Anesthesiology 2002; 96:1485–91

Effects of Xenon on In Vitro *and* In Vivo *Models of Neuronal Injury*

Stefan Wilhelm, M.D., Daqing Ma, M.D., Ph.D.,* Mervyn Maze, M.B., Ch.B., F.R.C.P., F.R.C.A.,† Nicholas P. Franks, Ph.D.‡*

Background: **Xenon, the "inert" gaseous anesthetic, is an antagonist at the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor. Because of the pivotal role that NMDA receptors play in neuronal injury, the authors investigated the efficacy of xenon as a neuroprotectant in both *in vitro* and *in vivo* paradigms.**

Methods: **In a mouse neuronal–glial cell coculture, injury was provoked either by NMDA, glutamate, or oxygen deprivation and assessed by the release of lactate dehydrogenase into the culture medium. Increasing concentrations of either xenon or nitrogen (10–75% of an atmosphere) were coadministered and maintained until injury was assessed. In separate *in vivo* experiments, rats were administered *N*-methyl-DL-aspartate and killed 3 h later. Injury was quantified by histologic assessment of neuronal degeneration in the arcuate nucleus of the hypothalamus.**

Results: **Xenon exerted a concentration-dependent protection against neuronal injury provoked by NMDA (IC_{50} = 19 ± 6% atm), glutamate (IC_{50} = 28 ± 8% atm), and oxygen deprivation (IC_{50} = 10 ± 4% atm). Xenon (60% atm) reduced lactate dehydrogenase release to baseline concentrations with oxygen deprivation, whereas xenon (75% atm) reduced lactate dehydrogenase release by 80% with either NMDA- or glutamate-induced injury. In an *in vivo* brain injury model in rats, xenon exerted a concentration-dependent protective effect (IC_{50} = 78 ± 8% atm) and reduced the injury by 45% at the highest xenon concentration tested (75% atm).**

Conclusions: **Xenon, when coadministered with the injurious agent, exerts a concentration-dependent neuroprotective effect at concentrations below which anesthesia is produced in rodents. Unlike either nitrous oxide or ketamine (other anesthetics with NMDA antagonist properties), xenon is devoid of both neurotoxicity and clinically significant adverse hemodynamic properties. Studies are proposed to determine whether xenon can be used as a neuroprotectant in certain clinical settings.**

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

* Postdoctoral Research Fellow, Chelsea & Westminster Hospital. † Sir Ivan Magill Professor of Anaesthetics, ‡ Professor of Biophysics and Anaesthetics, Chelsea & Westminster Hospital, and the Biophysics Group, The Blackett Laboratory, Imperial College of Science, Technology and Medicine.

Received from the Magill Department of Anaesthesia, Chelsea & Westminster Hospital, and the Biophysics Group, The Blackett Laboratory, Imperial College of Science, Technology and Medicine, London, United Kingdom. Submitted for publication October 3, 2001. Accepted for publication February 6, 2002. Supported by grants from the Medical Research Council, London, United Kingdom, the Chelsea and Westminster NHS Healthcare Trust, London, United Kingdom, and a research stipend from the Deutsche Forschungsgemeinschaft, Bonn, Germany (to Dr. Wilhelm). Drs. Franks and Maze are both board members and paid consultants of an Imperial College spin-out company (Protexeon Ltd.), which hopes to develop clinical applications for xenon. The work described in this article was not supported by Protexeon, although some work in the authors' laboratories is now being funded by this company.

Address reprint requests to Dr. Franks: Biophysics Group, The Blackett Laboratory, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BZ, United Kingdom. Address electronic mail to: n.franks@ic.ac.uk or m.maze@ic.ac.uk. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

IN marked contrast to most general anesthetic agents,[1,2] xenon, at surgically relevant concentrations, produces little or no potentiation at γ-aminobutyric acid synapses[3,4] or on γ-aminobutyric acid type A receptors in *in vitro* expression systems.[5,6] Rather, is an effective inhibitor of glutamatergic *N*-methyl-D-aspartate (NMDA) receptors, yet an apparently weak inhibitor of the non-NMDA subtypes of glutamate receptors.[3,4]

The NMDA subtype of the glutamate receptor is a target for many putative neuroprotective agents because its activation is required to sustain ongoing neuronal injury and death.[7] Notwithstanding their putative beneficial effect in animal models, the development of several NMDA receptor antagonists has had to be abandoned because clinical trials revealed that they possessed their own inherent neurotoxicity (characterized by a specific lesion in the retrosplenial cortex) or because of their failure to reach the site of injury.[8] Previous studies revealed that xenon, uniquely among anesthetics with NMDA receptor antagonist properties, does not appear to produce neurotoxicity in the retrosplenial cortex; rather, xenon protects against the neurotoxic injury produced by ketamine.[9] Furthermore, xenon readily partitions across the blood–brain barrier and can be expected to access the site of central nervous system injury rapidly.

