CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

PROFOUND SYSTEMIC HYPOTHERMIA INHIBITS THE RELEASE OF NEUROTRANSMITTER AMINO ACIDS IN SPINAL CORD ISCHEMIA

Profound hypothermia induced with cardiopulmonary bypass has a protective effect on spinal cord function during operations on the thoracoabdominal aorta. The mechanism of this protection remains unknown. It has been proposed that the release of excitatory amino acids in the extracellular space plays a causal role in irreversible neuronal damage. We investigated the changes in extracellular neurotransmitter amino acid concentrations with the use of in vivo microdialysis in a swine model of spinal cord ischemia. All animals underwent left thoracotomy and right atrium–femoral artery cardiopulmonary bypass with additional aortic arch perfusion. Lumbar laminectomies were then done and microdialysis probes were inserted stereotactically in the anterior horn of the second and fourth segments of the lumbar spinal cord. The probes were perfused with artificial cerebrospinal fluid at a rate of 2 μl/min and 15-minute samples were assayed by high-performance liquid chromatography. Group 1 animals (n = 6) underwent aortic clamping distal to the left subclavian artery and proximal to the renal arteries for 60 minutes at normothermia (37°C) and group 2 animals (n = 5) were cooled to a rectal temperature of 20°C before application of aortic clamps, maintained at this level during cardiopulmonary bypass until the aorta was unclamped, and then slowly rewarmed to 37°C. Seven amino acids were studied, including two excitatory neurotransmitters (glutamate and aspartate) and five putative inhibitory neurotransmitters (glycine, γ-aminobutyric acid, serine, adenosine, and taurine). Glutamate exhibited a threefold increase in extracellular concentration during normothermic ischemia compared with baseline values and remained elevated until 60 minutes after reperfusion. The increase in aspartate concentration was not significant. The extracellular concentrations of glycine and γ-aminobutyric acid also increased significantly during ischemia and reperfusion. Hypothermia uniformly prevented the release of amino acids in the extracellular space. Glutamate levels remained significantly decreased even after rewarining to normothermia whereas glycine levels returned to baseline values. These results are consistent with a role for excitatory amino acids in the production of ischemic spinal cord injury and suggest that the mechanism of hypothermic protection may be related to decreased release of these amino acids in the ischemic spinal cord. (J THORAC CARDIOVASC SURG 1995;110:27-35)

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Profound hypothermia has long been known to protect against experimentally induced cerebral ischemia.\textsuperscript{1-4} It has been recently shown that profound hypothermia induced with cardiopulmonary bypass (CPB) has a protective effect on spinal cord function after clamping of the thoracoabdominal aorta in baboons.\textsuperscript{5} Nevertheless, the mechanism by which hypothermia exerts its protective effect remains unknown.

It has been postulated that the release of excitatory neurotransmitters in the extracellular space of the central nervous system (CNS) by ischemic cells may contribute substantially to neuronal cell death in cerebral ischemic injury.\textsuperscript{6-9} Neurotransmitter release has been implicated in the pathophysiologic process of ischemic brain injury and in the mechanism of its modification by hypothermia because the concentration of excitatory amino acids in dialysis fluid sampled from ischemic brain regions increases during ischemia and decreases during hypothermic conditions. Decreased temperature inhibits the biosynthesis, release, and uptake of various neurotransmitters in the brain and through such a mechanism may affect the outcome of the ischemic insult.\textsuperscript{10, 11}

We used microdialysis in an in vivo swine model of spinal cord ischemia, analogous to the situation encountered clinically for resection of thoracoabdominal aneurysm, to measure extracellular neurotransmitter levels within the spinal cord before, during, and after ischemia. The hypothesis tested was that profound hypothermia affects the release of neurotransmitter amino acids in the spinal cord in this model.

