonists have diminished injury in animal models of both global and focal ischemia.^{23, 24}

In previous work we⁷ demonstrated that the topography of the selective neuronal necrosis associated with

Fig. 7. Photomicrographs of histologic sections of CA1 hippocampus. **A**, Pyramidal cells of the CA1 hippocampus of a normal (negative control) dog are shown (one is marked with black arrow). **B**, Corresponding hippocampal cells of group B dogs are shrunken, angular, and hypereosinophilic, indicative of severe ischemic injury. **C**, In contrast, the hippocampal cells in the CA1 region of dogs treated with dizocilpine (group A) are well preserved.

HCA corresponds closely with the distribution of EAA receptors. The hippocampus, cerebellum, and basal ganglia, which have high densities of glutamate receptors, are characteristically most vulnerable to this injury, implicating excitotoxicity as an underlying mechanism. The

present study has shown the efficacy of dizocilpine in reducing the neurologic injury associated with prolonged HCA. There was significant improvement in the functional recovery of dogs subjected to 2 hours of circulatory arrest at 18° C, and microscopy confirmed the ability of dizocilpine in limiting selective neuronal necrosis in the CA-1 hippocampus and related regions, the neocortex, the basal ganglia, and the cerebellum. Receptor autoradiography revealed significantly better preservation of NMDA glutamate receptor subtypes in dizocilpine-treated dogs. These results represent the first direct evidence of a role for excitotoxicity in the development of HCA-induced brain injury.

The characteristic neurologic sequelae after HCA may be explained on the basis of glutamate excitotoxicity. Choreoathetosis has been observed in up to 19% of infants undergoing intracardiac operations with HCA^{2,3}; it is a delayed phenomenon in that the patient may be neurologically normal initially but begins having choreoathetoid movements 24 hours or more after the operation. Glutamate excitotoxicity is also a delayed process, and excitotoxicity injury to the putative inhibitory γ -aminobutyric acidergic pathways within the basal ganglia and brain stem could permit uninhibited extrapyramidal activity, leading to the distinctive choreoathetoid movements. The transient overexpression of glutamate receptors in the developing neonate and infant brain would exaggerate the neuronal vulnerability to such excitotoxic injury.

The intelligence quotient in children with arrest times greater than 50 minutes is significantly lower in the late postoperative period than that of children with shorter arrest times. There is an inverse relationship between arrest time and intelligence quotient late in the postoperative period.²⁵ The severity of damage to the hippocampus and related regions seen after HCA in our model could explain the learning and memory deficits seen especially in children who have had circulatory arrest.

The neuroprotective capacity of dizocilpine demonstrated in this study suggests that selective glutamate receptor antagonists may represent an important pharmacologic strategy to prevent neuronal injury during HCA, particularly when prolonged arrest times are anticipated. In this context, the value of the present study is that it illustrates the principles by which glutamate receptor antagonists could be used safely in human beings in a clinical situation. Monitoring the EEG for electrical silence to determine the bolus doses proved critical in our model. We found that lower doses that did not suppress EEG activity were not protective. Furthermore, exposure of neurons to NMDA antagonists may sensitize the brain to EAA-mediated injury. Administration of dizocilpine

Fig. 8. NMDA-sensitive ³H-glutamate binding in the CA1 hippocampus, zones 1 and 2 (CA1-1 and CA1-2) of group A, B, and C dogs. For group B dogs only, there was a significant decline in receptor density in CA1-2, compared with controls, group C. Receptor expression was preserved with dizocilpine treatment in group A in this zone. In CA1-1, with low NMDA receptor density, there was no significant reduction in binding in group B dogs. *DPM*, Disintegrations per minute; *NS*, not significant.

Fig. 9. NMDA binding in the CA3 hippocampus, zones 1 and 2 (CA3-1 and CA3-2). Significant reduction in binding occurred in group B dogs in CA3-2. Receptor expression was preserved in group A dogs in this zone. No significant reduction in binding occurred in either group in the CA3-1, where NMDA receptor density is low. *DPM*, Disintegrations per minute; *NS*, not significant.

(MK-801) rapidly upregulates the number of glutamate receptors by 30% to 50% within 30 minutes.²⁶ This phenomenon may account for the paradoxically enhanced EAA-mediated injury noted previously.⁶ A gradual weaning regimen was therefore used in our experiments to obviate this potential problem. This strategy proved essential in our model, because we found the HCA-induced injury to be equivalent to or worse than that in control animals when the drug was abruptly stopped. A distinct advantage of a pharmacologic approach to reduction of HCA-induced injury is that it can be continued into the postoperative period when excitotoxic neuronal injury may still be evolving.