In a series of *in vitro* and *in vivo* models, we determined whether xenon protects against neuronal injury. The results demonstrate that xenon exerts potent neuroprotection at subanesthetic concentrations in each of the models examined.

Materials and Methods

This study conformed to the United Kingdom Animals (Scientific Procedures) Act of 1986.

In Vitro *Experiments*

Cell Culture. Mixed cortical cell cultures, containing both neuronal and glial elements, were prepared using the methods described previously[10,11] with minor modifications. Briefly, whole cerebral neocortices (devoid of the hippocampal formation and basal ganglia) were removed from fetal BALB/c mice at 14–16 days' gestation. After trypsination and resuspension, cells were plated on a confluent monolayer of astrocytes at a density of 1–1.25 × 10^5 cells/cm^2 on 24-multiwell plates (Costar, Cambridge, MA). Cortical glial cell cultures were prepared from early postnatal BALB/c mice (postnatal

days 1–3) and reached confluence within approximately 1 week after plating. The mixed glial–neuronal cocultures were used at 15 ± 1 days.

Exposure to Excitatory Amino Acids. For the experiments in which either NMDA or glutamate was exogenously applied, the culture medium had been carefully replaced by HEPES buffer (120 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 15 mM glucose, and 20 mM HEPES, titrated to pH 7.4 using 1 M NaOH). Agonist exposure was generally performed at room temperature in room air (22 ± 2°C). After 10-min exposure, NMDA or glutamate was washed out, and culture medium free of glutamate and lactate dehydrogenase (LDH; Eagle minimum essential medium augmented with 25 mM glucose and 38 mM bicarbonate) was maintained until the end of the experiment.

Preparation of Xenon Solutions. Solutions for the xenon experiments were prepared by first bubbling pure gases (oxygen, nitrogen, or xenon) through fine sintered glass bubblers in 250- or 500-ml Drechsel bottles filled with the above-described HEPES buffer. Solutions were bubbled for 20 min (in preliminary experiments, the xenon concentration was monitored using gas chromatography to confirm that this time was sufficient for equilibration). To minimize oxidation, the excitatory amino acids were absent in the fully oxygenated buffer but present at the appropriate concentrations in the xenon and nitrogen solutions. During bubbling, the solutions were continually stirred at room temperature. As a final step, these solutions were mixed to achieve the desired final concentrations of the respective gases. Immediately before the experiment, NMDA or glutamate was added to 15 ml of the gas-containing solutions and carefully mixed to obtain the desired NMDA and glutamate concentrations. Preliminary experiments using a dye indicated that good mixing was achieved.

Gas Exposure Chambers. To effect continuous delivery of xenon, a purpose-built, airtight, temperature-controlled cell-culture chamber, equipped with inlet and outlet valves and an internal electric fan, was constructed. When connected to calibrated flowmeters for xenon, oxygen, nitrogen, and carbon dioxide, the desired gas composition could be obtained. In all experiments, 20% oxygen and 5% carbon dioxide were used, with the remainder being xenon or nitrogen in various proportions. Total gas flow was 100 ml/min, and the chamber was flushed for 30 min before establishing a closed system. With this protocol, the final gas concentrations (determined using gas chromatography) were found to be stable, and there was no measurable leakage over 24 h.

Oxygen Deprivation. Oxygen deprivation was performed as described previously[12] with minor modifications. Briefly, the culture medium was replaced with prewarmed (37°C) deoxygenated balanced salt solution, pH 7.4 (NaCl 116 mM, KCl 5.4 mM, $MgSO_4$ 0.8 mM, NaH_2PO_4 1.0 mM, $CaCl_2$ 1.8 mM, $NaHCO_3$ 26 mM, glucose 5 mM, pH adjustment with 2 M HCl). Immediately after replacement of the medium, the cell cultures were transferred into the gas exposure chamber (containing 95% nitrogen, 5% carbon dioxide) for 75 min at 37°C. Oxygen deprivation was terminated by exchanging oxygenated minimum essential medium before the cultures were returned to a normoxic incubator (95% air, 5% carbon dioxide,) or into the xenon-containing gas exposure chamber (20% oxygen, 5% carbon dioxide, with the remainder being chosen concentrations of xenon and nitrogen) for the subsequent 24 h at 37°C.

Assessment of Neuronal Injury. The neuronal injury was quantified by the amount of LDH released into the medium over the appropriate time period. As previously reported,[13] this convenient and quantitative index correlated with morphologic estimate and was used as the index of injury. LDH activity in each sample was determined in triplicate by spectrophotometric measurements (Shimadzu UV 1201 photometer; Milton Keynes, United Kingdom) at room temperature using a standardized colorimetric enzyme kit (Sigma 500 C; Sigma-Aldrich, Poole, United Kingdom). The amount of LDH released by sham-washed controls was subtracted to give the LDH signal specific to the injury, neuronal protection was expressed as a percentage of the maximal LDH released, and the IC_{50} concentration was defined as the xenon concentration that reduced the release to 50% of the control value.

