Neuroprotective effects of xenon: a therapeutic window of opportunity in rats subjected to transient cerebral ischemia

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ABSTRACT Brain insults are a major cause of acute mortality and chronic morbidity. Given the largely ineffective current therapeutic strategies, the development of new and efficient therapeutic interventions is clearly needed. A series of previous investigations has shown that the noble and anesthetic gas xenon, which has low-affinity antagonistic properties at the N-methyl-D-aspartate (NMDA) receptor, also exhibits potentially neuroprotective properties with no proven adverse side effects. Surprisingly and in contrast with most drugs that are being developed as therapeutic agents, the dose-response neuroprotective effect of xenon has been poorly studied, although this effect could be of major critical importance for its clinical development as a neuroprotectant. Here, we show, using ex vivo and in vivo models of excitotoxic insults and transient brain ischemia, that xenon, administered at subanesthetic doses, offers global neuroprotection from reduction of neurotransmitter release induced by ischemia, a critical event known to be involved in excitotoxicity, to reduction of subsequent cell injury and neuronal death. Maximal neuroprotection was obtained with xenon at 50 vol%, a concentration at which xenon further exhibited significant neuroprotective effects in vivo even when administered up to 4 h after intrastriatal NMDA injection and up to at least 2 h after induction of transient brain ischemia.—David, H. N., Haelewyn, B., Rouillon, C., Lecoq, M., Chazalviel, L., Apiou, G., Risso, J.-J., Lemaire, M., Abraini, J. H. Neuroprotective effects of xenon: therapeutic window of opportunity in rats subjected to transient cerebral ischemia. FASEB J. 22, 000–000 (2008)

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Brain insults that follow disruption of cerebral blood flow, such as ischemic stroke or perinatal hypoxia-ischemia, remain a major cause of acute mortality and chronic morbidity. Given the largely ineffective current therapeutic strategies as well as the magnitude of the problem, in terms of disability for the affected patients and subsequent financial cost for society, new and efficient therapeutic interventions are needed to minimize the severity of disability and long-term neurological disorders that may result from such critical life events.

Disruption in cerebral blood flow results in oxygen and glucose deprivation for the cell, a condition that leads to neuronal death through necrotic and apoptotic mechanisms (1). This process is thought to depend largely on overstimulation of the N-methyl-D-aspartate (NMDA) glutamatergic receptor (2) and is called excitotoxicity. However, despite the beneficial effect of blocking the NMDA receptor in animal models of stroke and other ischemic insults, the clinical development of several NMDA receptor antagonists has had to be abandoned because these compounds possess an intrinsic neurotoxicity (3–5) and further fail to reach the site of brain injury (6). Interestingly, the noble gas xenon may be a promising agent with effective neuroprotective properties and minimal adverse side effects and neurotoxicity when administered at concentrations up to 70–75 vol% (7). Indeed, xenon, which possesses anesthetic properties at ~70% of 1 atm in humans (8), exhibits a pharmacological profile resembling that of the low-affinity NMDA receptor antagonists (9, 10), a class of agents that produce noncompetitive antagonism at the NMDA glutamate receptor and the nicotinic acetylcholine receptor and possess few neurotoxic properties (11). In addition, xenon readily crosses the blood-brain barrier and has low blood/gas solubility, which is advantageous in terms of rapid inflow and washout (12), conditions that may favor treatment and reduce risk of adverse side effects. Neuroprotection by xenon was first addressed by showing that it reduces...
neuronal injury induced by intraperitoneal administration of ketamine or N-methyl-DL-aspartate (13–16) and further attenuates excitotoxic neuronal death in neuronal cell cultures (15, 17, 18) and in rats given intrastriatal injections of NMDA (7). Then, it was demonstrated in animal models of diseases that xenon further reduces cardiopulmonary bypass-induced neurocognitive and neurological dysfunctions (19) as well as neuronal death induced by focal ischemia and perinatal hypoxia-ischemia in rodents (20–24). We first demonstrated (20), as confirmed recently (22, 24), that xenon reduces neuronal death not only when given before or during the insult phase (intraischemic neuroprotection) but also when administered after the ischemic insult (postischemic neuroprotection). Surprisingly, and in contrast with most drugs that are being studied and developed as potentially therapeutic agents, the dose-response neuroprotective effect of xenon has been very poorly investigated, although this effect could be of critical importance for the clinical use of xenon and its development as a neuroprotectant (7, 20, 25–28).

Here, we investigated the dose-response neuroprotective effects of xenon on three different ex vivo and in vivo models of excitotoxic insult including neurotransmitter release and cell injury produced by oxygen and glucose deprivation (OGD) in brain slices and neuronal death induced by intrastriatal administration of NMDA in rats. We found that maximal neuroprotection against cell injury and neuronal death occurred at the subanesthetic concentration of 50 vol% xenon. Given this result, we further investigated the therapeutic window of opportunity after which treatment with 50 vol% xenon may be futile for treating ischemic insults in rats subjected to middle cerebral artery occlusion (MCAO), a clinically relevant model of brain ischemia.

**MATERIALS AND METHODS**

**Animals**

All animal-use procedures were performed in accordance with the Declaration of Helsinki and were within the framework of the French legislation for the use of animals in biomedical experimentation. Adult male Sprague-Dawley rats (Janvier, Le Genest Saint-Ise, France) weighing 250–280 g were used. Before being used, rats were housed at 21 ± 0.5°C in Perspex home cages with free access to food and water. Light was maintained on a light/dark reverse cycle, with lights on from 8:00 PM to 8:00 AM.