**Material and methods**

Fifteen adult pigs weighing 30 to 35 kg were used. Humane care was provided in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). In addition, the study protocol was approved by the Washington University Animal Studies Committee. The animals were anesthetized with acetylmeprazine (1.1 mg/kg) and atropine (0.5 mg/kg) administered intramuscularly and thiopental (2.5%) administered intravenously. Endotracheal intubation was done and general anesthesia was maintained with halothane (0.5% to 1%) and continuous intravenous infusion of fentanyl citrate (5 μg/kg per hour). Pancuronium bromide (0.1 mg/kg intravenously) was used as necessary for paralysis. **Instrumentation.** A peripheral venous line was inserted in the ear vein for administration of fluids and drugs and an arterial line was placed in the right femoral artery. A second arterial line was placed in the right carotid artery nonocclusively under direct visualization and a venous line was inserted into the right internal jugular vein. Rectal and nasopharyngeal temperature probes (series 400; Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) were inserted and were connected to a thermometer (model 43TD; Yellow Springs Instrument). Proximal and distal arterial pressures and a three-lead electrocardiogram were continuously recorded.

**Surgical preparation.** The animals were initially placed in the prone position. Lumbar laminectomies were done and the second and fourth lumbar spinal segments were exposed. After placement of the animal in the right lateral decubitus position with the pelvis rotated 45 degrees to the left, a left thoracotomy was done through the fourth intercostal space. The abdominal aorta was exposed through a separate left retroperitoneal incision. After systemic anticoagulation with porcine-derived heparin sulfate (300 U/kg intravenous bolus) was accomplished, cannulation was done. A single cannula was inserted in the right atrium for return of venous blood to the pump oxygenator. Two arterial lines (one in the left femoral artery and one in the aortic arch) attached to separate roller pumps and two membrane oxygenators and filters were used.

The animals were then turned to a full right lateral decubitus position and the microdialysis probes were implanted. A spinal temperature probe (model 511; Yellow Springs Instrument) was inserted intrathecally and was connected to the thermometer. CPB was established at 100 ml/kg per minute with one third of total flow directed to the upper and two thirds to the lower part of the body, as previously described.\textsuperscript{9} The aorta was clamped immediately distal to the left subclavian artery and just proximal to the renal arteries for 60 minutes. The isolated aortic segment was vented to atmospheric pressure.

**Experimental design.** The animals were randomly assigned to two groups. In group 1 animals (n = 6) the rectal temperature was maintained at 37°C by the perfusate during the 60-minute interval of aortic occlusion (control group). The group 2 animals (n = 5) were cooled by the perfusate to a rectal temperature of 20°C before application of the aortic clamps (hypothermia group). The duration of CPB required to achieve this temperature averaged 20 ± 4 minutes. Flow was gradually reduced during cooling to 60 ml/kg per minute (20 ml/kg per minute through the upper cannula and 40 ml/kg per minute through the lower cannula). The rectal temperature and the total flow were maintained at these levels until the aorta was unclamped. Flow was then gradually increased and the animals were warmed to 37°C (rectal). The rewarming period averaged 38 ± 7 minutes. In addition to the animals in these experimental groups, two animals were treated identically the same as the group 1 animals (control sham) and two the same as the group 2 animals (hypothermia sham) but did not undergo aortic clamping. All animals were maintained on CPB throughout the duration of the experiment with mean arterial pressure between 60 and 80 mm Hg. Determinations of levels of arterial blood gases, serum potassium, and hematocrit were made at 15-minute intervals. A hemato-
were assayed for amino acids (glutamate, aspartate, glycine, 7-aminobutyric acid \[GABA\], serine, adenosine, and taurine) by the use of phenyl isothiocyanate derivatization, high-performance liquid chromatography reverse phase separation, and ultraviolet detection at a wave-length of 254 nm following the method of Cohen, Bidlingmeyer, and Tarvin.\(^\text{15}\) Dialysate samples were derivatized with 30 \(\mu\)L of phenyl isothiocyanate, methanol, and triethylamine (TEA) and were dried under vacuum. These samples were then reconstituted in solvent consisting of 0.14 mol/L sodium acetate, 0.05% triethylamine, and 6% acetonitrile and were brought to pH 6.4 with glacial acetic acid. Samples were run in the same solvent with the column being washed between each sample run in 60% acetonitrile and 40% water. Amino acids were quantified by use of area integration and comparison to amino acid standards. Recovery of amino acids by the dialysis probes varied between 30% and 40%.