Although electrocerebral silence was used to determine dosing of dizocilpine, the EEG suppression itself is

unlikely to have contributed to the observed neuroprotection. Other drugs that depress electrical activity of the brain, such as barbiturates, do so by enhancing the inhibitory γ -amino-n-butyric acid pathways and have not proved to provide neuronal protection.²⁷ By inhibiting the excitatory effects of glutamate and, as a result, suppressing EEG activity, dizocilpine, however, does afford neuroprotection.

In this study, densitometric receptor autoradiography illustrated the capacity of dizocilpine to reduce the HCA-induced loss of NMDA receptors in critical brain regions. Overall, preservation was significant only for neuronal populations with high densities of NMDA receptors. Such discriminating protection by a selective glutamate antagonist provides further evidence that exci-

Fig. 10. NMDA binding in the molecular layer (mol) of the dentate nucleus and molecular layer (mol) of the cerebellum. Significant reduction occurred in group B dogs only. DPM, Discintillations per minute.

Fig. 11. AMPA binding in the CA1 hippocampus, zone 2 (CA1-2), the CA3 hippocampus, zone 2 (CA3-2), and the molecular layer (mol) of the dentate nucleus. Significant reduction in binding was observed in group B dogs only. DMP, Discintillations per minute.

toxicity represents an underlying mechanism of HCA-induced brain injury. The preservation of AMPA binding by dizocilpine only in regions with high NMDA binding suggests that the process of arresting the excitotoxicity cascade for neurons expressing NMDA receptors may indirectly preserve other juxtaposed neurons. Because EAAs play a vital role in the developing central nervous system, regulating synaptogenesis, neuronal circuitry, and cytoarchitecture and activity-dependent synaptic plasticity, the ability to preserve glutamate receptor expression after arrest may prove crucial when HCA is used in neonates and infants undergoing cardiac procedures.

Although glutamate antagonists protect against excitotoxic neuronal injury, these compounds can cause widespread central nervous system depression and learn-

ing impairment, together with pathologic changes in defined populations of neurons.^{28,29} Olney, Labruyere, and Price²⁹ reported morphologic damage to neurons in the cerebral cortex of rats treated with dizocilpine, stating that this injury could be prevented by simultaneous administration of anticholinergic agents and barbiturates. We found no evidence of pathomorphologic neuronal injury in dogs receiving dizocilpine. Such effects may have been prevented by the use of the anesthetic thiamylal sodium in our model.

Gangliosides, in particular the monosialoganglioside GM₁, have been demonstrated to be efficacious in limiting calcium-dependent EAA neurotoxicity in cerebellar and hippocampal cell cultures³⁰ without interfering with the EAA receptor recognition sites; this is termed receptor abuse-dependent antagonism and is not associated

Fig. 12. Receptor autoradiograms demonstrating NMDA-sensitive ^3H -glutamate binding in the hippocampus of group C dogs (*top*), group B dogs (*middle*), and group A dogs (*bottom*). Note the color scale for receptor density. Receptor density is diminished in group B dogs and preserved in group A dogs.

with morphologic neuronal injury. We⁷ have previously demonstrated that GM₁ can significantly reduce HCA-induced neuronal injury with preservation of glutamate receptor expression.

Although this study has delineated the benefits of inhibiting the NMDA receptor during prolonged HCA, the potential value of antagonizing the non-NMDA receptor subtypes remains to be fully evaluated, by means

Fig. 13. Receptor autoradiograms depicting AMPA binding in the hippocampus of group C dogs (*top*), group B dogs (*middle*), and group A dogs (*bottom*). There is loss of receptor density in group B dogs in regions where NMDA receptor density is high.

of selective non-NMDA antagonists. In addition, the long-term effects of HCA on glutamate receptor expression and central nervous system development warrant further elucidation.

We are grateful to Mary Lang for her expert assistance in preparing the autoradiographic data. We also thank David Goldsborough for his help in statistical analysis and Barbara

Dobbs and Lynn DiMarcantonio for their help in preparing the manuscript.