**Preparation and incubation of brain slices**

Rats were killed by decapitation. The brains were carefully removed and placed in ice-cold freshly prepared artificial cerebrospinal fluid (aCSF). Then, coronal brain slices (400-μm thickness) including the striatum (anteriority: from +1.2 to +2 mm from bregma) were cut using a tissue chopper (Mickie Laboratory Engineering Co., Gomshall, Surrey, UK).

**Measurement of dopamine release**

Before being used, brain slices were gently transferred in an isolated brain slice chamber containing freshly prepared oxygenated aCSF containing 4.9 mM KCl, 118 mM NaCl, 1.18 mM MgCl₂, 1.25 mM NaH₂PO₄, 1.25 mM CaCl₂, 3.6 mM NaHCO₃, 10 mM d-glucose, and 30 mM HEPES and allowed to recover at room temperature for at least 45 min. Then, brain slices were placed in a recording chamber (1 ml volume) at 34.5 ± 0.5°C and superfused continuously at a flow rate of 1 ml/min with oxygenated aCSF. After a 30- to 45-min period of stabilization, control values of carrier-mediated- and depolarization-dependent (KCl: 100 mM, 1 min) dopamine release were recorded for 15 min; then brain slices were superfused for an additional 15-min period with a glucose-free aCSF saturated and continuously bubbled with either 1) medical air (controls); 2) 100 vol% nitrogen (OGD); or 3) 15–75 vol% xenon, with the remainder being nitrogen (OGD + xenon). The number of slices per group was n = 5, except control slices, for which the number of slices was n = 8.

Carrier-mediated and KCl-evoked striatal dopamine release were monitored using a Biopulse polarograph (Radiometer, Villeurbanne, France) and electrochemically pretreated glass-encased Nafion-precoated carbon fiber recording microelectrodes of 10 μm diameter and 50 μm length (World Precision Instruments, Aston-Stevenage, Hertfordshire, UK), as detailed previously (10). Just before each experiment, the oxidation peak potential of the carbon fiber recording microelectrode for dopamine was determined in 0.5 μM dopamine in aCSF using differential normal pulse voltammetry. Then, the polargraph was switched from the differential normal pulse voltammetry to the differential pulse amperometry mode and set at the dopamine oxidation peak potential, enabling real-time analysis of dopamine release. Signals were fed to a Y-t chart recorder and digitized using an analog to digital converter. The tip of the carbon fiber recording microelectrode was positioned ~70 μm below the slice surface within the striatum, and the platinum auxiliary and Ag/AgCl reference electrodes were positioned at a convenient position on the slice and used to maintain it in the bath.

Changes in carrier-mediated and KCl-evoked dopamine responses were calculated using each slice as its own control. After each experiment, the dopamine concentration was quantified by postexperimental calibration of the carbon fiber recording electrode: the oxidation peak potential for dopamine was checked in 0.5 μM dopamine in aCSF by differential normal pulse voltammetry. Comparison of the postexperimental and pre-experimental current signals showed no major change or shift of the dopamine oxidation peak potential. Amperometric responses were calibrated in 0–0.5 μM dopamine. Neither the amperometric response nor the dopamine oxidation peak potential was altered by the presence of air, nitrogen, or xenon in the bath.

**Measurement of cell injury with lactate dehydrogenase activity assay**

Before being used, brain slices were gently transferred into individual vials with 1.3 ml of freshly prepared oxygenated aCSF containing 120 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 26 mM NaHCO₃, 1.19 mM MgSO₄, 1.18 mM KH₂PO₄, 11 mM d-glucose, and 30 mM HEPES and allowed to recover at room temperature for 45 min. Then, brain slices were placed at 36 ± 0.5°C into individual vials containing 1.3 ml of freshly prepared aCSF, saturated, and continuously bubbled with 100 vol% oxygen (25 ml/min per vial). After a 30-min period of stabilization, the incubation aCSF solution was renewed with...
oxygenated aCSF maintained at 36°C; the slices were then incubated for 1 h to allow recording of basal levels of lactate dehydrogenase (LDH). Whereas control slices were incubated for an additional 20-min period in the same conditions, those corresponding to the ischemic group were incubated in a glucose-free solution, saturated, and continuously bubbled with 100 vol% nitrogen (OGD slices). After this 20-min period of OGD, to mimic reperfusion and treatment, the medium was replaced in all groups with freshly prepared aCSF solution, saturated and continuously bubbled with either medical air or xenon at 15–75 vol%, with the remainder being oxygen at 25 vol% completed with nitrogen when necessary.

To better assess the mechanisms by which xenon exhibits neuroprotective properties, additional experiments were performed with the prototypical NMDA receptor antagonist MK-801 and the low-affinity NMDA receptor antagonist memantine in aCSF solution, saturated and continuously bubbled with medical air. The doses used were chosen on the basis of previous data that have shown in cultured neurons that anoxia or NMDA-induced LDH release is totally blocked by MK-801 in the dose range of 0.1–1 μM (IC100/IC50 = 6.6; refs. 29, 30) and reduced by memantine in the dose range of 0.1–1 μM (31). For information and comparison, similar results with total neuroprotection against OGD- or NMDA-induced LDH release have been obtained in neuronal cell cultures with xenon at 75 vol% (IC100/IC50 = 5.4–7.5; ref. 15).