**Spinal cord microdialysis.** The spinal cord microdialysis technique used allows direct sampling of spinal cord interstitial fluid.\(^\text{12-14}\) This technique involves the implantation of a hollow fiber within the spinal cord tissue and the perfusion of this fiber with artificial cerebrospinal fluid (CSF). During perfusion of the fiber, exchange of molecules occurs across the fiber membrane between the spinal cord interstitial fluid outside the fiber and the artificial CSF inside the fiber. Because the artificial CSF is amino acid free, amino acids enter the fiber and can be recovered in the perfusate. Two concentric microdialysis probes (CMA/10; Bioanalytical Systems, Inc., West Lafayette, Ind.) were implanted, one in the second and the other in the fourth lumbar spinal cord segment. The probes were placed in a micromanipulator (model 1761; Kopf, Inc., Tujunga, Calif.) and were lowered into the spinal cord tissue so that the 4 mm length of dialysis fiber rested primarily in the anterior horn. The probes were then cemented into place with carboxylate cement. Artificial CSF was infused through the probes throughout the experiment at a flow rate of 2 \(\mu\)L/min with a microinjection pump (CMA/100; Bioanalytical Systems). The CSF was bubbled with 95% \(\text{N}_2/5\% \text{CO}_2\) to achieve a pH of 7.2 and \(\text{O}_2\) tension of 25 to 39 mm Hg. A 90-minute recovery period was allowed between the implantation of the microdialysis probes and initiation of experimental protocols (aortic clamping in group 1 or initiation of cooling in group 2) to achieve steady-state amino acid levels. The dialysis samples were collected every 15 minutes with a microfraction collector (CMA/140; Bioanalytical Systems) and stored in a freezer before analysis. Six 15-minute samples were collected during the 90-minute period of recovery from implantation. The final 15-minute collection served as the baseline sample.

**Sample processing and analysis.** Dialysate samples were assayed for amino acids (glutamate, aspartate, glycine, \(\gamma\)-aminobutyric acid \[GABA\], serine, adenosine, and taurine) by the use of phenyl isothiocyanate derivatization, high-performance liquid chromatography reverse phase separation, and ultraviolet detection at a wave-length of 254 nm following the method of Cohen, Bidlingmeyer, and Tarvin.\(^\text{15}\) Dialysate samples were derivatized with 30 \(\mu\)L of phenyl isothiocyanate, methanol, and triethylamine (TEA) and were dried under vacuum. These samples were then reconstituted in solvent consisting of 0.14 mol/L sodium acetate, 0.05% triethylamine, and 6% acetonitrile and were brought to pH 6.4 with glacial acetic acid. Samples were run in the same solvent with the column being washed between each sample run in 60% acetonitrile and 40% water. Amino acids were quantified by use of area integration and comparison to amino acid standards. Recovery of amino acids by the dialysis probes varied between 30% and 40%.

**Evoked potentials.** Spinal cord function was assessed intraoperatively by evoked potential monitoring. Somatosensory and motor evoked potentials were measured in all animals with a clinical evoked potential system (Nicolet Pathfinder; Nicolet Instruments, Madison, Wis.) as previously described.\(^\text{16}\) Somatosensory evoked traces were generated by stimulation of the sciatic nerve with a bipolar input electrode and impulses were recorded at the sixth thoracic level. Motor evoked traces were generated by direct cord stimulation at the sixth thoracic level and anterior spinal conduction was recorded at the fourth thoracic interspace. Recordings were obtained at baseline, during cooling, and after aortic clamping.

