Anesthesiology 2003; 98:690–8 © 2003 American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins, Inc.

Xenon Attenuates Cardiopulmonary Bypass–induced Neurologic and Neurocognitive Dysfunction in the Rat

Daqing Ma, M.D., Ph.D., Hong Yang, M.D.,† John Lynch, M.D.,‡ Nicholas P. Franks, Ph.D.,§ Mervyn Maze, M.B., Ch.B., F.R.C.P., F.R.C.A.,‖ Hilary P. Grocott, M.D., F.R.C.P.C.#*

Background: **With clinical data suggesting a role for excitatory amino acid neurotransmission in the pathogenesis of cardiopulmonary bypass (CPB)–associated brain injury, the current study was designed to determine whether xenon, an *N*-methyl-D-aspartate receptor antagonist, would attenuate CPB-induced neurologic and neurocognitive dysfunction in the rat.**

Methods: **Following surgical preparation, rats were randomly divided into four groups: (1) sham rats were cannulated but did not undergo CPB; (2) CPB rats were subjected to 60 min of CPB using a membrane oxygenator receiving a gas mixture of 30% O_2, 65% N_2, and 5% CO_2; (3) CPB + MK801 rats received MK801 (0.15 mg/kg intravenous) 15 min prior to 60 min of CPB with the same gas mixture; and (4) CPB + xenon rats underwent 60 min of CPB using an oxygenator receiving 30% O_2, 60% xenon, 5% N_2, and 5% CO_2. Following CPB, the rats recovered for 12 days, during which they underwent standardized neurologic and neurocognitive testing (Morris water maze).**

Results: **The sham and CPB + xenon groups had significantly better neurologic outcome compared to both the CPB and CPB + MK801 groups on postoperative days 1 and 3 ($P < 0.05$). Compared to the CPB group, the sham, CPB + MK801, and CPB + xenon groups had better neurocognitive outcome on postoperative days 3 and 4 ($P < 0.001$). By the 12th day, the neurocognitive outcome remained significantly better in the CPB + xenon group compared to the CPB group ($P < 0.01$).**

Conclusion: **These data indicate that CPB-induced neurologic and neurocognitive dysfunction can be attenuated by the administration of xenon, potentially related to its neuroprotective effect *via* *N*-methyl-D-aspartate receptor antagonism.**

XENON has been used experimentally in clinical anesthetic practice for more than 50 yr.[1] Despite xenon's similar (and arguably better) safety and superior ecologic profile compared to other conventional anesthetics, its high cost has precluded its more widespread clinical use. Concerns over cost have been mitigated to some extent by technological developments in the delivery and recycling of xenon that permit much less total gas to be expended during each administration. Still, its use is likely to be restricted to settings in which there is a clear cost–benefit advantage.

Xenon is an inhibitor of glutamatergic *N*-methyl-D-aspartate (NMDA) receptors.[2,3] Because activation of the NMDA receptor appears to be crucial to the initiation of neuronal injury and death from a variety of insults, we have previously examined xenon's putative neuroprotective effects in a series of *in vitro* and *in vivo* studies.[4] Using a primary culture of neuronal and glial cells from the cerebral cortex of neonatal mice, we demonstrated that xenon, in a concentration-dependent fashion, reduced neuronal injury produced by NMDA, glutamate, and oxygen deprivation. In an *in vivo* model of brain injury in rats, xenon concentration-dependently reduced neuronal degeneration in the arcuate nucleus of the hypothalamus provoked by NMDA administration. However, functional outcome was not assessed.

To further explore xenon's possible clinical utility as a neuroprotectant, we have chosen a rat model of cardiopulmonary bypass (CPB)[5] in which functional neurocognitive deficits extend into the remote postoperative period much the same as that which occurs following cardiac surgery in humans. We hypothesized that xenon, at a subanesthetic concentration, would provide long-lasting protection against the functional neurocognitive deficits that occur following CPB in rats.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Methods

The protocol was approved by the Duke University Animal Care and Use Committee (Durham, North Carolina), and all procedures met the guidelines of the National Institutes of Health for animal care (*Guide for the Care and Use of Laboratory Animals*).[6]

Surgical Preparation and Cardiopulmonary Bypass

The methodology of the CPB model used in the current study has been previously reported.[5] Briefly, anesthesia was induced in male Sprague-Dawley rats (age, 12–14 weeks; weight, 350–380 g; Harlan, Indianapolis, IN) with 5% isoflurane in oxygen-enriched air in a plastic box. Following orotracheal intubation with a 14-gauge cannula, the lungs were mechanically ventilated (40% O_2/balance N_2) to maintain an arterial carbon dioxide tension ($Paco_2$) of 36–42 mmHg. During surgical prep-

* Research Associate, § Professor of Biophysics and Anaesthetics, ‖ Sir Ivan Magill Professor of Anaesthetics, Departments of Anesthetics and Biological Sciences, Imperial College of Science, Technology and Medicine, London, United Kingdom. † Research Associate, # Associate Professor of Anesthesiology, Department of Anesthesiology (Multidisciplinary Neuroprotection Laboratories), ‡ Assistant Professor of Medicine, Department of Medicine (Neurology), Duke University Medical Center.

Received from the Department of Anesthesiology (Multidisciplinary Neuroprotection Laboratories), Duke University, Durham, North Carolina. Submitted for publication May 31, 2002. Accepted for publication October 25, 2002. Supported by the Medical Research Council, London, United Kingdom, and by a grant from Protexeon Ltd., London, United Kingdom. Drs. Franks and Maze are both board members and paid consultants of the Imperial College spin-out company, Protexeon Ltd. Dr. Grocott was also a paid consultant to Protexeon Ltd. Presented in part at the Outcomes 2002 meeting, Key West, Florida, May 23, 2002.