During the reperfusion period, the incubation aCSF solution was renewed every 1 h, thereby allowing samples to be obtained from the removed solution to determine the time-course of LDH release. OGD-induced neuronal injury was quantified by the amount of LDH released in the incubation solution samples every 1 h; values were summed to determine total levels of LDH released during the 3-h post-OGD reperfusion period. LDH activity was measured using a spectrophotometer at 340 nm in 50 μl of incubation medium by following the oxidation (decrease in absorbance) of 100 ml of β-NADH (3 mg in 10 ml of PBS) in 20 μl of sodium pyruvate (6.25 mg in 10 ml of PBS) using a microplate reader (32). LDH effluxes induced by OGD were expressed as the amount of LDH measured in the incubation solution and as a percentage of OGD control slices. The number of slices per condition was n = 20–26.

NMDA-induced neuronal death in vivo

On the day of surgery, rats were anesthetized with 1.5% halothane in oxygen alone in an anesthesia box and mounted on a stereotactic apparatus with the incisor bar set at 3.9 mm below the horizontal zero. A burr hole was drilled and a micropipette (~10 μm at the tip) was lowered into the right striatum [according to the rat brain stereotactic atlas of Paxinos and Watson (33); anterior, 0.6 mm; lateral, 3.0 mm; ventral, 5.8 mm, from bregma] to allow injection of 70 nmol (6.25 mg in 10 ml of PBS) using a microplate reader (32).

Assessment of motor functions and neurological outcome in MCAO rats

The motor functions and neurological outcome of the rats were investigated by recording their horizontal locomotor activity, rearing activity, and motor coordination, according to the blinded procedure described above. The horizontal locomotor activity and rearing activity of the rats were quantified using a bank of eight individual activity cages of 30 × 20 × 20 cm, equipped with infrared beams (Imetronic, Pessac, France). The motor coordination of the rats was quantified using a bank of eight individual activity wheels of 34 cm in diameter, equipped with 1/8 rotation sensors (Imetronic). Beam interruptions and rotation signals were detected through an electrical interface and recorded over 5-min intervals on a PC. On day 4 and day 3 before MCAO, the rats were familiarized with the experimental procedure and environment. On day 2 (48 h) before MCAO and on day 1 (24 h) and day 2 (48 h) after MCAO, the animals were placed in the activity cages and the activity wheels and recorded for 15 min in each type of environment. Post-MCAO scores of horizontal locomotor activity, rearing activity, and motor coordination recorded on day 1 and day 2 after MCAO
were expressed as a percentage change of the pre-MCAO scores recorded on day 2 before MCAO using each animal as its own control.

**Effects of xenon on brain temperature and other physiological parameters**

General anesthetics are known to produce hypothermia, a condition that is neuroprotective by itself (34). To assess the effects of xenon on brain temperature, additional experiments were performed in four groups of rats \((n=4 \text{ per group})\) that were implanted with an intracerebral brain cannula. Brain temperature was measured at the end of a 3-h period of exposure to medical air or xenon at 37.5, 50, or 75 vol%, by introducing a temperature probe in the brain cannula. Blood samples were also taken to assess arterial pH, pCO\(_2\), pO\(_2\), and SaO\(_2\).

**Gas treatment and pharmacology**

Oxygen, nitrogen, and xenon of medical grade were obtained from Air Liquide. Gas mixtures containing 75 vol% nitrogen and 25 vol% oxygen (medical air, controls), or xenon at 15–75 vol%, with the remainder being oxygen at 25 vol% completed with nitrogen when necessary, were obtained using calibrated flowmeters and gas analyzers. In vivo, freely moving rats were treated with xenon for 3 h in a closed chamber (10-L volume) fitted with a viewing window with either medical air or xenon, according to a blinded and randomized procedure, at a flow rate of 10 L/min. This allows maintenance of carbon dioxide levels at <0.03 vol% and humidity between 60% and 70%.

**Histological analysis**

Fifty hours after intrastriatal injection of NMDA or induction of MCAO, the rats were killed by decapitation under halothane anesthesia. The brain was rapidly removed, frozen in isopentane, and placed at −80°C. Coronal brain sections (20 μm) were cryostat-cut, mounted on slides, and stained with thionin. Brain sections colored with thionin were digitized on a PC. Brain sections stained with thionin, dehydrated with serial alcohol and cleared with xylene, and coverslipped with Eukitt mounting. Thionin as follows: slices were briefly immersed in water, stained with thionin, dehydrated with serial alcohol and cleared with xylene, and coverslipped with Eukitt mounting. Brain sections colored with thionin, dehydrated with serial alcohol and cleared with xylene, and coverslipped with Eukitt mounting. Histological analysis

**Statistical analysis**

Data are given as mean ± se. According to the size of samples, the significance of differences was determined by parametric ANOVA and/or the post hoc Student’s unpaired \(t\) test \((n≥8\) for each group; OGD-induced LDH release) or by the Kruskall-Wallis nonparametric ANOVA and/or the post hoc Mann-Whitney unpaired \(U\) test \((n=8\); brain temperature, OGD-induced dopamine release, NMDA-induced neuronal death, and MCAO-induced brain infarction and behavioral alterations).