In Vivo *Experiments*

Four groups of female Sprague-Dawley rats (weight, 240–260 g) were treated as follows: group 1 (control, n = 6) received 0.8 ml/100 g saline subcutaneously; group 2 (n = 7) received 100 mg/kg *N*-methyl-DL-aspartate (NMA) subcutaneously; group 3 (n = 5) received 75% xenon in oxygen; group 4 was subdivided into four treatment subgroups and exposed to 20% xenon (n = 5), 40% xenon (n = 5), 60% xenon (n = 5), or 75% xenon (n = 8), with the remainder being nitrogen in various proportions and 25% oxygen for 15 min before subcutaneous injection of 100 mg/kg NMA. The dose of NMA was selected on the basis of a previous study,[15] and it produced no obvious behavioral effects. Similarly, xenon induced no behavioral effects, either on its own or after NMA injection, with the exception of a mild and short-lived (1–2 min) period of motor hyperexcitability that was observed at the highest concentration used (75% xenon).

During exposure to 20, 40, 60, and 75% xenon (as previously mentioned), the gas mixture was introduced into a chamber (International Market Supply, Cheshire, United Kingdom) using calibrated flowmeters. After a 3-min flush at a flow rate of 4 l/min, the flow rate was reduced to 40 ml/min for the remaining 3 h. The carbon dioxide level was kept less than 0.6% with soda lime, and

the humidity was kept between 40 and 60% using silica gel (Merck, Leicestershire, United Kingdom).

Histology. Three hours after treatment was initiated, animals were killed by an overdose of pentobarbital, perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer, and the whole brain was removed, sliced, and embedded in paraffin. Three sections (4 μm) cut midway between the rostral and caudal boundaries of the arcuate nucleus were stained with cresyl violet for light microscopic evaluation. To determine the amount of neuronal damage in the arcuate nucleus, degenerated neurons were identified by their pyknotic nuclei and edematous swelling of the surrounding cytoplasmic compartment, giving the appearance of a bull's eye. The sum of degenerated neurons of three representative sections was used.

To determine whether xenon induces toxicity, layers three and four of the retrosplenial cortex were examined for intracytoplasmic vacuolization using the criteria established by Fix *et al.*[14] The sum of vacuolated neurons of six snapshots (acquired by Zeiss AxioCam Digital Camera; Zeiss, Welwyn Garden City, United Kingdom) of the retrosplenial cortex from each of four rats was used.

Reagents and Solutions

Minimal essential medium, fetal bovine serum, horse serum, and murine epidermal growth factor were obtained from GibcoBRL, Life Technologies Ltd. (Paisley, United Kingdom). NMDA, NMA, glutamate, and the colorimetric kit for the determination of LDH were purchased from Sigma-Aldrich (Poole, United Kingdom). Unless otherwise stated, all other chemicals were obtained from Merck (Leicestershire, United Kingdom). Beside xenon, which was obtained from Air Products (Crewe, United Kingdom), all other gases were supplied by BOC Gases (Guildford, Surrey, United Kingdom).

Data Analysis

In vitro data were tested using analysis of variance with *post hoc t* test and correction for multiple comparisons using Bonferroni correction. For the *in vivo* data, statistical analysis was performed by analysis of variance followed by the Newman-Keuls test.

Results

In control experiments, brief (10 min) exposures of the cell cultures to either NMDA (fig. 1A) or glutamate (fig. 1B) resulted in a concentration-dependent increase in the LDH released into the culture medium. Microscopic examination of the cell cocultures showed widespread neuronal disintegration, whereas the morphology of the underlying astroglia bed appeared relatively unaffected (not shown). The NMDA- or glutamate-induced