**Histologic analysis.** The animals were killed at the conclusion of the experiment with an intravenous dose of saturated potassium chloride and were perfused first with 4 L of normal saline solution to remove the blood and then with 1 L of a solution of formaldehyde. The spinal cords were carefully removed, the dura and the leptomeninges were dissected away, and the spinal cords were immersed in formaldehyde overnight. Representative sections of each segment of the spinal cord were stained with hematoxylin-eosin stain, Luxol fast blue and periodic acid-Schiff stains for myelin,\(^\text{17,18}\) and a silver stain for axons.\(^\text{19}\) The sections were reviewed by a neuropathologist who was blinded to the experimental conditions.

**Statistical analysis.** The data obtained were analyzed with the Personal Computer Statistical Analysis Software package (PC SAS, Cary, N.C.). A two-way analysis of

| Table I. Concentrations of amino acids in spinal cord microdialyses in micromoles per liter |
|----------------------------------------|--------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Baseline**                           | **Ischemia (60 min)** | **Reperfusion (15 min)** | **Reperfusion (60 min)** |
| Group 1                                | Group 2             | Group 1          | Group 2          | Group 1          | Group 2          | Group 1          | Group 2          |
| Glutamate                              | 15 ± 2              | 13 ± 3           | NS               | 43 ± 6\(^*\)   | 6 ± 3\(^*\)     | 0.02            | 38 ± 6\(^*\)   | 7 ± 2.4\(^*\) | 0.01            | 16 ± 4          | 9 ± 2\(^*\) | 0.04          |
| Aspartate                              | 3.5 ± 1             | 4.5 ± 2.1        | NS               | 9 ± 3           | 2 ± 0.8         | 0.01            | 7.5 ± 3.2      | 2.6 ± 0.9      | NS              | 1.5 ± 0.8       | 1.1 ± 0.3 | NS           |
| Glycine                                | 28 ± 5              | 34 ± 7           | NS               | 64 ± 12\(^*\)  | 15 ± 4\(^*\)    | 0.01            | 54 ± 11\(^*\)  | 24 ± 3\(^*\)   | 0.03            | 23 ± 8          | 36 ± 6 | NS           |
| GABA                                   | 2.4 ± 0.5           | 2.9 ± 0.7        | NS               | 11.5 ± 2\(^*\) | 1.2 ± 0.4       | 0.04            | 9.5 ± 1\(^*\)  | 10 ± 0.5       | 0.001           | 2.5 ± 0.9       | 0.7 ± 0.5 | NS           |
| Serine                                 | 5 ± 2               | 4 ± 1            | NS               | 9 ± 2           | 2.5 ± 1.0       | 0.04            | 7.2 ± 2        | 2.8 ± 1.0      | 0.03            | 2 ± 0.3         | 4 ± 1   | NS           |
| Adenosine                              | 0.4 ± 0.1           | 0.6 ± 0.3        | NS               | 1.8 ± 0.6       | 0.16 ± 0.05     | 0.04            | 0.2 ± 0.1      | 0.17 ± 0.1     | NS              | 0.2 ± 0.1       | 0.1 ± 0.08 | NS           |
| Taurine                                | 1.1 ± 0.2           | 1.1 ± 0.6        | NS               | 2.1 ± 0.5       | 0.6 ± 0.5       | NS              | 1.4 ± 0.8      | 0.9 ± 0.5      | NS              | 1.1 ± 0.5       | 0.9 ± 0.5 | NS           |

\(^*\)Comparison with baseline: \(p < 0.05\).
Results

Neurotransmitter amino acid levels (Table I). Data obtained from the microdialysis probes implanted in the fourth lumbar spinal cord segment are reported. There was no statistically significant difference between these data and the data obtained from the probe in the second lumbar segment.