REFERENCES

- Niazi SA, Lewis FJ. Profound hypothermia: report of a case. *Ann Surg* 1957;147:264-6.
- Kirklin JW, Barratt-Boyes BG. In: Kirklin JW, Barratt-Boyes BG, eds. *Cardiac surgery*. New York: John Wiley, 1986:29-82.
- Brunberg JA, Doty DB, Reilly EL. Choreoathetosis in infants following cardiac surgery with deep hypothermia and circulatory arrest. *J Pediatr* 1974;84:232-5.
- Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1988;1:623-34.
- Olney JW, Ho OL, Rhee V, et al. Neurotoxic effects of glutamate. *N Engl J Med* 1973;289:1374-5.
- McDonald JW, Johnston MV. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res Rev* 1990;15:41-70.
- Redmond JM, Gillinov AM, Blue ME, et al. The monosialoganglioside, GM₁, reduces neurologic injury associated with hypothermic circulatory arrest. *Surgery* 1993;114:324-33.
- Tisherman SA, Safar P, Radvosky A, et al. Profound hypothermia (<10° C) compared with deep hypothermia (15° C) improves neurologic outcome in dogs after two hours' circulatory arrest to enable resuscitative surgery. *J Trauma* 1991;31:1-11.
- McDonald JN, Johnston MV, Young AB. Differential ontogenic development of three receptors comprising the NMDA receptor/channel complex in the rat hippocampus. *Exp Neurol* 1990;110:237-47.
- Arroyo S, Lesser RP, Redmond JM, et al. EEG predicts neurological recovery of dogs undergoing profound hypothermic circulatory arrest. *Ann Neurol* 1992;32:235.
- Tharion J, Johnson DC, Celermajer JM, Hawker RM, Cartmill TB, Overton JH. Profound hypothermia with circulatory arrest: nine years' clinical experience. *J THORAC CARDIOVASC SURG* 1982;84:66-72.
- Davis EA, Gillinov AM, Cameron DE, Reitz BA. Hypothermic circulatory arrest as a surgical adjunct: a 5-year experience with 60 adult patients. *Ann Thorac Surg* 1992;53:402-7.
- Griep RB, Ergin MA, Lansman SL, et al. The physiology of hypothermic circulatory arrest. *Semin Thorac Cardiovasc Surg* 1991;3:188-93.
- Bloodwell RD, Hallman GL, Cooley DA. Aneurysm of the ascending aorta with aortic valvular insufficiency: surgical management. *Arch Surg* 1966;92:588-99.
- Asano K, Sakurai Y, Irisawa T, Aoki E, Amano K. Separate cardiopulmonary bypass as an aid of surgery of thoracic aortic aneurysms. *Jpn J Thorac Surg* 1979;23:77-82.
- Ueda U, Miki S, Kusuhara K, et al. Surgical treatment of the aneurysm or dissection involving ascending aorta and aortic arch utilizing circulatory arrest and retrograde perfusion. *J Jpn Assoc Thorac Surg* 1988;36:161-6.
- Takamoto S, Matsuda T, Harada M, et al. Simple hypothermic retrograde cerebral perfusion during aortic arch replacement. *J THORAC CARDIOVASC SURG* 1992;104:1106-9.
- Robbins RC, Balaban RS, Swain JA, McDonald TJ Jr, Schneider B, Groom RC. Intermittent hypothermic asanguineous cerebral perfusion (cerebroplegia) protects the brain during prolonged circulatory arrest. *J THORAC CARDIOVASC SURG* 1990;99:878-84.
- Bachet J, Guilmet D, Goudot B, et al. A new technique of cerebral protection during operations on the transverse aortic arch. *J THORAC CARDIOVASC SURG* 1991;102:85-94.
- Rothman SM. Synaptic release of excitatory amino acids neurotransmitter mediates anoxic neuronal death. *J Neurosci* 1984;4:1884-91.
- Weiss JH, Goldberg MP, Choi DW. Ketamine protects cultured neocortical neurons from hypoxic injury. *Brain Res* 1986;380:186-90.
- Goldberg MP, Weiss JW, Pham PC, Choi DW. *N*-Methyl-D-aspartate receptors mediate hypoxic neuronal injury in cortical culture. *J Pharmacol Exp Ther* 1987;243:784-91.
- Gill R, Foster AC, Woodruff GN. Systemic administration of MK-801 protects against ischemia-induced hippocampal neurodegeneration in the gerbil. *J Neurosci* 1987;7:3343-9.
- McDonald JW, Silverstein FS, Cardona D, Hudson C, Chen R, Johnston MV. Systemic administration of MK-801 protects against *N*-methyl-D-aspartate and quisqualate mediated neurotoxicity in perinatal rats. *Neuroscience* 1990;36:589-99.
- Wells FC, Coghill S, Caplan HL, Lincoln C, Kirklin JW. Duration of circulatory arrest does influence the psychological development of children after cardiac operations in early life. *J THORAC CARDIOVASC SURG* 1983;86:823-31.
- McDonald JW, Silverstein FS, Johnston MV. MK-801 pretreatment enhances *N*-methyl-D-aspartate-mediated brain injury and increases brain *N*-methyl-D-aspartate recognition site binding in rats. *Neuroscience* 1990;38:103-13.
- Steen PA, Milde JH, Michenfelder JD. No barbiturate protection in a dog model of complete cerebral ischemia. *Ann Neurol* 1979;5:343-9.
- Morris RGM, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature* 1986;319:774-6.
- Olney JW, Labruyere J, Price MT. Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* 1989;244:1360-2.
- Skaper SD, Leon A, Facci L. Death of cultured hippocampal pyramidal neurons induced by pathological activation of *N*-methyl-D-aspartate receptors is reduced by monosialogangliosides. *J Pharm Exp Ther* 1991;259:452-7.