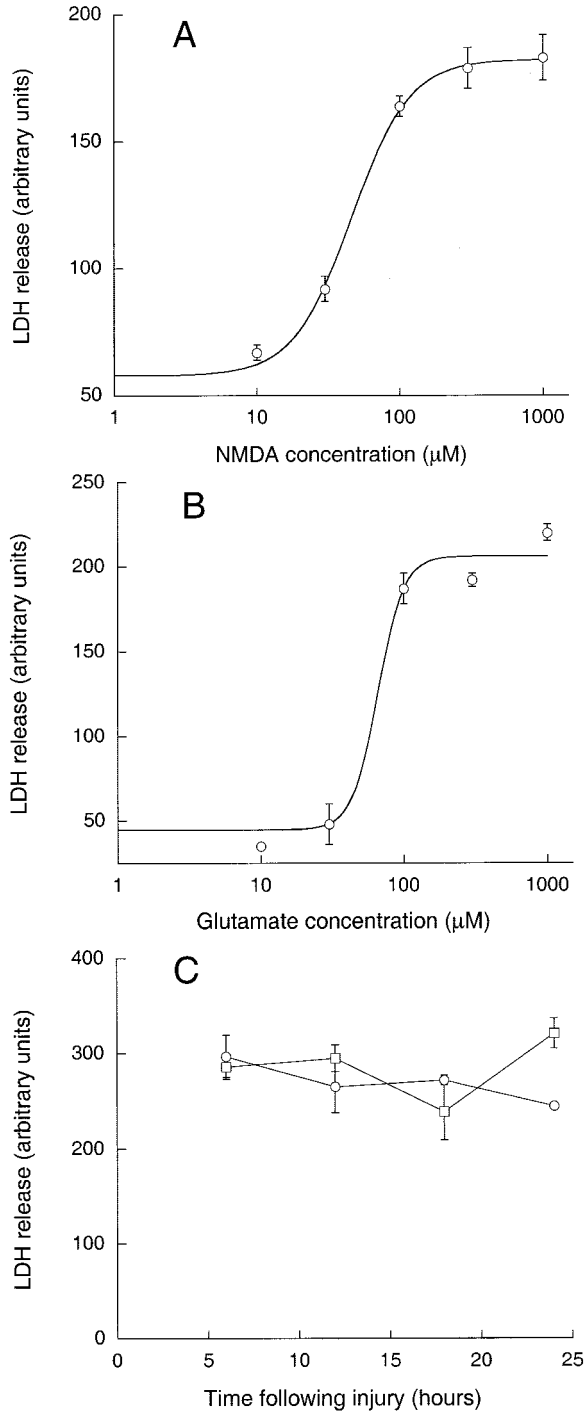

Fig. 1. Concentration–response relation for *N*-methyl-D-aspartate (NMDA) and glutamate neurotoxicity and time course for injury. Cortical neuronal cultures were exposed for 10 min to either NMDA (*A*) or glutamate (*B*), and injury was quantified by the amount of lactate dehydrogenase (LDH) released into the bathing medium after 24 h. The EC_{50} concentrations for NMDA- and glutamate-induced injury were 46 ± 8 and 66 ± 19 μM, respectively (mean ± SEM). (*C*) Neuronal damage provoked by 250 μM NMDA (squares) or 100 μM glutamate (circles) was essentially complete 6 h after injury and did not change greatly up to 24 h after injury. Baseline LDH release was typically about 50 units. Data are presented as mean ± SEM (n = 3).

LDH release was markedly larger than baseline release (*i.e.*, in the absence of added NMDA or glutamate) and saturated at NMDA and glutamate concentrations in excess of approximately 100 μM. The EC_{50} concentrations for NMDA- and glutamate-induced injury were 46 ± 8 and 66 ± 19 μM, respectively (mean ± SEM). LDH assays performed at times between 6 and 24 h (fig. 1C) showed a similar degree of injury.

To assess the possible neuroprotective effects of xenon, LDH assays were performed 6 h after brief exposures to either NMDA or glutamate (at, or close to, concentrations that induced maximal LDH release) in the presence of increasing concentrations of xenon. LDH release was significantly reduced at all concentrations tested (fig. 2), with xenon IC_{50} concentrations for neuroprotection being 19 ± 6% and 28 ± 8% for NMDA and glutamate-induced injury, respectively (mean ± SEM). Xenon at 75% atm had no significant effect ($P > 0.7$) on baseline LDH release (*i.e.*, in the absence of added NMDA or glutamate), indicating no intrinsic neurotoxicity of xenon alone. Xenon was also effective (IC_{50} = 10 ± 4% atm) in protecting against the injury caused by depriving the cell cultures of oxygen (fig. 2C) for 75 min.

We went on to investigate whether the neuroprotection that was evident *in vitro* could be observed *in vivo*. We used a well-established *in vivo* model of neurotoxicity that involved the subcutaneous injection of NMA and the subsequent neuronal degeneration that occurs in the arcuate nucleus of the hypothalamus.[15] No degenerated neurons were found in either control animals or in those tested with xenon alone. We confirmed that characteristic and reproducible neuronal degeneration occurs after NMA injection that is easily distinguishable from sham-injected animals (figs. 3A and B). Figure 3C shows that, in the presence of 75% xenon, the number of degenerated neurons (characterized by cytoplasmic swelling and pyknotic nuclei) is greatly reduced. We quantified the extent of neuroprotection by counting the number of degenerated neurons after the animals had been exposed to increasing concentrations of xenon. The number of degenerated neurons decreased with increasing xenon concentration, with an IC_{50} concentration of 78 ± 8% (mean ± SEM; fig. 3D).

We observed no significant change in the percentage of neurons in the retrosplenial cortex showing intracytoplasmic vacuolations in rats treated with 75% xenon (3.4 ± 0.7%; mean ± SD) when compared with rats administered normal saline (4.1 ± 1.1%; mean ± SD).