No statistically significant difference was found between the two groups with regard to baseline concentrations in any of the measured amino acid neurotransmitters. Amino acid levels were generally lower during ischemia in group 2 (hypothermia) animals when compared with those in group 1 (control) animals.

Glutamate exhibited a threefold increase in extracellular concentration during normothermic ischemia compared with baseline values and progressively returned to baseline levels within 60 minutes after reperfusion. Hypothermia prevented the release of glutamate in the extracellular space during cooling and ischemia. However, in contrast with the pattern exhibited by glutamate, glycine levels returned to baseline values within 45 minutes after reperfusion, soon after normothermia was achieved. The time course of glutamate and glycine concentrations in a representative experiment is depicted in Fig. 1.

GABA exhibited a sixfold increase in extracellular concentration during normothermic ischemia compared with baseline values and progressively returned to baseline levels within 60 minutes after reperfusion. There was a statistically significant difference between the two groups in the extracellular concentration of GABA during aortic crossclamping. Serine, adenosine, and taurine were detected in small amounts and the changes in concentration were subtle and not statistically significant.

The control sham animals demonstrated stable baseline values of amino acid concentrations for at
least 150 minutes. The hypothermia sham animals (hypothermia without aortic crossclamping) exhibited time-course changes in dialysate amino acid levels that were indistinguishable from the changes observed in group 2 animals (hypothermia and aortic crossclamping).

**Evoked potentials** (Fig. 2). Group 1 (control animals) lost motor and sensory evoked responses within 15 minutes after aortic clamping. These responses failed to return with reperfusion. Group 2 (hypothermia) animals demonstrated evoked potentials with increased latency during cooling and these remained unchanged during aortic clamping. Motor and sensory evoked potentials returned to normal after reperfusion and rewarming.

**Histologic analysis.** Definite evidence of early ischemic neuronal damage such as microvacuolation or eosinophilic neuronal necrosis was not found in the paraffin sections in either group 1 or group 2 animals. Nonspecific changes, including minor petechial hemorrhages and focal microscopic perivascular chronic inflammatory changes, were seen in the spinal cords of animals in both groups.

**Discussion**

Profound systemic hypothermia induced with CPB is recognized as a potent means of conferring organ protection during cardiovascular and neurosurgical procedures. The protective effect of hypothermia on ischemic neural tissue has been documented in experimental studies. The mechanism by which hypothermia exerts this protective effect is thought to be related to a reduction in metabolic rate and thus in the oxygen requirements of neurons.

Recently it has been demonstrated that even mild to moderate degrees of brain cooling are capable of conferring dramatic cerebroprotection. Busto and associates showed in rats that neuronal injury after global brain ischemia can be reduced by mild lowering of brain temperature. Furthermore, cooling of the brain by a few degrees substantially improves the number of surviving neurons, particularly in the most vulnerable areas. Okada, Tanimoto, and Yoneda demonstrated in vitro that deep hypothermia (21°C) substantially prolonged the survival time of the neurons during long-lasting anoxia. Other studies have documented evidence of improved functional outcome after hypothermic ischemia.

The precise mechanism responsible for ischemic neuronal injury is not known. Experimental studies indicate that the amino acids glutamate and aspartic acid are the most abundant excitatory transmitters in the mammalian CNS. In fact, the spinal cord was one of the first CNS areas in which it was demonstrated that amino acids such as glutamate depolarize neurons. It is now established that spinal cord neurons use either glutamate or aspartate as principal excitatory transmitters. It may therefore be somewhat surprising that glutamate is also a neurotoxin. Under normal circumstances, protective mechanisms limit neuronal glutamate exposure and prevent toxicity, but pathologic conditions may alter the potential for neuronal exposure to glutamate. Glutamate-induced neuronal damage may contribute substantially to neuronal death in several neurologic diseases, including cerebral hypoxia-ischemia. At least in part, the beneficial effect of hypothermia against hypoxia may be a result of inhibition of the biosynthesis, release, and uptake of various neurotransmitters. Therefore systemic hypothermia can be considered to protect neurons by means of the inhibition of increase in...
intracellular free calcium through the reduction of extracellular glutamate concentration.