Address reprint requests to Dr. Grocott: Department of Anesthesiology, Duke University Medical Center, Box 3094, Durham, North Carolina 27710. Address electronic mail to: h.grocott@duke.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

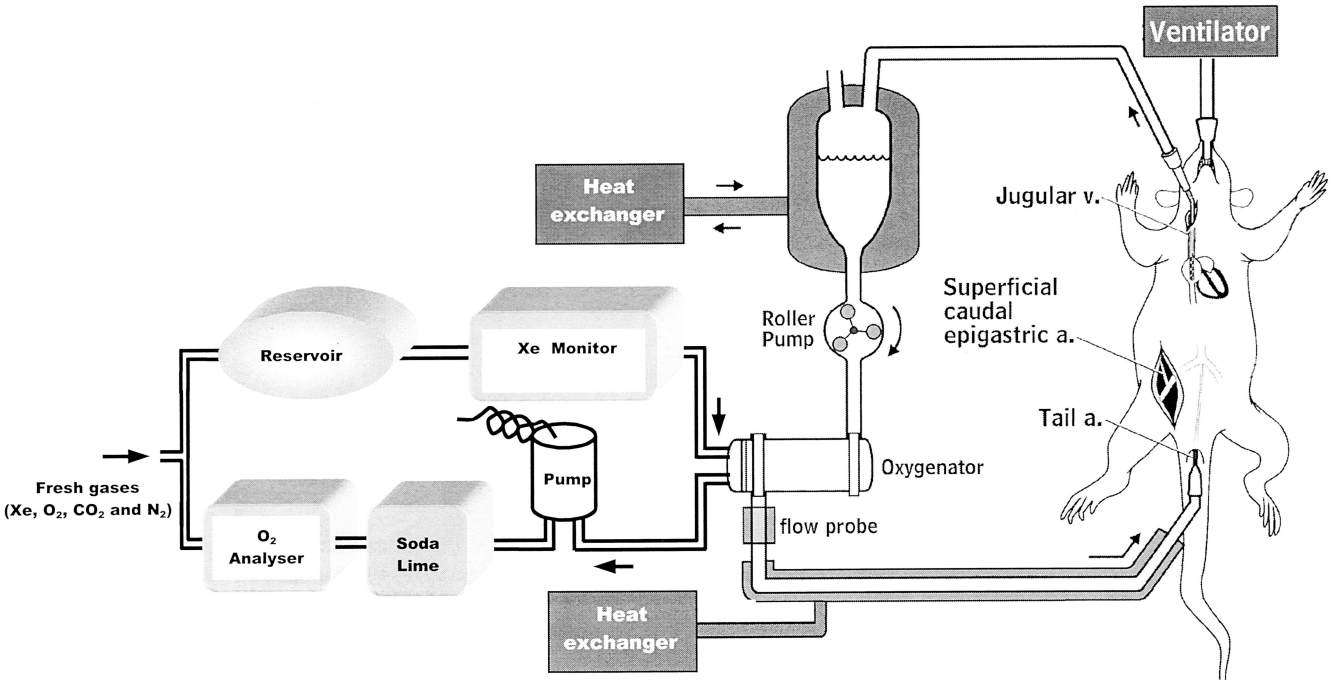

Fig. 1. Schematic diagram of the rat cardiopulmonary bypass (CPB) model and xenon gas delivery system (modified with permission from Grocott *et al.*[25]).

aration, anesthesia was maintained with 1.5–2.0% isoflurane, and the rectal and pericranial temperatures were monitored (YSI 400 series thermistor and 73ATA Indicating controller; YSI, Yellow Springs, OH) and servo-controlled to 37.5 ± 0.1°C with a heating blanket and a convective forced-air heating system. The right superficial caudal epigastric artery, a branch of a femoral artery, was cannulated with PE-10 tubing for monitoring mean arterial pressure. During CPB, rats were anesthetized with fentanyl (30 μg/kg intravenous), midazolam (0.4 mg/kg intravenous), and atracurium (0.5 mg/kg intravenous) as a bolus injection and followed by a continuous infusion of the mixture of three with a syringe pump (2.3–2.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ intravenous fentanyl, 0.027–0.03 $mg \cdot kg^{-1} \cdot min^{-1}$ intravenous midazolam, 0.076–0.08 $mg \cdot kg^{-1} \cdot min^{-1}$ intravenous atracurium). Pilot studies confirmed that with this anesthetic regimen, the animals (when unparalyzed) did not move in response to noxious stimuli during CPB. Venous return blood was drained *via* a 4.5-French multiorifice cannula inserted into the external jugular vein *via* a neck incision and advanced into the right heart. Blood was returned to the animals from the CPB circuit *via* a 20-gauge 1.1-in catheter sited in the ventral tail artery.

The CPB circuit (fig. 1) consisted of a venous reservoir, a peristaltic pump, and a membrane oxygenator, all of which were connected with 1.6-mm-ID silicone tubing (Tygon®; Cole-Parmer Instrument Co., Vernon Hills, IL). The CPB circuit was primed with approximately 40 ml whole blood obtained prior to the start of the experiment from two heparinized (100 IU/rat, intravenous) donor rats (weight, 275–320 g) that were exsanguinated under isoflurane anesthesia. The venous return blood drained to a warmed venous reservoir (jacketed with circulating water from a heat pump) and then to a peristaltic pump (Masterflex®; Cole-Parmer Instrument Co.) that pumped the blood through a membrane oxygenator (Micro® neonatal oxygenator; Cobe Cardiovascular, Inc., Arvada, CO). A closed-circuit gas-delivery system fed the appropriate gas into the oxygenator, after which blood was infused back into the rat (fig. 1). An in-line flow probe (2N806 flow probe and T208 volume flowmeter; Transonics Systems, Inc., Ithaca, NY) was used to continuously measure CPB flow. Arterial line inflow temperature was maintained at 37.5°C using a circulating water bath system. The venous oxygen saturation from the venous return line was measured continuously using an Oximetrix® Monitor and an Opticath® Catheter (Abbot Laboratories, North Chicago, IL). Arterial blood gases were performed using an IL 1306 blood gas analyzer (Instrument Laboratories, Inc., Lexington, MA) with hemoglobin determined using an OSM3 Hemoximeter® (Radiometer Inc., Copenhagen, Denmark). Baseline physiologic measurements, including mean arterial pressure, temperatures, and blood gases, were made 10 min prior to commencement of CPB. The targeted CPB flow was 160–180 $ml \cdot kg^{-1} \cdot min^{-1}$ corresponding to the normal cardiac output in the rat.[7] The animals were weaned from CPB without the need for inotropes or vasopressors; heparin-induced anticoagulation was allowed to dissipate spontaneously. Following decannulation, rats gradually emerged from the effects