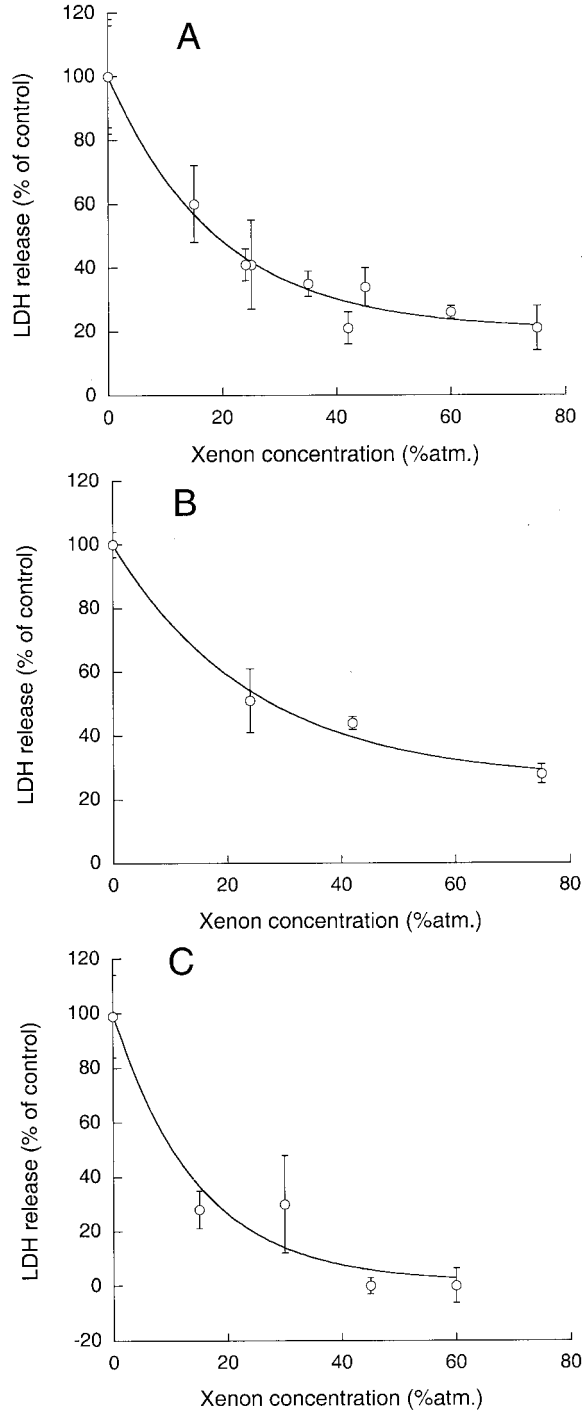

Fig. 2. Xenon exerts a concentration-dependent neuroprotective effect *in vitro*. Neuronal cultures were treated with either (*A*) 250 μM NMDA or (*B*) 100 μM glutamate for 10 min in the presence of increasing concentrations of xenon. The xenon IC_{50} concentrations for neuroprotection were 19 ± 6 and 28 ± 8% for NMDA and glutamate-induced injury, respectively (mean ± SEM). (*C*) Xenon was also neuroprotective in cultures deprived of oxygen (circles) for 75 min before rescue with medium containing 20% oxygen. In all cases, neuronal injury is expressed as a percentage of the maximum lactate dehydrogenase (LDH) release after 6 h determined in the absence of xenon. The points represent mean ± SEM (n = 3).

Discussion

Using a primary culture of neuronal and glial cells from the cerebral cortex of neonatal mice, predictable neuronal injury (as reflected by the release of LDH into the culture medium) is produced by NMDA, glutamate, and