In experiments involving microdialysis in rats, Busto and associates\textsuperscript{10} found that animals whose intraischemic brain temperature was maintained at 36°C demonstrated a sevenfold increase in extracellular glutamate concentrations when subjected to 20 minutes of ischemia. When brain temperature was maintained between 30°C and 33°C, the release of glutamate was completely inhibited. There was no difference between the control and the experimental group in the degree of intraischemic local cerebral blood flow reduction and the tissue accumulation of free fatty acids. They concluded that mild hypothermia does not affect the severity of the ischemic insult as measured by the magnitude of blood flow reduction or energy depletion and suggested that hypothermia may exert its protective effect by reducing the ischemia-induced glutamate release. Similarly, Mitani and Kataoka\textsuperscript{40} showed in gerbils that when brain temperature was maintained at 31°C during ischemia, the release of glutamate attenuated to 25% of the concentration in ischemic normothermic animals and there was no neuronal damage.

In our study, the clinical situation of resection of the thoracoabdominal aorta was simulated in a swine model of spinal cord ischemia. The swine spinal cord has blood supply similar to that of human beings and has been used extensively in metabolic studies of the spinal cord.\textsuperscript{41-43} In a primate model of spinal cord ischemia similar to the model used in this study, we demonstrated that systemic profound hypothermia (15°C) has a protective effect on spinal cord function after 60 minutes of aortic crossclamping.\textsuperscript{5} In the present study, animals were only cooled to a systemic temperature of 20°C in anticipation of marked reduction in extracellular neurotransmitter concentrations even with small temperature decreases.\textsuperscript{10} Because excitotoxicity appears to be a major pathogenetic mechanism in neuronal ischemic injury, moderate systemic hypothermia (20°C to 30°C) may prove to provide adequate neuroprotection during cardiovascular procedures that involve hypothermic CPB or circulatory arrest for brain or spinal cord protection.

Glutamate is thought to be the primary excitatory amino acid in the spinal cord and is normally found in considerably higher concentrations than those of aspartate.\textsuperscript{34, 45} In our study, the extracellular concentration of glutamate exhibited a threefold increase during ischemia and progressively normalized within 60 minutes from reperfusion in control (group 1) animals whereas the increase in aspartate levels was not statistically significant. These results are in agreement with the findings of Simpson, Robertson, and Goodman,\textsuperscript{10} who performed microdialysis in the spinal cords of rabbits submitted to 20 minutes of ischemia. Brain microdialysis studies in rats have shown similar patterns in time-course changes but a much greater (6- to 15-fold) increase in the extracellular concentration of glutamate.\textsuperscript{9, 10, 12, 40, 47} This difference may explain the higher vulnerability of the brain to ischemia when compared with the vulnerability of the spinal cord.

Hypothermia prevented the release of glutamate in the extracellular fluid in group 2 animals. Furthermore, there was no increase in this reduced concentration throughout the 60-minute period of ischemia. In this regard, group 2 animals were indistinguishable from sham animals that underwent cooling without aortic clamping. Our finding that extracellular glutamate levels remain significantly decreased for at least 60 minutes after reperfusion and rewarming to normothermia may imply that hypothermia continues to have a protective effect on neuronal function for some time after normothermia is achieved. Clinically, this may provide some protection from ischemic spinal cord insults during intraoperative or immediate postoperative episodes of hypotension and hypoperfusion, potentially reducing the incidence of delayed paraplegia.