**RESULTS**

**Effect of xenon on OGD-induced dopamine release in vitro**

Brain slices were exposed in vitro to experimental ischemia in the form of OGD to determine the effect of xenon on OGD-induced neurotransmitter release. Figure 1 shows the effects of OGD on carrier-mediated- and KCl-evoked dopamine release. Using the protocol described above (Fig. 1A), exposure to OGD resulted in a sustained increase in carrier-mediated striatal dopamine release \((U=0, \ P<0.01)\) (Fig. 1B) but showed no effect on KCl-evoked dopamine release \([U=16, \ NS]\), compared with control slices exposed to air (data not shown). The presence of xenon during OGD led to significant changes in the increase in carrier-mediated dopamine release induced by OGD \((H=13.038, \ P<0.05)\), so that in the presence of 25–75 vol% xenon, carrier-mediated dopamine levels were lower than those recorded in the presence of nitrogen alone \((U=36–39, \ P<0.005–0.02)\) (Fig. 1C). Maximal reduction of OGD-induced carrier-mediated dopamine release was obtained with 37.5 vol% xenon. Whatever the concentration used, xenon showed no effect on KCl-evoked dopamine release \((H=2.284, \ NS, \ data \ not \ shown)\).

**Effect of xenon on OGD-induced cell injury in vitro**

Brain slices were exposed ex vivo to experimental ischemia in the form of OGD to determine the effect of xenon on OGD-induced neuronal injury as assessed by the release of LDH. Figure 2 illustrates the effects of xenon on LDH release induced by OGD. Exposure to OGD led to a sustained and progressive increase in LDH release compared with control slices \((t=-11.471, \ P<0.0001)\) (Fig. 2A). The presence of xenon after OGD, i.e., during the postischemic reperfusion period, resulted in significant changes in OGD-induced LDH release \((F=48.660, \ P<0.0001)\), so that in the presence of 25–75 vol% xenon, LDH levels were lower than those recorded in control slices exposed to medical air \((t=5.791–18.005, \ P<0.0001)\) (Fig. 2A). Maximal neuroprotection was obtained with 50–75 vol% xenon. At these concentrations, xenon not only blocked OGD-induced LDH release but also further decreased LDH release below LDH levels in control slices \((t=5.741–10.016, \ P<0.001)\); we infer that this may result from a reduction by xenon of LDH release induced by the brain cutting procedure in addition to its action on OGD. As shown in Fig. 2B, neuroprotection by xenon increased as a function of time \((e.g., \ with \ 50\% \ vol\% \ xenon, \ from \ 44\% \ at \ 1 \ h \ to \ 79\% \ at \ 3 \ h)\).

For comparison, additional experiments were performed with the low-affinity use-dependent NMDA re-
Receptor antagonist memantine and the prototypical non-competitive NMDA receptor antagonist MK-801. Memantine at 10 μM (t=3.331, P<0.002) but not at 1 μM (t=−0.055, NS), exhibited mild neuroprotective action on OGD-induced LDH release, reaching a level of neuroprotection less than that produced by xenon at 25 vol% (Fig. 2A). In contrast with memantine and xenon, MK-801 at 0.1 μM (t=−0.055, NS) or 1 μM (t=−1.546, NS) showed no effect on OGD-induced LDH release (Fig. 2A).

**Figure 1.** A) Representative experimental profile showing the effects of OGD on carrier-mediated and KCl-evoked dopamine release. The inset illustrates the typical protocol used. Carrier-mediated and KCl-evoked (100 μM, 1 min) dopamine release was measured by differential pulse amperometry set at the dopamine oxidation peak potential. Changes in dopamine release were estimated as follows, using each slice as its own control: changes in carrier-mediated dopamine release were calculated as B3 − B1 and B2 − B1; and peak 2 (P2) KCl-evoked dopamine release was compared with peak 1 (P1) KCl-evoked dopamine release taken as a 100% value. Brain slices were exposed to OGD in the presence of nitrogen (OGD slices) or xenon alone or in combination with nitrogen (xenon-treated slices); control slices were exposed to medical air. B) OGD (n=8) caused a sustained increase in carrier-mediated dopamine release compared with control slices exposed to medical air (n=5). C) Effects of xenon on changes in carrier-mediated dopamine release induced by OGD. Xenon at 25–75 vol% (n=5 per group) reduced the OGD-induced increase in carrier-mediated dopamine release. Data are expressed as mean ± SE. *P < 0.05 vs. controls (saline+O2).

**Figure 2.** A) Dose-response effects of xenon on OGD-induced LDH release. Exposure to OGD led to an increase in LDH release compared with control slices. Xenon at 25–75 vol% significantly blocked LDH release induced by OGD; xenon at 75 vol% and 50 vol% not only blocked OGD-induced LDH release, but further decreased LDH release below LDH basal levels measured in control slices. Memantine (Mem) at 10 μM exhibited mild neuroprotective action on OGD-induced LDH release. In contrast with memantine and xenon, MK-801 (MK) had no effect on OGD-induced LDH release. B) Time course effects of OGD-induced cell injury and xenon-induced neuroprotection. Cell injury induced by OGD as well as neuroprotection by xenon increased as a function of time during the 3-h reperfusion period. For instance, neuroprotection by 50 vol% xenon increased from 44% at 1 h after reperfusion to 79% at 3 h after reperfusion. Data are expressed as mean ± SE. *P < 0.05 vs. controls (saline+O2). +P < 0.05 vs. OGD.
Effect of xenon on NMDA-induced neuronal death