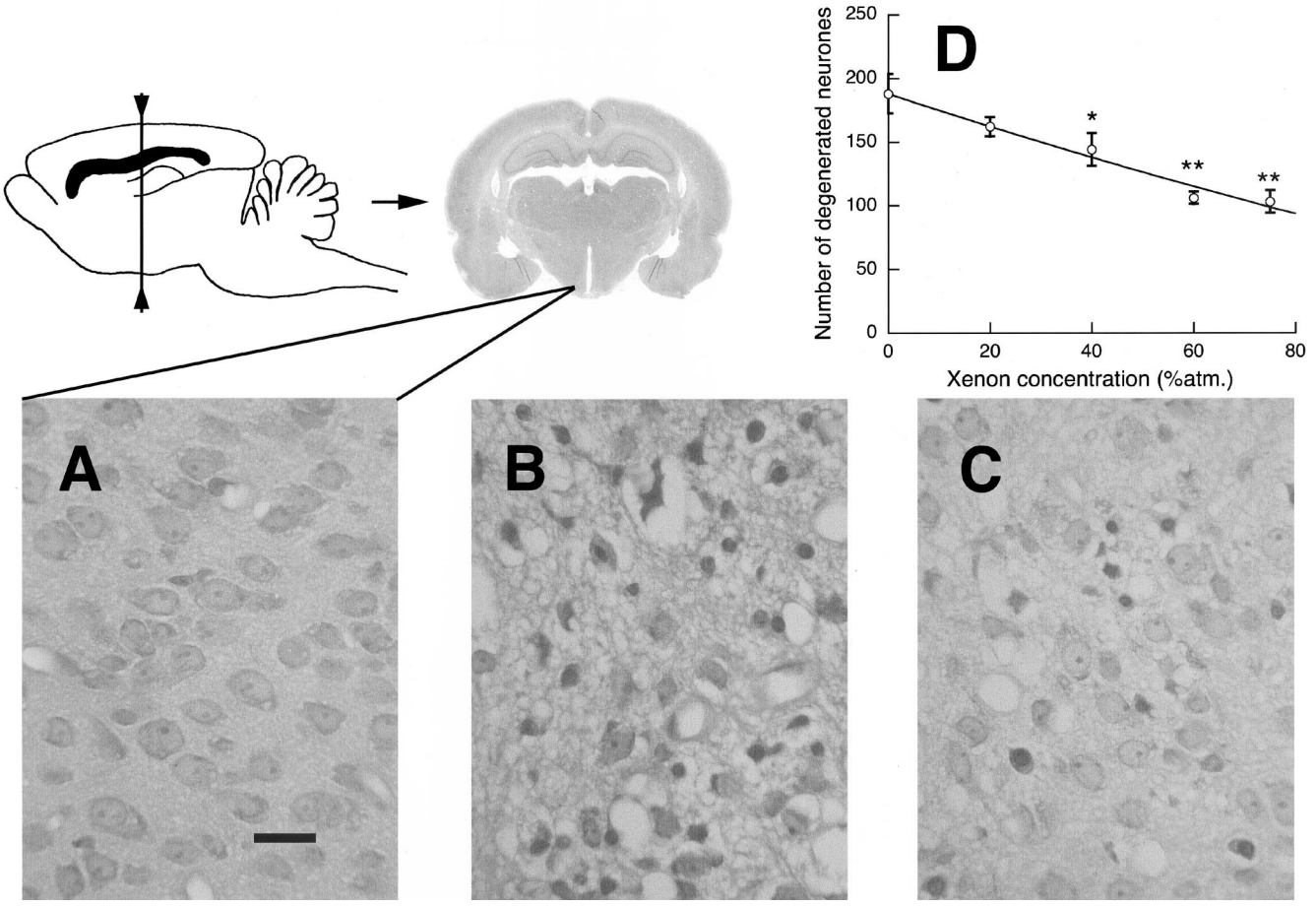

Fig. 3. Xenon exerts a concentration-dependent neuroprotective effect *in vivo*. Coronal sections containing the arcuate nucleus of the hypothalamus were stained with cresyl violet and visualized at a magnification of 200 times. A control section is shown (*A*) where the scale bar represents 20 μM. Subcutaneous injection of 100 mg/kg *N*-methyl-DL-aspartate induced a characteristic neurodegeneration in the arcuate nucleus of the hypothalamus (*B*), and this was greatly reduced in the presence of 75% xenon (*C*). The neuroprotective effect of xenon was concentration-dependent (*D*) and was quantified by counting the number of degenerated neurons in both arcuate nuclei over a range of xenon concentrations. At greater than 40% xenon, the number of degenerated neurons was significantly reduced at the 5% level (*) and at and above 60% xenon, protection was significant at the 1% level (**). The points represent mean ± SEM (n = 5–8).

oxygen deprivation, as reported previously (fig. 1).[10–12,16] Xenon, in a concentration-dependent manner, reduced neuronal injury produced by each of NMDA (fig. 2A), glutamate (fig. 2B), and oxygen deprivation (fig. 2C). Neuronal protection was complete for the injury produced by oxygen-deprivation studies and up to 80% complete for the injury produced by each of NMDA and glutamate. In a validated *in vivo* model of brain injury in rats,[15] xenon, in a concentration-dependent manner, reduced neuronal degeneration in the arcuate nucleus of the hypothalamus provoked by NMA (fig. 3). In each of the *in vitro* and *in vivo* paradigms, xenon exerted its neuroprotective effect at subanesthetic concentrations.[17,18] This might be clinically relevant because other anesthetics with known neuroprotective properties, such as propofol or pentobarbital, need to be administered at far higher concentrations to mitigate oxidative stress in primary astrocyte cultures[19] or to protect against ischemic brain injury in animal models.[20,21]

Xenon has been in anesthetic use for more than 50 years and has been demonstrated to have a remarkably safe clinical profile.[18] It is regarded as an anesthetic agent with minimal cardiorespiratory and renal effects.[22,23] A major obstacle to its widespread clinical application is its scarcity (representing no more than 0.00000875% of the atmosphere) and, as a consequence, the cost associated with its extraction. Therefore, it is anticipated that the use of xenon as a clinical anesthetic will be confined to settings in which cost–benefit analysis justifies its application.