Discussion

Dr. James L. Cox (St. Louis, Mo.). I must compliment you on this superb study. This is a classic example of what can be done if experimental research is done correctly.

Dr. Thomas H. Wareing (*St. Louis, Mo.*). I congratulate Dr. Redmond and his colleagues on this set of experiments, recognizing how difficult it is to get these animals through such a preparation. This study and others are currently accumulating evidence that the release of excitatory neurotransmitters can become toxic to neurons. This is new and exciting information in this field and something that we should pay attention to, because this mechanism of injury is potentially treatable.

I have two questions for you: First, have you considered trying to measure glutamate directly in cerebrospinal fluid effluent during the course of these experiments? Second, why did you select 2 hours of HCA for this study when it is clearly beyond the level of comfort for most clinical operations?

Dr. John Kennedy (*Cambridge, England*). I too enjoyed the study. Can you be sure that the neurologic deficits that you have presented, and indeed are partly corrected by treatment, are the result of only this problem? Have you excluded, for example, the problem of reflow injury caused by hyperoxide release as the cause for the disability or for microbubbles during the rewarming phase from extracorporeal circulation? Did you have a monitor, for example, for microbubbles in the line?

Dr. Alfonso T. Miyamoto (*Kitakyushu, Japan*). Because ketamine, which is approved by the Food and Drug Administration, also has blocking action on the NMDA receptors, similar to those described for MK-801, have you considered evaluating its possible protective effects?

Dr. Redmond. I thank the discussants for their comments. We have not yet attempted to measure glutamate levels in the cerebrospinal fluid. This has been done by several groups in

patients with epilepsy and hyperthermia-induced seizures and in a variety of models of hypoxic brain injury. The observed elevation in glutamate levels supports the excitotoxicity theory. We are, however, developing this assay in our laboratory for use in our spinal cord ischemia models.

We chose 2 hours of HCA for several reasons. First, 120 minutes of arrest produces a substantial and consistent neurologic injury at 18° C, whereas shorter arrest times yield less reliable brain damage. Second, from a clinical standpoint, our primary objective has been to extend the safe period of HCA well beyond 45 minutes.

Although several mechanisms could have contributed to the neurologic injury in our model, including microembolic phenomena, reperfusion injury with free radical generation, and the no-reflow phenomenon, these could not satisfactorily explain the selective neuronal necrosis observed in our model, which is our particular interest. Although no dog in either group had any focal neurologic signs clinically, on microscopic examination we did find evidence of very limited reperfusion injury.

The anesthetic ketamine is indeed a glutamate-receptor antagonist. We have not used it in our model, although it has been shown to have neuroprotective properties in treating hyperglycemia by Ohya and colleagues (*Pediatr Neurol* 1991;7:66-68) and has been used in head injury cases by McCollagh's group in Britain. It does have profound effects on cerebral blood flow and intracranial pressure, which, in addition to its pharmacokinetics, render it less useful for the purposes of our work.