Because xenon is believed to exert neuroprotection in the central nervous system through antagonism at the NMDA glutamate receptor, we further investigated its neuroprotective action in animals that were given an excitotoxic insult by intrastriatal infusion of 70 nmol of NMDA. Figure 3 shows the dose-response neuroprotective effects of xenon on NMDA-induced neuronal death. Administration of NMDA led to significant brain damage compared with sham rats injected with saline (U=0, P<0.0005) (Fig. 3A). Rats treated with 15–75 vol% xenon exhibited reduced neuronal death, compared with control animals treated with medical air (H=14.073, P<0.05), so that in rats treated with xenon at 25, 37.5, 50, and 75 vol%, neuronal death was lower than that recorded in control animals (25 vol% xenon: U=15, P<0.02; 37.5 vol% xenon: U=15, P<0.02; 50 vol% xenon: U=8, P<0.005; 75 vol% xenon: U=10.5, P<0.01) (Fig. 3A). As described above for OGD-induced cell injury, maximal neuroprotection was obtained with 50 vol% xenon.

To determine the therapeutic window of opportunity after which treatment with xenon may be futile to reduce NMDA-induced neuronal death, three additional groups of rats were studied according to the same procedure of NMDA-induced neuronal death and treatment with xenon at 50 vol%. Figure 3B shows the neuroprotective effects of xenon when given 2, 3, or 4 h after NMDA injection. Compared with control animals treated with medical air, xenon at 50 vol% still reduces neuronal death significantly (H=13.714, P<0.01), even when administered 2 h (U=11, P<0.01), 3 h (U=17, P<0.05) or 4 h (U=20, P<0.05) after NMDA injection.

Effects of xenon on MCAO-induced neuronal death and behavioral motor alterations

MCAO control rats treated with medical air exhibited neuronal death at the cortical (U=0, P<0.0005) and subcortical levels (U=0, P<0.0005); sham animals showed no lesion (Fig. 4A). Control rats subjected to MCAO further exhibited behavioral deficits (Fig. 4B). This resulted in a decrease in motor coordination (U=5.5, P<0.005), rearing activity (U=11, P<0.005), and horizontal locomotor activity (U=21.5, P<0.05) on day 1 after MCAO-induced cerebral ischemia. On day 2 after MCAO, rats subjected to MCAO still showed a decrease in motor coordination (U=0, P<0.0005) and rearing activity (U=4, P<0.001), but exhibited spontaneous recovery in horizontal locomotor activity, so that no significant difference was found between scores of basal locomotor activity in MCAO control rats treated with medical air and sham animals (U=32, NS). Such a spontaneous recovery in locomotor activity clearly indicated that locomotor activity does not constitute a reliable index of a MCAO-induced behavioral deficit. Thus, locomotor activity was not taken into account in the experiments below on neuroprotection provided by xenon.

When administered 2 h after MCAO induction, xenon at 50 vol% reduced cortical volumes of infarction by ~85%, compared with control rats treated with medical air (Fig. 5A). Cortical volumes of infarction in MCAO rats treated with xenon were significantly lower than those measured in control animals treated with air (U=10, P<0.0001); in contrast, xenon at 50 vol% did not.
not reduce subcortical neuronal death (U=89.5, NS).

At the behavioral level, MCAO rats treated with xenon showed a progressive and significant recovery from day 1 to day 2 in scores of motor coordination (day 1, U=63, NS; day 2, U=27.5, P<0.005) and rearing activity (day 1, U=42, P<0.02; day 2, U=27.5, P<0.005) compared with MCAO control rats treated with air (Fig. 5B) but still exhibited a significant deficit in motor coordination (day 1, U=10, P<0.005; day 2, U=15, P<0.02) but not in rearing activity (day 1, U=30, NS; day 2, U=42, NS) compared with sham animals (Fig. 5C).

When given 3 h after induction of MCAO, instead of 2 h, xenon at 50 vol% reduced neither cortical (U=47, NS) nor subcortical (U=29, NS) infarction compared with MCAO control rats treated with air (Fig. 6A). As shown in Fig. 6B, MCAO rats treated with xenon 3 h after MCAO induction showed a behavioral profile similar to that of MCAO control rats treated with air (motor coordination: day 1, U=46.5, NS; day 2, U=53, NS; rearing activity: day 1, U=46, NS; day 2, U=41, NS) and, as a consequence, still exhibited a decrease in motor coordination (day 1: U=3, P<0.005; day 2: U=5, NS).

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**Figure 4.** A) MCAO-induced cortical and striatal neuronal death in MCAO control rats (treated with medical air). Sham (Sh) animals showed no brain damage. B) Compared to sham rats (open square), MCAO control rats (filled circle) exhibited behavioral deficits in motor coordination and rearing activity on day 1 and day 2 after MCAO-induced ischemia, as well as a decrease in basal locomotor activity on day 1 but not on day 2 after MCAO-induced ischemia. This finding indicated that locomotor activity does not constitute a reliable index of an MCAO-induced behavioral deficit. Thus, locomotor activity was not taken into account in the experiments below on neuroprotection provided by xenon. Data are expressed as mean ± se (mm³). *P < 0.05.