In each of the experimental paradigms, xenon was administered before the injury was provoked; therefore, if they are able to be extrapolated to clinical settings, these data should be considered analogous to preemptive therapy in anticipation of a forthcoming neurologic insult (such as cardiopulmonary bypass or surgery for intracranial arteriovenous malformations or neoplasms). Although each of the models that were used has been

validated in previous studies,[10,12,15] it must be recognized that these models may not necessarily be the neuropathologic correlates of disease processes involved in ischemia or trauma. It is also possible that the neuroprotective effect of xenon may not necessarily be caused by its well-defined antagonism[3,4] of the NMDA receptor; *e.g.*, it is theoretically possible that the *in vivo* neuroprotective effect may be caused by a decrease in brain temperature that was not monitored.

Previously, several NMDA receptor antagonists have shown remarkable efficacy against neurologic injury in preclinical models but failed to live up to their promise when subsequently investigated in clinical settings.[7,24] There are several possible reasons for the ineffective clinical translation of NMDA receptor antagonists. First, many of the NMDA receptor antagonists do not readily penetrate the blood–brain barrier, requiring large doses to be administered systemically; this, in turn, can produce systemic toxicity.[24] Others have been shown to block certain serotonin receptors and calcium channels in addition to NMDA receptors, which limits their clinical usefulness.[25] It is notable that xenon, a small uncharged atom, readily crosses the blood–brain barrier, producing a rapid onset of central nervous system effects in humans within the first minute of administration. Even for NMDA antagonists that do traverse the blood–brain barrier, central nervous system neurotoxicity may evolve.[24] Xenon is devoid of intrinsic neurotoxicity, as evidenced by the lack of vacuolization in layers three and four of the retrosplenial cortex, a region particularly susceptible to injury by NMDA antagonists.[14] In fact, xenon itself may prevent the toxicity associated with other NMDA antagonists.[9] Of course, one cannot discount the possibility that the reason for the clinical failures of NMDA antagonists may be that blockade of this glutamate receptor subtype may be insufficient to protect against injury.

Apart from anesthetics with NMDA receptor antagonist properties, other anesthetics have also been tested for their ability to mitigate neuronal injury in various paradigms.[19–21,26–28] Recently, Kudo *et al.*[28] examined the neuroprotective effects of several volatile anesthetic agents in a similar *in vitro* model of NMDA excitotoxicity. Remarkably, in the clinical anesthetic range (*i.e.*, approximately 1 minimum alveolar concentration), the neuroprotective effect was only approximately 20%, which is more than fourfold lower than both their observation with MK-801 and our observations with xenon. However, it is notable that volatile anesthetics may exert a more potent effect *in vivo* than is seen *in vitro*,[27] possibly because of the additional neuroprotective effect that activation of γ-aminobutyric acid type A receptors may confer.[29] In an *in vivo* experimental paradigm, which is roughly comparable to that used by Harada *et al.*,[27] xenon does appear to be a more potent neuroprotectant (fig. 3) than isoflurane.

Xenon may represent a useful neuroprotectant for preemptive use in clinical settings, in which NMDA receptor activation is pivotal to the production of injury, because of its known lack of toxic side effects and the relative ease with which it can be delivered to the brain. We are now embarking on clinical trials to investigate whether cognitive deficits, which develop in patients undergoing cardiopulmonary bypass in the course of their cardiac surgery,[30,31] can be prevented by administering xenon as part of the anesthetic regimen.

The authors thank Vesna Jevtovic-Todorovic, M.D., Ph.D. (Department of Anesthesiology, Washington University, St. Louis, MO), for helpful advice on the histological procedures.