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

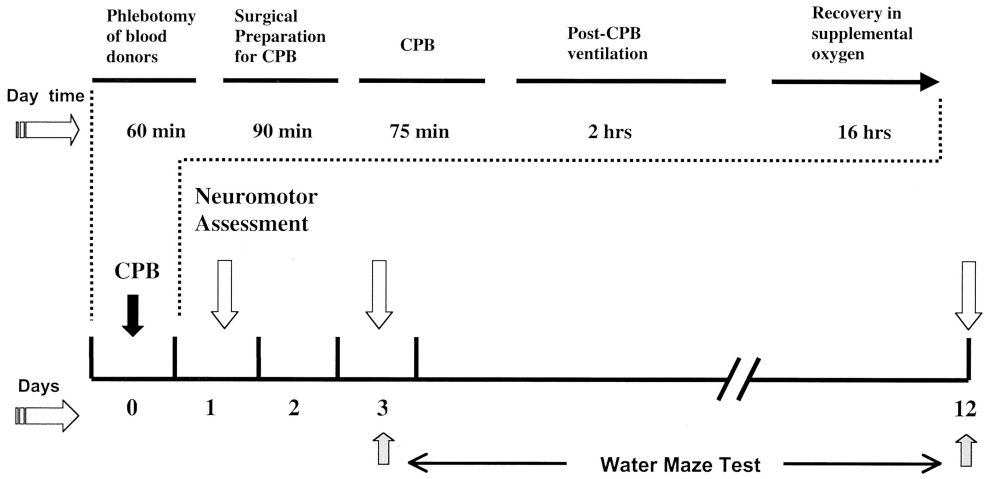

Fig. 2. Schematic diagram of the experimental schedule. Rats underwent cardiopulmonary bypass (CPB) on experimental day 0. Details of experimental procedures are indicated in top part of the figure. Following recovery, they underwent neurologic function testing on the post-CPB days 1, 3, and 12, indicated by open arrows. Neurocognitive testing, using the Morris water maze to evaluate visuospatial learning, was also performed on a daily basis from post-CPB day 3.

of the anesthetic and neuromuscular blockade over a period of 2 h. When adequate spontaneous breathing resumed, the animals were extubated. The animals were recovered in an oxygen-enriched enclosure for 24 h with free access to water and food; thereafter, they were returned to their cages and housed individually.

Experimental Groups

Rats were randomized into four groups (n = 12 or 13 per group). In the sham group, rats were cannulated but did not undergo CPB. In the CPB group, rats were subjected to 60 min of nonpulsatile CPB, during which the oxygenator received a gas mixture of 30% O_2, 65% N_2, and 5% CO_2. Carbon dioxide was added because of the efficiency of the oxygenator in ventilation, which without the additional carbon dioxide would have lead to unphysiologically low $Paco_2$ values. In the CPB + MK801 group, rats received MK801 (0.15 mg/kg intravenous) 15 min prior to 60 min of CPB with similar concentrations of gases as in the CPB group. The dose of MK801 was based on other studies demonstrating its neuroprotective efficacy[8] and on our own pilot studies designed to find a dose with minimal behavioral side effects. In the CPB + xenon group, rats received a gas mixture during CPB of 60% xenon with the balance comprising 30% O_2, 5% N_2, and 5% CO_2.

Neurologic and Neurocognitive Testing

The timeline of the testing protocol is outlined in figure 2. On the 1st, 3rd, and 12th postoperative days, animals underwent standardized functional neurologic testing using an established neuromotor protocol, which included assays of prehensile traction, strength, and balance beam performance graded on a 0–9 scale (best score = 9).[9,10] At the each time point, each animal was tested twice, and the sum of those two values was used for data analysis. In addition to the neurologic evaluation, neurocognitive outcome was evaluated daily (starting on the third postoperative day) in the Morris water maze[11] using a computerized video tracking system (EthoVision®; Noldus, Wageningen, The Netherlands). Briefly, the Morris water maze consisted of a 1.5-m-diameter, 30-cm-deep pool of water (26.5 ± 0.2°C) with a hidden submerged (1 cm below surface) platform in one quadrant. Rats were placed in the water in a dimly lit room with various visual clues around the maze. The time to locate the submerged platform (defined as the latency) was measured to test for impairment in visuospatial learning and memory components of neurocognition. Rats underwent daily testing in the water maze with four trials per testing period, each limited to a 90-s water exposure. Each of the trials was begun from a separate quadrant. The testing was consecutively repeated for 10 days. On the final post-CPB day, immediately after the final trial, each animal was subjected to a probe trial (60-s cutoff), in which they were placed in the maze that had the platform removed. The time spent in the quadrant that previously contained the submerged platform was recorded and represented an index of memory.[12]

Histologic Examination

After completion of the testing on the final day, the animals were anesthetized with 3% halothane and were perfused transcardially with 100 ml heparinized phosphate buffer, 0.1 M, followed by 500 ml paraformaldehyde, 4%, in 0.1 M phosphate buffer. The whole brain was immediately removed and fixed in 4% paraformaldehyde for 24 h. Thereafter, slices of middle brain (4 mm thick) were postfixed in formalin and embedded in paraffin. Three sections (4 μm) were harvested from −3.3 to −3.6 mm of the bregma in each of five animals from

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

Table 1. Mean Arterial Blood Pressure and Cardiopulmonary Bypass Flow

		CPB				Post-CPB	
	Baseline	10 min	20 min	40 min	60 min	30 min	120 min
MAP, mmHg	—	—	—	—	—	—	—
Sham	79 ± 11	77 ± 11	72 ± 10	76 ± 11	76 ± 7	83 ± 8	83 ± 7
CPB	77 ± 10	67 ± 11	73 ± 9	69 ± 9	67 ± 8	79 ± 7	86 ± 10
CPB + MK801	74 ± 8	62 ± 6	65 ± 9	64 ± 5	65 ± 9	68 ± 5	76 ± 5
CPB + xenon	74 ± 5	67 ± 7	69 ± 8	69 ± 9	71 ± 6	77 ± 6	77 ± 4
CPB flow, ml · kg^{-1} · min^{-1}	—	—	—	—	—	—	—
Sham	—	—	—	—	—	—	—
CPB	—	163 ± 16	159 ± 17	156 ± 18	159 ± 19	—	—
CPB + MK801	—	153 ± 15	153 ± 13	158 ± 11	156 ± 13	—	—
CPB + xenon	—	156 ± 16	156 ± 17	154 ± 14	157 ± 14	—	—
Rectal temperature, °C	—	—	—	—	—	—	—
Sham	37.4 ± 0.2	37.5 ± 0.03†	37.5 ± 0*	37.5 ± 0.03	37.5 ± 0	37.5 ± 0.08	37.5 ± 0.07
CPB	37.5 ± 0.1	37.2 ± 0.2	37.3 ± 0.2	37.5 ± 0.1	37.5 ± 0.1	37.5 ± 0.1	37.5 ± 0.1
CPB + MK801	37.5 ± 0.1	37.2 ± 0.2	37.4 ± 0.1	37.5 ± 0.05	37.5 ± 0.04	37.5 ± 0.1	37.5 ± 0
CPB + xenon	37.5 ± 0.04	37.2 ± 0.2	37.5 ± 0.1	37.5 ± 0.1	37.5 ± 0	37.5 ± 0	37.5 ± 0
Pericranial temperature, °C	—	—	—	—	—	—	—
Sham	37.3 ± 0.3	37.5 ± 0.1†	37.5 ± 0.1*	37.5 ± 0.1	37.5 ± 0.03	37.5 ± 0.03	37.5 ± 0.07
CPB	37.5 ± 0.1	37.1 ± 0.3	37.3 ± 0.2	37.5 ± 0.1	37.5 ± 0.1	37.5 ± 0.1	37.5 ± 0.1
CPB + MK801	37.5 ± 0.1	37.2 ± 0.2	37.3 ± 0.2	37.4 ± 0.2	37.4 ± 0.1	37.5 ± 0.1	37.5 ± 0
CPB + xenon	37.5 ± 0.03	37.5 ± 0.5	37.5 ± 0.5	37.4 ± 0.2	37.4 ± 0.2	37.5 ± 0	37.5 ± 0.04

Values are shown as mean ± SD; n = 10 in each group.