**Figure 5.** Neuroprotection provided by xenon when administered 2 h after MCAO induction. A) MCAO rats treated with xenon exhibited a reduction of ~85% in cortical neuronal death induced by MCAO compared with control rats treated with medical air. No neuroprotective effect of xenon was found on subcortical neuronal death induced by MCAO. B) MCAO rats treated with xenon (gray triangle) showed a progressive and significant recovery from day 1 to day 2 and exhibited higher scores of motor coordination and rearing activity than control animals treated with medical air (filled circle). C) Compared with sham animals (open square), MCAO rats treated with xenon (gray triangle) still exhibited a significant deficit in motor coordination, but not in rearing activity. Data are expressed as mean ± se (mm³). *P < 0.05.
and rearing activity (day 1: U=5, P<0.01; day 2: U=0, P<0.002) compared with sham rats (Fig. 6C). As reported in MCAO control rats, MCAO rats treated with xenon 3 h after induction of MCAO showed a progressive and spontaneous recovery in locomotor activity, so that no significant difference was found on day 2 between scores of locomotor activity in MCAO-treated rats and sham animals (day 1: U=6.5, P<0.02; Day 2: U=14, NS; data not shown).

Taken together with previous data performed in our laboratory in rats that were treated with xenon immediately after the end of the MCAO period (20), these findings allow illustration of the time course of the neuroprotection of xenon. As shown in Fig. 7, whereas cortical neuroprotection provided by xenon remained unchanged when this gas is administered up to 2 h after induction of MCAO, subcortical neuroprotection by xenon rapidly decreases.

**Effects of xenon on brain temperature and other physiological parameters**

Detailed values of brain temperature and arterial pH, pCO$_2$, pO$_2$, and SaO$_2$ at the end of a 3-h exposure to medical air or xenon at 37.5, 50, or 75 vol% are shown in Table 1. Xenon reduced brain temperature (U=0–0.5, P<0.02–0.05) but had no effect on arterial pH, pCO$_2$, pO$_2$, and SaO$_2$ (H=1.3–3.5, NS) compared with medical air.

**DISCUSSION**

**Summary**

We first examined the dose-response neuroprotective effects of 15–75 vol% xenon on OGD-induced neurotransmitter release and cell injury in brain slices and NMDA-induced neuronal death *in vivo*. Maximal neuroprotection with xenon was obtained at the subanesthetic doses of 37.5 vol% (OGD-induced carrier-mediated dopamine release) and 50 vol% (OGD-induced cell injury and NMDA-induced neuronal death). Given this result, we further examined the therapeutic window after which treatment with 50 vol% xenon may be futile for reducing brain damage induced by intrastri-
Xenon, but not medical air, reduced brain temperature by 0.7 to 1.3°C. Neither medical air nor xenon produced any significant change in arterial pH, pCO₂, pO₂, and SaO₂. Values are mean ± se. pO₂, arterial oxygen partial pressure; pCO₂, arterial carbon dioxide partial pressure; SaO₂, arterial hemoglobin saturation of oxygen. *p < 0.05 vs. air.

Comparison with previous data

The dose-response effects obtained in the present study demonstrating that maximal neuroprotection with xenon occurred in the subanesthetic range of 37.5–50 vol% are in line with previous investigations demonstrating that the dose of 50 vol% xenon is sufficient to produce actual neuroprotection (7, 20, 22, 24). In contrast, others have reported dose-dependent effects and maximal neuroprotection with xenon at 75 vol% (15, 22), the highest concentration that can be administered under normobaric conditions without compromising oxygenation. However, at such concentrations xenon may produce adverse side effects in addition to its neuroprotective properties (7, 10, 20, 27, 28). As suggested previously, these discrepancies in the optimal concentration of xenon to be used may be accounted for by the different models of brain injury used and their relative severity in terms of excitotoxicity (20): 1 h of MCAO in the present study (a condition that produces total ischemia focally and thereby focal anoxia and glucose deprivation) vs. systemic NMDA injection-induced neuronal changes in the arcuate nucleus (15) or perinatal hypoxia-ischemia in rat pups (a model that combines partial ischemia and moderate hypoxia in terms of oxygen level; ref. 22). Thus, it has been demonstrated previously that xenon at 75 vol% reduced Ca²⁺ influxes in neuronal cell cultures induced by low doses of NMDA (up to 15 μM) but potentiated those produced by high doses of NMDA [50–100 μM (20)], thereby indicating that, depending on its concentration and the level of the glutamatergic input at the NMDA receptors, xenon may exhibit neuroprotective and/or neurotoxic properties as is known for other NMDA receptor antagonists such as the inert gas nitrous oxide (at supramaximal doses reached in hyperbaric conditions) and the prototypical NMDA receptor antagonist MK-801 (35). In fact, there is probably an ideal balance between the concentration of xenon to be used and the type and degree of severity of brain injury. Support for this hypothesis are physiological studies demonstrating that, in contrast with its lack of effects on circulatory functions and metabolism at concentrations <70 vol% (25, 26), xenon at higher concentrations increases cerebral blood flow that contraindicates its clinical use at high concentrations for the treatment of severe cerebrovascular diseases (27, 28).