References

1. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 1994; 367:607–14
2. Krasowski MD, Harrison NL: General anaesthetic actions on ligand-gated ion channels. Cell Mol Life Sci 1999; 55:1278–303
3. de Sousa SL, Dickinson R, Lieb WR, Franks NP: Contrasting synaptic actions of the inhalational general anesthetics isoflurane and xenon. ANESTHESIOLOGY 2000; 92:1055–66
4. Franks NP, Dickinson R, de Sousa SLM, Hall AC, Lieb WR: How does xenon produce anaesthesia? (letter). Nature 1998; 396:324
5. Hapfelmeier G, Zieglgansberger W, Haseneder R, Schneck H, Kochs E: Nitrous oxide and xenon increase the efficacy of GABA at recombinant mammalian GABA(A) receptors. Anesth Analg 2000; 91:1542–9
6. Yamakura T, Harris RA: Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels: Comparison with isoflurane and ethanol. ANESTHESIOLOGY 2000; 93:1095–101
7. Dingledine R, Borges K, Bowie D, Traynelis SF: The glutamate receptor ion channels. Pharmacol Rev 1999; 51:7–61
8. Arrowsmith JE, Harrison MJ, Newman SP, Stygall J, Timberlake N, Pugsley WB: Neuroprotection of the brain during cardiopulmonary bypass: A randomized trial of remacemide during coronary artery bypass in 171 patients. Stroke 1998; 29:2357–62
9. Nagata A, Nakao Si S, Nishizawa N, Masuzawa M, Inada T, Murao K, Miyamoto E, Shingu K: Xenon inhibits but N(2)O enhances ketamine-induced c-Fos expression in the rat posterior cingulate and retrosplenial cortices. Anesth Analg 2001; 92:362–8
10. Choi DW, Maulucci-Gedde M, Kriegstein AR: Glutamate neurotoxicity in cortical cell culture. J Neurosci 1987; 7:357–68
11. Choi DW, Koh JY, Peters S: Pharmacology of glutamate neurotoxicity in cortical cell culture: Attenuation by NMDA antagonists. J Neurosci 1988; 8:185–96
12. Bruno VM, Goldberg MP, Dugan LL, Giffard RG, Choi DW: Neuroprotective effect of hypothermia in cortical cultures exposed to oxygen-glucose deprivation or excitatory amino acids. J Neurochem 1994; 63:1398–406
13. Koh JY, Choi DW: Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay. J Neurosci Methods 1987; 20:83–90
14. Fix AS, Horn JW, Wightman KA, Johnson CA, Long GG, Storts RW, Farber N, Wozniak DF, Olney JW: Neuronal vacuolization and necrosis induced by the noncompetitive N-methyl-D-aspartate (NMDA) antagonist MK(+)801 (dizocilpine maleate): A light and electron microscopic evaluation of the rat retrosplenial cortex. Exp Neurol 1993; 123:204–15
15. Jevtovic-Todorovic V, Todorovic SM, Mennerick S, Powell S, Dikranian K, Benshoff N, Zorumski CF, Olney JW: Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nat Med 1998; 4:460–3
16. Goldberg MP, Choi DW: Combined oxygen and glucose deprivation in cortical cell culture: Calcium-dependent and calcium-independent mechanisms of neuronal injury. J Neurosci 1993; 13:3510–24
17. 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
18. Lynch C 3rd, Baum J, Tenbrinck R: Xenon anesthesia. ANESTHESIOLOGY 2000; 92:865–8
19. Peters CE, Korcok J, Gelb AW, Wilson JX: Anesthetic concentrations of propofol protect against oxidative stress in primary astrocyte cultures: Comparison with hypothermia. ANESTHESIOLOGY 2001; 94:313–21
20. Pittman JE, Sheng H, Pearlstein R, Brinkhous A, Dexter F, Warner DS: Comparison of the effects of propofol and pentobarbital on neurologic outcome and cerebral infarct size after temporary focal ischemia in the rat. ANESTHESIOLOGY 1997; 87:1139–44
21. Yamaguchi S, Midorikawa Y, Okuda Y, Kitajima T: Propofol prevents

delayed neuronal death following transient forebrain ischemia in gerbils. Can J Anaesth 1999; 46:593–8

22. Luttropp HH, Romner B, Perhag L, Eskilsson J, Fredriksen S, Werner O: Left ventricular performance and cerebral haemodynamics during xenon anaesthesia: A transoesophageal echocardiography and transcranial Doppler sonography study. Anaesthesia 1993; 48:1045–9

23. 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

24. Muir KW, Lees KR: Clinical experience with excitatory amino acid antagonist drugs. Stroke 1995; 26:503–13

25. Lynch DR, Gallagher MJ: Inhibition of N-methyl-D-aspartate receptors by haloperidol: Developmental and pharmacological characterization in native and recombinant receptors. J Pharmacol Exp Ther 1996; 279:154–61

26. Miura Y, Grocott HP, Bart RD, Pearlstein RD, Dexter F, Warner DS: Differential effects of anesthetic agents on outcome from near-complete but not incomplete global ischemia in the rat. ANESTHESIOLOGY 1998; 89:391–400

27. Harada H, Kelly PJ, Cole DJ, Drummond JC, Patel PM: Isoflurane reduces N-methyl-D-aspartate toxicity in vivo in the rat cerebral cortex. Anesth Analg 1999; 89:1442–7

28. Kudo M, Aono M, Lee Y, Massey G, Pearlstein RD, Warner DS: Effects of volatile anesthetics on N-methyl-D-aspartate excitotoxicity in primary rat neuronal-glial cultures. ANESTHESIOLOGY 2001; 95:756–65

29. Schwartz-Bloom RD, Miller KA, Evenson DA, Crain BJ, Nadler JV: Benzodiazepines protect hippocampal neurons from degeneration after transient cerebral ischemia: An ultrastructural study. Neuroscience 2000; 98:471–84

30. Brillman J: Central nervous system complications in coronary artery bypass graft surgery. Neurol Clin 1993; 11:475–95

31. Sotaniemi KA: Long-term neurologic outcome after cardiac operation. Ann Thorac Surg 1995; 59:1336–9