* $P < 0.05$, † $P < 0.01$ *vs.* CPB at the same time point.

MAP = mean arterial pressure; CPB = cardiopulmonary bypass.

each group, and sections were stained with hematoxylin and eosin for light microscopic evaluation of necrotic neurons. The mean total number of both normal and necrotic neurons in hippocampus from the three slides was recorded.

Statistical Analysis

Parametric data are summarily presented as mean ± SD with nonparametric data presented as median (interquartile range). Physiologic values and the outcome from water maze testing were compared between groups using repeated measures analysis of variance followed by Newman-Keuls test for multiple comparisons as appropriate. Neurologic outcomes between groups on post-CPB days 1, 3, and 12 were compared using the Kruskal-Wallis nonparametric analysis of variance followed by the Dunn multiple comparisons test. Statistical significance was assumed when P was less than 0.05.

Results

There were two or three deaths in each of CPB groups, mostly due to vascular perforation during cannulation or excessive blood loss during decannulation. A single animal died postoperatively (CPB + MK801) after exhibiting marked pulmonary congestion. These animals were all excluded from the analysis, leaving a total of 10 animals in each group for complete data analysis.

Physiologic Parameters

There were no significant differences in mean arterial pressure and flow rates in the three CPB groups (table 1). The rectal and pericranial temperatures were well maintained near 37.5°C except for a brief period following the commencement of CPB; no significant differences were noted among the three CPB groups (table 1). The pH and blood gas measurements are tabulated (table 2) and were maintained within normal limits throughout. Hemoglobin gradually decreased in the sham group (likely due to repeated blood sampling), while it gradually increased to near baseline levels after an initial drop in the three groups undergoing CPB (table 2). No differences in blood glucose among the four groups were found (table 2). The animals' body weights decreased to a nadir on the 3rd postoperative day and thereafter increased to above baseline by the end of the experiment on the 12th postoperative day (table 3). No differences were found among the four groups with respect to weight.

General Behavioral Changes

All animals that had received MK801 exhibited hyperactivity, head weaving, and related disturbances of motor coordination lasting for 1 to 2 h after they emerged from anesthesia. However, all animals were able to drink and to eat after emergence.

Neuromotor Functional Testing

Both the sham and CPB + xenon groups had improved neurologic outcome compared to the CPB group on the first and third postoperative days: on the first day: 15.5 (13.5–16.5), sham ($P < 0.01$), or 17 (15–17), CPB + xenon ($P < 0.001$), *versus* 11 (7.5–12.5), CPB; on the

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

Table 2. Blood Gases during the Course of the Study

		CPB				Post-CPB	
	Baseline	10 min	20 min	40 min	60 min	30 min	120 min
Ph	—	—	—	—	—	—	—
Sham	7.38 ± 0.04	7.40 ± 0.04	7.41 ± 0.04	7.40 ± 0.06	7.43 ± 0.03	7.40 ± 0.03	7.41 ± 0.06
CPB	7.39 ± 0.02	7.39 ± 0.04	7.36 ± 0.04	7.39 ± 0.04	7.41 ± 0.06	7.42 ± 0.03	7.41 ± 0.03
CPB + MK801	7.40 ± 0.04	7.39 ± 0.06	7.43 ± 0.07	7.44 ± 0.11	741 ± 0.04	7.43 ± 0.04	7.45 ± 0.03
CPB + xenon	7.41 ± 0.03	7.38 ± 0.05	7.46 ± 0.04	7.41 ± 0.06	7.45 ± 0.05	7.45 ± 0.04	7.45 ± 0.05
$Paco_2$, mmHg	—	—	—	—	—	—	—
Sham	37 ± 2.4	37 ± 2.1	36 ± 2.3	37 ± 3.5	35 ± 1.9	34 ± 3.6*	35 ± 5.8
CPB	36 ± 3.0	40 ± 4.0	40 ± 2.0	37 ± 6.0	39 ± 3.0	39 ± 2.0	39 ± 2.0
CPB + MK801	35 ± 3.0	42 ± 5.0	36 ± 6.0	40 ± 6.0	41 ± 4.0	37 ± 5.0	36 ± 5.0
CPB + xenon	35 ± 1.8	39 ± 2.8	38 ± 3.0	39 ± 3.3	36 ± 3.8	37 ± 3.1	38 ± 6.1
Pao_2, mmHg	—	—	—	—	—	—	—
Sham	276 ± 50	213 ± 48	219 ± 53	225 ± 46	254 ± 51	237 ± 52	272 ± 53
CPB	225 ± 53	250 ± 35	231 ± 22	244 ± 35	227 ± 22	253 ± 70	281 ± 71
CPB + MK801	240 ± 69	229 ± 15	215 ± 34	217 ± 17	207 ± 16	254 ± 81	270 ± 64
CPB + xenon	261 ± 62	215 ± 15	206 ± 18	219 ± 22	211 ± 36	257 ± 68	290 ± 39
Svo_2, %†	—	—	—	—	—	—	—
Sham	—	—	—	—	—	—	—
CPB	—	56 ± 5	53 ± 7	54 ± 6	54 ± 5	—	—
CPB + MK801	—	59 ± 9	54 ± 8	57 ± 9	57 ± 9	—	—
CPB + xenon	—	57 ± 7	54 ± 6	54 ± 4	53 ± 6	—	—
Hemoglobin, g/dl	—	—	—	—	—	—	—
Sham	15.1 ± 1.6	14.1 ± 1.4	14.0 ± 1.7**	13.9 ± 2.1*	13.3 ± 1.7	13.5 ± 1.8	12.9 ± 1.6
CPB	15.9 ± 1.2	11.2 ± 0.9	11.7 ± 0.9	12.1 ± 1.1	11.9 ± 1.2	12.7 ± 2.0	14.3 ± 0.9
CPB + MK801	16.1 ± 1.0	11.6 ± 0.9	12.1 ± 1.0	12.3 ± 0.8	12.3 ± 0.8	13.7 ± 0.9	14.4 ± 1.4
CPB + xenon	15.8 ± 1.2	11.3 ± 0.8	12.1 ± 0.7	12.3 ± 0.9	11.9 ± 1.1	13.6 ± 0.9	14.4 ± 1.1
Glucose, mg/dl	—	—	—	—	—	—	—
Sham	111 ± 23	—	—	88 ± 20	—	—	86 ± 12
CPB	99 ± 11	—	—	106 ± 13	—	—	87 ± 13
CPB + MK801	109 ± 29	—	—	106 ± 14	—	—	88 ± 15
CPB + xenon	90 ± 14	—	—	101 ± 14	—	—	86 ± 13

Values are shown as mean ± SD; n = 10.