The therapeutic window of opportunity for xenon against NMDA-induced neuronal death reported in the present study is in good agreement with recent findings demonstrating in rat pups subjected to hypoxia-ischemia that xenon at 60 vol% can offer neuroprotection when given up to 4 h after the induction of the insult (22). Contrasting with these findings, we found that 50 vol% xenon only offered neuroprotection against MCAO-induced cortical infarction and behavioral alterations when administered up to 2 h after induction of MCAO. Taken together, these data indicate that the excitotoxic processes involved in cerebral ischemia are only one part of the mechanisms that lead to neuronal death and further suggest that these processes may play a greater role in brain damage produced by hypoxia-ischemia in rat pups than in brain damage induced by MCAO in adult rats. In addition, brain plasticity in rat pups may be greater than that in adult rats, which may also explain the larger therapeutic window of opportunity of xenon in rats pups exposed to hypoxia-ischemia compared with adult rats submitted to MCAO-induced ischemia. Alternatively, we further found that xenon at 50 vol% did not reduce subcortical infarction when administered either 2 or 3 h after induction of MCAO. This finding contradicts previous data from our laboratory demonstrating that xenon at 50 vol% did reduce subcortical brain damage when given immediately after cerebral blood flow was restored in the middle cerebral artery (20), but appears consistent with the fact that the striatum, which constitutes the main part of the subcortical areas that were damaged, is known to be difficult to protect against stroke because of its lack of collateral

<table>
<thead>
<tr>
<th>Group</th>
<th>PaO₂ (mmHg)</th>
<th>PaCO₂ (mmHg)</th>
<th>SaO₂ (%)</th>
<th>Arterial pH</th>
<th>Brain temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>95 ± 11</td>
<td>34 ± 4</td>
<td>97 ± 1</td>
<td>7.4 ± 0.025</td>
<td>37.5 ± 0.1</td>
</tr>
<tr>
<td>Xenon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.5 vol%</td>
<td>96 ± 1</td>
<td>38.3 ± 1.45</td>
<td>97 ± 0.5</td>
<td>7.42 ± 0.025</td>
<td>36.8 ± 0.05*</td>
</tr>
<tr>
<td>50 vol%</td>
<td>95 ± 3.5</td>
<td>38 ± 1.95</td>
<td>97 ± 0.5</td>
<td>7.42 ± 0.025</td>
<td>36.2 ± 0.45*</td>
</tr>
<tr>
<td>75 vol%</td>
<td>88 ± 3.5</td>
<td>43.8 ± 2.05</td>
<td>96 ± 0.5</td>
<td>7.37 ± 0.005</td>
<td>36.7 ± 0.2*</td>
</tr>
</tbody>
</table>

TABLE 1. Brain temperature, arterial pH, pCO₂, pO₂, and SaO₂ before and after a 3-h exposure to medical air or xenon.
vasculature compared with the cortex. Despite this fact, as stated above, xenon did produce behavioral neuroprotection, which therefore can be attributed to its ability to reduce cortical brain damage. This finding may be related to the fact that most silent brain infarctions, which are ischemic episodes free of neurological deficit, are known to occur in deep brain structures including the basal ganglia, pons, and subcortical white matter (36).

The in vivo findings above, which demonstrated the existence of therapeutic windows for reducing excitotoxic processes and treating ischemia-induced brain damage, agree with the general view that the excitotoxic processes that lead from cell injury to neuronal death and irreversible brain damage occur relatively slowly for a period of time after the primary initial step of the ischemic-excitotoxic insult (1, 37). Further evidence for this view in the present study is the time course of LDH release induced by OGD, which indicates that cell injury progressively increases with time during the reperfusion and recovery period (Fig. 2B). Despite this increase, neuroprotection by xenon of OGD-induced LDH release also increased as a function of time (e.g., with 50 vol% xenon, neuroprotection increased from 44% at 1 h to 79% at 3 h); this time course of LDH release in the presence of xenon may be in line with recent studies that have demonstrated medium- and long-term neuroprotection by xenon in models of perinatal hypoxia (22, 24).

In the present study, we found that xenon slightly reduced brain temperature. This finding obtained in awake, freely moving, adult rats is in agreement with previous data in rat pups subjected to perinatal hypoxia-ischemia (22) but is in contrast with results of others who have reported that xenon may produce a slight but significant hyperthermia (24). In the latter study, although the mean temperature did not differ between the control group and the xenon-treated group, as revealed by the authors’ data, the conclusion that xenon may produce hyperthermia was obtained by comparing the difference between the rats’ mean rectal temperature, instead of an appropriate brain temperature, and the corresponding mean environmental chamber temperature for the control and xenon-treated groups. Therefore, because hypothermia is known to be neuroprotective by itself (34), the possibility that xenon at 50 vol% may have offered neuroprotection by reducing the rats’ brain temperature to 36.2°C must be examined. However, this possibility is unlikely to be true, because previous studies have shown that brain temperature needs to be decreased to at least 34°C to provide neuroprotection in adult rats (38). In line with these data, other investigations have shown that a reduction of brain temperature to 35°C is not sufficient to provide neuroprotection in rat pups (22). Taken together, these results suggest that xenon offers neuroprotection through its pharmacological rather than physiological properties.