Aspartate was found in low concentrations in the spinal cord and the increase in extracellular concentration was not significantly altered during ischemia or reperfusion, in agreement with the findings of other investigators.\textsuperscript{46}

Recent studies have established that nonglutamatergic neurotransmitters are also intimately involved in ischemic neuronal injury.\textsuperscript{48, 49} Other neurotransmitters that may play a role in modulating the excitotoxic effects of glutamate include glycine, the most abundant inhibitory neurotransmitter in the spinal cord,\textsuperscript{44, 45} and GABA, another putative inhibitory neurotransmitter. It has been postulated that an imbalance between excitation and inhibition consequent to the release of multiple neuromodulators into the extracellular fluid during and after ischemia may constitute a major determinant of ischemic neuronal damage.\textsuperscript{48, 49}

Glycine concentrations were the highest of any amino acid measured at baseline in our study. Previous reports have established that glycine has the highest concentration of all amino acids in the ventral gray matter,\textsuperscript{45} whereas the same is true for
glutamate in the dorsal gray matter. Furthermore, our results show that the extracellular concentrations of glycine and GABA increased significantly during ischemia, and after reperfusion they followed a course of normalization similar to that of glutamate. These findings are consistent with previously reported data from studies on the spinal cord and the areas of the brain that are least vulnerable to ischemia.\textsuperscript{48, 49}

Profound systemic hypothermia prevented the release of glycine and GABA in the extracellular space during cooling and ischemia in our study. However, in contrast to concentrations of glycine, extracellular concentrations of GABA remained decreased long after reperfusion and rewarming. Although the measured GABA concentrations were generally low and this difference did not achieve statistical significance when postischemic concentrations were compared with baseline values, the importance of this finding is unclear at present and may deserve further investigation.

The changes in other amino acids measured in our study were not significant in either group of animals. The most intriguing of these amino acids is taurine, which is one of the most abundant free amino acids in the brain: only glutamate is consistently higher in concentration.\textsuperscript{50} The role of taurine in the spinal cord is not clear but it does not appear that taurine plays any significant role in modulating the ischemic injury.

The functional outcome of the animals in our study is not known, because they were not allowed to survive. However, in a similar experimental preparation in baboons we showed that profound systemic hypothermia induced with CPB has a protective effect on spinal cord function.\textsuperscript{5} Monitoring of sensory and motor evoked potentials in group 1 animals confirmed the induction of spinal cord ischemia in our experimental preparation, because the evoked responses disappeared shortly after aortic clamping, as also documented in previous studies.\textsuperscript{10} Group 2 animals demonstrated increased latency of evoked responses as a result of cooling but they never showed isoelectric potentials during cooling or aortic crossclamping. This observation is consistent with the results of Coles and associates,\textsuperscript{51} who found that spinal evoked potentials are only abolished at temperatures lower than 10{\degree}C. Furthermore, there was no change in the amplitude or latency of evoked potentials during the 60-minute period of ischemia. In fact, the time course of these potentials in group 2 animals was identical to that in sham animals that underwent cooling for a comparable period without aortic clamping. The lack of histologic findings is probably related to the lack of time for histologic changes to mature.

In summary, our evidence indicates that the increase in the extracellular concentrations of excitatory amino acids, particularly glutamate, is consistent with the hypothesis that excitotoxins mediate neuronal damage in the ischemic spinal cord during ischemia produced by aortic clamping. Furthermore, we have shown that the protective effect of hypothermia on the spinal cord during operation on the thoracoabdominal aorta is related to decreased release of neurotransmitter amino acids in the ischemic spinal cord.

From our data, as well as from data in the literature, it is unclear whether the same degree of functional protection of the spinal cord that can be achieved with profound hypothermia can also be achieved with cooling to moderate degrees of hypothermia. Further studies to define the protective effect of systemic cooling to temperatures between 20{\degree} and 30{\degree}C may be warranted. Nevertheless, better understanding of the mechanisms involved in neuronal death opens new horizons in the field of pharmacoprotection. Pharmacologic antagonism of glutamate receptor-mediated neurotoxicity appears to be an appealing concept and it may provide a novel approach to amelioration of the effects of spinal cord ischemia.\textsuperscript{52}

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