* $P < 0.05$ *vs.* CPB at the same time point.

† SvO_2, mixed venous oxygen saturation, which was monitored *via* the outlet from the venous reservoir, was unavailable in the sham group.

CPB, cardiopulmonary bypass.

third day: 17 (15.5–18), sham ($P < 0.05$), or 18 (17–18), CPB + xenon ($P < 0.001$), *versus* 12.5 (8.5–15), CPB. No difference was found between CPB and CPB + MK801 groups: on the first day: 11 (9.5–12.5), CPB, *versus* 12 (10–15), CPB + MK801 ($P > 0.05$); on the third day: 12.5 (8.5–15), CPB, *versus* 16.5 (12–17.5), CPB + MK801 ($P > 0.05$). On the 12th postoperative day, no difference was found among the four groups (fig. 3). Qualitative analysis of the individual components within the functional neurologic testing suggested that this difference was predominantly attributable to worse performance on the balance beam and prehensile traction ability (data not shown).

Table 3. Animal Body Weight during the Course of the Study

	Baseline	24 hr	72 hr	12 d
Sham	369 ± 30	360 ± 29	363 ± 33	390 ± 26
CPB	378 ± 25	372 ± 29	370 ± 30	388 ± 30
CPB + MK801	371 ± 33	358 ± 28	363 ± 31	394 ± 29
CPB + xenon	371 ± 13	357 ± 18	360 ± 12	391 ± 13

Values are shown as mean ± SD; n = 10. Body weight values are in grams. No difference was found among the groups at the corresponding time point.

CPB, cardiopulmonary bypass.

Morris Water Maze Testing

The latencies, denoting the cumulative time taken by animals to find the platform based on four trials of each day, were longer in the CPB group compared to the sham, CPB + MK801, and CPB + xenon groups. There was a statistically significant difference when each group was compared with the CPB group (CPB *vs.* sham: $F = 18.2$, $P < 0.0001$; CPB *vs.* CPB + MK801: $F = 20.7$, $P < 0.0001$; CPB *vs.* CPB + xenon: $F = 21.6$, $P < 0.0001$). Repeated measurements with the Student-Newman-Keuls test showed a significant difference when compared with the CPB group on the third and fourth days postoperatively (fig. 4). Swimming speeds varied from 4.7 to 6.2 in/s throughout 10 postoperative days and were not significantly different among the four groups (fig. 5). In the probe trial, the time spent in the quadrant of the former platform was significantly longer in both the sham and CPB + xenon groups when compared with the CPB group (39 ± 10 and 44 ± 7 *vs.* 28 ± 9 s;

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

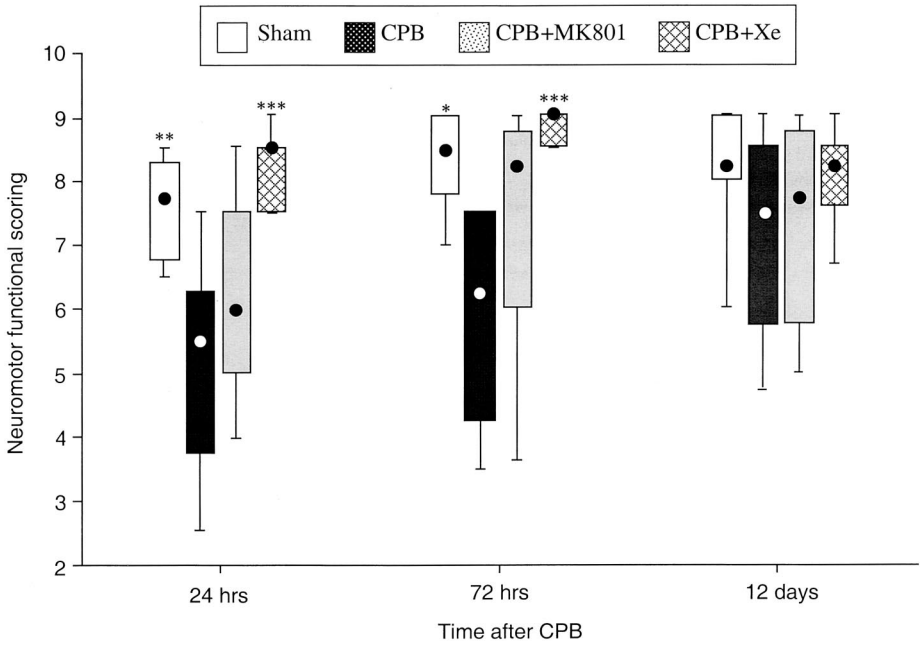

Fig. 3. Neurologic function was evaluated using a neuromotor functional testing protocol that included assays of prehensile traction, strength, and balance beam performance. A score of 3 in any category indicates normal performance. Values are presented in a box-and-whisker plot (boxes are constructed with 25% and 75% confident intervals; closed or open circles in the boxes are medians; positive or negative bars are maximum or minimum individual values in that group, respectively; n = 10). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with cardiopulmonary (CPB) group at the same time point.

$P < 0.05$ and < 0.01, respectively; fig. 6). There was also a significant difference in the probe trial between the CPB + MK801 and CPB + xenon groups (30 ± 11 *vs.* 44 ± 7 s, $P < 0.05$).

Histology

No differences were found among groups with respect to total number of normal or dead neurons in CA1–2 and CA3 regions of the hippocampus. Although the mean total number of dead neurons in the dentate gyrus was highest in the CPB group, only the sham group reached statistical significance when compared with the CPB group (8 ± 3 sham *vs.* 17 ± 6 cells CPB, $P < 0.05$; table 4).

Discussion

Cerebral injury, representing a spectrum from subtle neurocognitive dysfunction to overt stroke, continues to complicate present-day cardiac surgery. Stroke, the incidence of which varies considerably depending upon

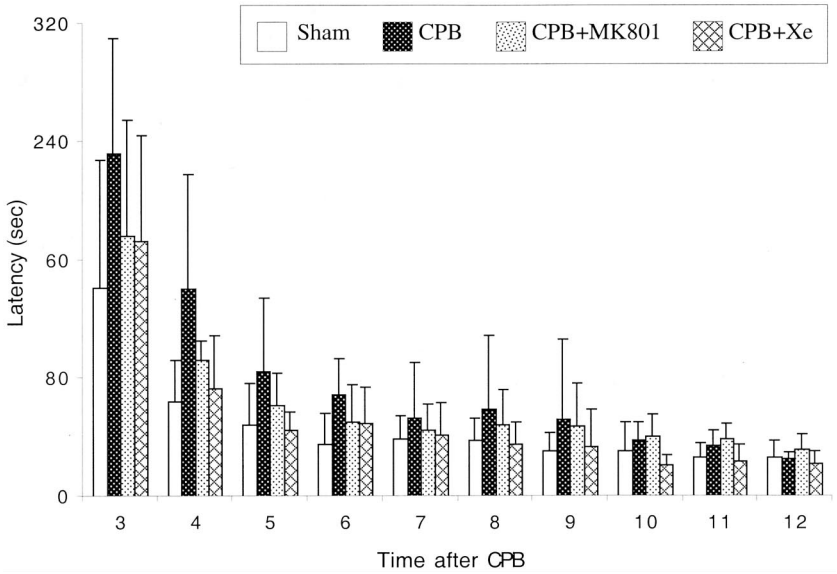

Fig. 4. Neurocognitive outcome as evaluated daily from postexperimental days 3–12 after cardiopulmonary bypass (CPB) by visuospatial learning with the Morris water maze. The results are the sum of four latencies, which are the time for animals to find the platform based on four trials of each day (mean ± SD, n = 10). The analysis of variance shows that the sham, CPB + MK801, and CPB + xenon groups have a significant statistically difference when compared with CPB group, respectively (CPB *vs.* sham: $F = 18.2$, $P < 0.0001$; CPB *vs.* CPB + MK801: $F = 20.7$, $P < 0.0001$; CPB *vs.* CPB + xenon: $F = 21.6$, $P < 0.0001$). Multiple comparisons measured with the Student-Newman-Keuls test, followed by analysis of variance, show a significant difference when compared with the CPB group at 3 and 4 days after postoperative day ($P < 0.001$).

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

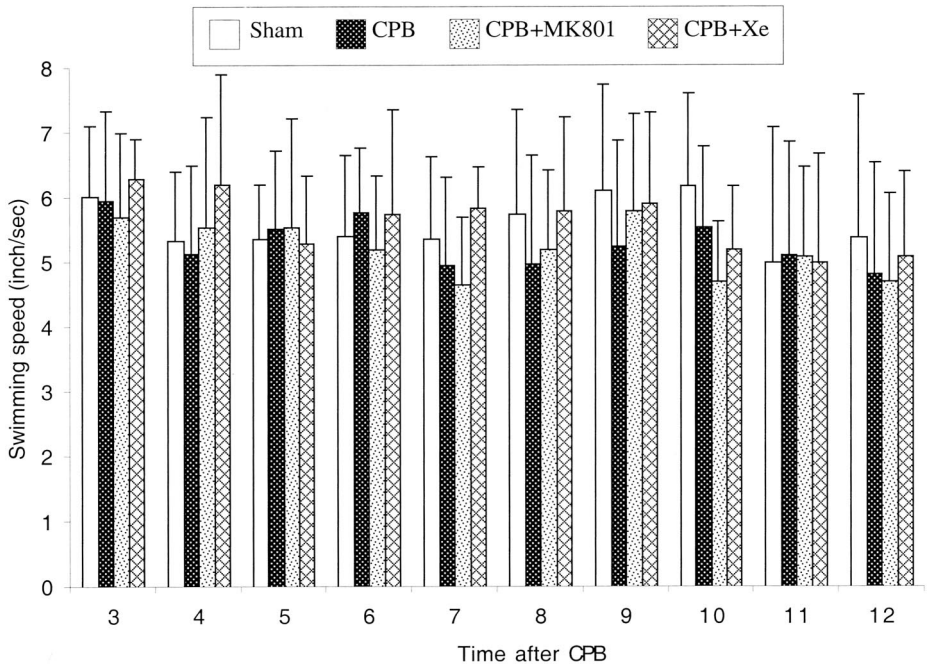

Fig. 5. Swimming speed in the Morris water maze as evaluated daily from postexperimental days 3–12 after cardiopulmonary bypass (CPB). Results are mean ± SD (n = 10). No differences were found among groups on the same day.

both patient and surgical factors, occurs in approximately 2–5% of patients.[13,14] Far more common than stroke, however, is neurocognitive dysfunction, which similarly varies due to patient factors, the sensitivity of the neurocognitive assessment battery, and importantly, the timing of the neurocognitive interrogation. In the early postoperative period after cardiac surgery, the incidence is as high as 80–90%; thereafter, it decreases over the ensuing months to approximately 30–40% at 3 months and approximately 15–25% at 1 yr.[13,14] However, recent longitudinal studies have demonstrated that up to 5 yr after cardiac surgery, the incidence of cognitive decline increases again to over 40%.[15] Although the disability incurred by stroke is easier to quantify and its presentation is often more dramatic, the impact of neurocognitive dysfunction both on patient quality of life,[16] as well as healthcare resource utilization,[17] is substantial.

Because of the magnitude of this clinical problem, serious consideration needs to be directed toward the understanding of its etiology and the development of neuroprotective strategies. To date, few preventive strategies aimed at reducing the cerebral complications of cardiac surgery have been sufficiently efficacious to be commonly accepted into clinical practice. Specifically, there are no approved (by regulatory agencies such as the Food and Drug Administration) pharmacologic

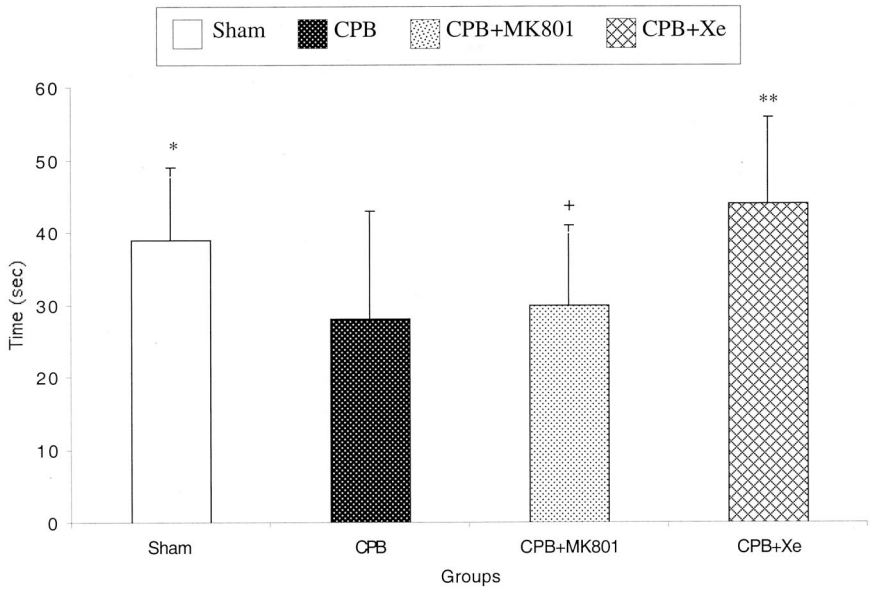

Fig. 6. On 12th day of postexperiment, immediately after the final escape trial, each animal was subjected to a probe trial (with a 60-s cutoff), in which there was no platform present. The time spent in the quadrant of the former platform position was obtained as a measure for spatial memory. The animals in the sham and CPB + xenon groups had longer probe trial times, indicating superior spatial memory function. The results are mean ± SD (n = 10). *$P < 0.05$, **$P < 0.01$ when compared with the values in CPB group. +$P < 0.05$ when compared with CPB + xenon group.

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

Table 4. Histology Results

	Normal Neurons			Dead Neurons		
	CA1–2	CA3	DG	CA1–2	CA3	DG
Sham	563 ± 99	264 ± 60	1163 ± 187	10 ± 6	6 ± 2	8 ± 3*
CPB	593 ± 73	282 ± 16	1285 ± 173	12 ± 4	7 ± 2	17 ± 6
CPB + MK801	599 ± 59	270 ± 32	1105 ± 198	12 ± 4	8 ± 1	13 ± 4
CPB + xenon	542 ± 50	254 ± 47	1165 ± 162	9 ± 3	6 ± 2	11 ± 3

Values are mean (SD). Cell counts (normal and dead neurons) in the hippocampal segments of CA1–2 and CA3 and also dentate gyrus (DG).

* $P < 0.05$ *vs.* CPB.

CPB, cardiopulmonary bypass.

agents for the treatment or prevention of neurocognitive dysfunction following cardiac surgery—this despite nearly two dozen pharmacologic agents having been studied in this setting. To date, one of the largest studies (albeit having enrolled less than 200 patients) of pharmacologic neuroprotection in cardiac surgery demonstrating positive results was an investigation of a noncompetitive NMDA receptor antagonist, remacemide.[18] Accepting that there are likely multiple pathways that culminate in cerebral injury in the setting of CPB, this positive trial identified a potentially important pathway in neuroprotection in cardiac surgery: antagonism of the NMDA receptor. Although there are most likely numerous reasons (including many not pertaining to efficacy) why this particular drug has not been further developed for this indication, the NMDA receptor is a promising target to pursue.

Xenon, an inert noble gas in anesthetic use for over 50 yr, also has NMDA receptor antagonist effects.[2,3,19] It is regarded as a reasonably safe anesthetic agent (similar to most other current anesthetic gases) with minimal cardiorespiratory and renal effects.[20,21] Major obstacles to its widespread clinical application are its scarcity (representing no more than 8.75×10^{-6}% of the atmosphere) and, as a consequence, the cost associated with its extraction. Therefore, it is anticipated that xenon's use will be confined to those settings in which cost–benefit analysis justifies its application.

Previously, several NMDA receptor antagonists have shown remarkable efficacy against neurologic injury in preclinical models of cerebral injury but have failed to live up to their promise when subsequently investigated in clinical settings.[22,23] In some instances, this is because of unfavorable pharmacologic properties preventing rapid transfer of the NMDA antagonist across the blood–brain barrier. In addition, other NMDA antagonists, such as MK801, have exhibited some central nervous system neurotoxicity that exceeds its putative beneficial properties.[24] Our data show that although MK801 did attenuate CPB-induced functional neurologic deterioration in a qualitatively similar fashion to xenon, it was quantitatively less efficacious (fig. 6). However, one of the limitations in making comparisons between MK801 and xenon is that their relative ED_{50}s are not known. It is entirely possible that the dose of MK801 used did not have equal potency as that of xenon, thereby explaining its differing efficacy. However, its dose was chosen based on a balance of visible neurobehavioral side effects while still maintaining it in a dose range that has been reported to be neuroprotective.[8]

One of the major limitations in developing neuroprotective strategies, specifically pharmacologic agents, in the cardiac surgery setting has been the lack of a reproducible preclinical model of cerebral dysfunction following cardiac surgery. Such a model could serve both to identify important targets and to screen protective compounds. With the development of our recently described rat model of CPB-associated neurocognitive dysfunction,[5,25] this study further highlights the utility of developing smaller animal models of CPB with which to investigate this condition. The fact that this model can be manipulated by pharmacologic intervention, as described here, further improves its validity.

There are, however, several limitations to our study. Whereas we did find a functional improvement in neurocognitive performance (defined by the temporal changes in the Morris water maze latency), we were not able to find a corresponding morphologic correlate. Reasons for this discrepancy include the possibility that the study was "underpowered" to elicit the changes and, second, that the changes are more subtle than can be elicited by our histologic criteria of cell necrosis. It is possible that the morphologic correlate of the CPB-induced changes in function are reflected by alterations in neuronal plasticity, which we are exploring further. In addition, our investigative group,[26] as well as others,[27] have identified changes in cerebral gene expression, both inflammatory and apoptotic. These changes could affect cerebral function without leading to necrotic changes.

A further limitation of our study involves the relevance of the small animal model to the clinical condition. For example, we did not use a sternotomy, which has some distinct benefits in this model, including the ability for long-term recovery of these animals. However, sternotomy itself may be a factor in inducing neurologic injury during cardiac surgery by contributing to the debris present in the shed blood that is returned to the venous

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

reservoir that likely adds to the cerebral embolic load.[28] Due to the size of the animal model, there are certain considerations with respect to the proportion of blood-foreign surface interaction compared to body weight. The rat CPB circuit volume is approximately 40 ml, with a surface area in the oxygenator of 0.3 m^2. This is a higher proportion of exposure to foreign surface than is seen clinically in humans, where the circuit volume is approximately 1.5–2 l with an oxygen surface area of 3.0 m^2. Despite this, a number of factors suspected to induce neurologic injury during cardiac surgery, including the inflammatory response,[28] are replicated by this model. Lastly, the sham-operated group was not exposed to donor rat blood, and it is theoretically possible that some of the deficits experienced by the CPB group may have been do to blood transfusion and not CPB itself. The current technologic limitations of rat CPB preclude performing CPB without additional donor blood, but it is hoped that with the development of even smaller circuits, this problem will be overcome.

Xenon rapidly penetrates the blood–brain barrier, appears devoid of intrinsic neurotoxicity,[4] and may represent an ideal neuroprotectant for preemptive use in clinical settings, in which NMDA receptor activation may be central in the pathogenesis of injury. We are currently embarking on clinical trials to investigate whether neurocognitive deficits after cardiac surgery can be prevented by administering xenon during the peri-CPB period.

The authors thank Betty Smith (Research Technician, Duke University Multidisciplinary Neuroprotection Laboratories) for her expert technical assistance. The authors also thank John Dingley, M.B., Ch.B. (Consultant Anesthetist, Department of Anaesthetics, Morriston Hospital, Swansea, United Kingdom), for his design efforts on the closed-circuit gas delivery system.

References

1. Cullen SC, Eger EI II, Cullen BF, Gregory P: Observations on the anesthetic effect of the combination of xenon and halothane. ANESTHESIOLOGY 1969; 31:305–9
2. Franks NP, Dickinson R, de Sousa SLM, Hall AC, Lieb WR: How does xenon produce anaesthesia? (letter). Nature 1998; 396:324
3. de Sousa SLM, Dickinson R, Lieb WR, Franks NP: Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. ANESTHESIOLOGY 2000; 92:1055–66
4. Wilhelm S, Ma D, Maze M, Franks NP: Effects of xenon on *in vitro* and *in vivo* models of neuronal injury. ANESTHESIOLOGY 2002; 96:1485–91
5. Mackensen GB, Sato Y, Nellgard B, Pinedia J, Newman MF, Warner DS, Grocott HP: Cardiopulmonary bypass induces neurologic and neurocognitive dysfunction in the rat. ANESTHESIOLOGY 2001; 95:1485–91
6. Guide for the Care and Use of Laboratory Animals. Health and Human Services, National Institutes of Health publication No. 86-23, revised 1996
7. Li SG, Randall DC, Brown DR: Roles of cardiac output and peripheral resistance in mediating blood pressure response to stress in rats. Am J Physiol 1998; 274:R1065–9
8. Rejdak K, Rejdak R, Sieklucka-Dziuba S, Stelmasiak Z, Grieb P: The effects of citicoline and/or MK801 on survival, neurological and behavioral outcome of mice exposed to transient hyperglycemia and oligemic hypoxia. Eur Neuropsychopharmacol 2001; 11:333–41
9. Combs D, D'Alecy L: Motor performance in rats exposed to severe forebrain ischemia: Effect of fasting and 1,3-butanediol. Stroke 1987; 18:503–11
10. Gionet T, Thomas J, Warner D, Goodlett C, Wasserman E, West J: Forebrain ischemia induces selective behavioral impairments associated with hippocampal injury in rats. Stroke 1991; 22:1040–7
11. Morris R: Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984; 11:47–60
12. Block F, Schwarz M: Correlation between hippocampal neuronal damage and spatial learning deficit due to global ischemia. Pharmacol Biochem Behav 1997; 56:755–61
13. Nussmeier N: Adverse neurologic events: Risks of intracardiac versus extracardiac surgery. J Cardiothorac Vasc Anesth 1996; 10:31–7
14. Borowicz L, Goldsborough M, Selenes O, McKhann G: Neuropsychological changes after cardiac surgery: A critical review. J Cardiothorac Vas Anesth 1996; 10:105–12
15. Newman MF, Kirchner JL, Phillips-Bute B, Gaver V, Grocott H, Jones RH, Mark DB, Reves JG, Blumenthal JA: Longitudinal assessment of neurocognitive function after coronary artery bypass. N Engl J Med 2001; 344:395–402
16. Newman MF, Grocott HP, Mathew JP, White WD, Landolfo K, Reves JG, Laskowitz JP, Mark DB, Blumenthal JA: Report of the substudy assessing the impact of neurocognitive function on quality of life 5 years after cardiac surgery. Stroke 2001; 32:2874–81
17. Roach G, Kanchuger M, Mora Mangano C, Newman MF: Adverse cerebral outcome after coronary bypass surgery. N Engl J Med 1996; 335:1857–63
18. Arrowsmith JE, Harrison MJG, Newman SP, Stygall J, Timberlake N, Pugsley WB: Neuroprotection of the brain during cardiopulmonary bypass: A randomised trial of remacemide during coronary artery bypass in 177 patients. Stroke 1998; 29:2357–62
19. Lynch C III, Baum J, Tenbrinck R: Xenon anesthesia. ANESTHESIOLOGY 2000; 92:865–8
20. Luttropp HH, Romner B, Perhag L, Eskilsson J, Fredriksen S, Werner O: Left ventricular performance and cerebral haemodynamics during xenon anaesthesia: A transoesphageal echocardiography and transcranial Doppler sonography study. Anaesthesia 1993; 48:1045–9
21. Hettrick DA, Pagel PS, Kersten JR, Tessmer JP, Bosnjak ZJ, Georgieff M, Warltier DC: Cardiovascular effects of xenon in isoflurane-anesthetized dogs with dilated cardiomyopathy. ANESTHESIOLOGY 1998; 89:1166–73
22. Dingledine R, Borges K, Bowie D, Traynelis SF: The glutamate receptor ion channels. Pharmacol Rev 1999; 51:7–61
23. Muir KW, Lees KR: Clinical experience with excitatory amino acid antagonist drugs. Stroke 1995; 26:503–13
24. Olney JW, Labruyere J, Price MT: Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 1989; 244: 1360–2
25. Grocott H, Mackensen G, Newman M, Warner D: Neurological injury during cardiopulmonary bypass in the rat. Perfusion 2001; 16:75–81
26. Sato Y, Laskowitz D, Warner D, Newman M, Grocott H: Cardiopulmonary bypass induces cerebral apoptotic gene expression in the rat. Anesth Analg 2001; 92:S38
27. Hindman BJ, Moore SA, Cutkomp J, Smith T, Ross-Barta SE, Dexter F, Brian JE Jr: Brain expression of inducible cyclooxygenase 2 messenger RNA in rats undergoing cardiopulmonary bypass. ANESTHESIOLOGY 2001; 95:1380–8
28. Brooker RF, Brown WR, Moody DM, Hammon JW, JR, Reboussin DM, Deal DD, Ghaziy HS, Stump DA: Cardiotomy suction: A major source of brain lipid emboli during cardiopulmonary bypass. Ann Thorac Surg 1998; 65:1651–5

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/98/3/690/337037/0000542-20030300-00017.pdf by guest on 20 March 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.