Possible mechanisms of action of xenon

Brain damage induced by ischemic insults is thought to depend largely on the activation of the NMDA receptor. However, in line with previous data in adult rats (39–41), we found that the prototypical NMDA receptor antagonist MK-801 but not memantine failed to provide neuroprotection against OGD-induced cell injury. Interestingly, it is now well known that xenon has a pharmacological profile that resembles that of memantine (9, 10), a low affinity use-dependent NMDA receptor antagonist that shows fast association and dissociation rates (11) and noncompetitive antagonism at both the nicotinic cholinergic receptors and the NMDA receptors containing the NR2D subunit (42, 43). Unlike the NMDA receptors expressing NR2A/NR2B subunits, NMDA receptors containing the NR2D subunit have a long offset decay and weak Mg2+ blockade (44). The low-affinity properties of xenon, together with its selectivity for NMDA receptors that contain the NR2D subunit, a condition reported to be a critical factor for helping to determine the therapeutic profile and tolerance of NMDA receptor antagonists (45), may avoid the prolonged channel block that is responsible for the neurotoxic effects of prototypical NMDA receptor antagonists (46). Accordingly, xenon at 50 vol% only reduces NMDA-evoked Ca2+ influx in cultured neurons (20), a process that is well known to be a major critical event in excitotoxic cell injury and neuronal death, by ~30% (47). In addition, conditions of hypoxia-ischemia result in a reversal of the membrane neurotransmitter transporters (48, 49), which leads to excessive neurotransmitter release that constitutes the primary step for receptor overstimulation and subsequent neuronal injury and death. In this study, the reversal of the membrane dopamine transporter by OGD manifested as an increase in carrier-mediated dopamine release was blocked by xenon, an effect thought to result from its antagonistic properties at the NMDA receptors containing the NR2D subunit (10), which are receptors appropriate for integrating presynaptic inputs (44). Interestingly, unlike xenon and memantine, the prototypical NMDA receptor antagonist MK-801 fails to block carrier-mediated dopamine release (10). These pharmacological properties of xenon (and memantine) at the NMDA receptor containing the NR2D subunit and the nicotinic acetylcholine (ACh) receptor, together with its low blood-gas partition coefficient (12) that is with no doubt advantageous in term of rapid inflow and washout and fast association and dissociation rates, may allow this noble gas to exhibit potent neuroprotective action associated with no or little side effects when given at nonanesthetic doses.

However, as shown in the present study (Fig. 2A), memantine was much less efficient in reducing OGD-induced LDH release than 50 vol% xenon. Therefore, it is likely that other mechanisms, apart from the antagonistic properties of xenon at the NMDA and nicotinic ACh receptors, could also have contributed to
the neuroprotective action of this noble gas. Thus, xenon is a potent activator of the two-pore domain K\(^+\) channel (50), which has been recently shown to play an important role in neuroprotection (51); it also has antagonistic action at non-NMDA glutamatergic receptors (52) and further acts at other cellular proteins that can modulate both extracellular and intracellular signaling (53). In addition, given the simple structure of gases, it is highly probable that future studies will identify other cellular and molecular targets of therapeutic interest by which xenon may produce, at least in part, its neuroprotective action.

**Possible therapeutic implications**

By showing that xenon at 40–50 vol% offers global neuroprotection from reduction of ischemia-induced neurotransmitter release, a critical event well known to be involved in excitotoxic neuronal death, to reduction of subsequent cell injury and neuronal death up to 2 h after MCAO, this study extends previous data and confirms that acute xenon given alone at appropriate concentrations could be considered a practical therapy for the treatment of brain insults involving excitotoxic processes, such as brain ischemia (7, 13–24).

To make interspecies comparisons of inhalation general anesthetic potency, a reliable and useful index is the minimum anesthetic concentration (MAC). Scales that assess the in vivo potency of inhaled anesthetics are based on the MAC, which is associated with well-defined behavioral end points. For example, MAC-awake defines the MAC that prevents voluntary responses to spoken commands, *i.e.*, the impairment of perceptive awareness to environmental stimuli (hypnosis), and MAC-immobility defines the MAC that produces deep sedation and suppresses purposeful movement to a standard noxious stimuli. The MAC-awake of xenon is 86 vol% in rats (20) and 33 vol% in humans (54), and the MAC-immobility of xenon is 161 vol% in rats (55) and 71 vol% in humans (8). In the present study, neuroprotection by xenon against MCAO-induced brain damage was obtained at the nonanesthetic dose of 50 vol%. Thus, if xenon at 86 vol% in rats is equivalent to xenon at 33 vol% in humans, then 50 vol% in rats will correspond to ~19 vol% in humans. Such low concentrations of xenon would further allow the use of mild to moderate hyperoxic concentrations, if proven to be beneficial, in addition of xenon for the treatment of hypoxic-ischemic insults. Alternatively, whereas the evolving processes involved in excitotoxicity take no more than 24 h in rodents as demonstrated in comparative studies using magnetic resonance imaging, triphenyltetrazolium chloride, thionin, and neuronal nuclei immunohistochemical staining techniques (56, 57), they are thought to take several days (or weeks) in humans. Thus, by assuming a reasonably reasonable evolving period of 4–6 days in humans and given our findings that xenon remains efficient in reducing ischemia-induced brain damage when administered up to at least 2 h after induction of ischemia, it can be estimated that the therapeutic window of opportunity of xenon may be between ~8 and 12 h in humans. However, caution in needed because there is as yet no evidence that this latter type of extrapolation may actually be predictive.

Given the profound impact and consequences of brain damage for affected patients, the largely ineffective current therapeutic interventions, and the subsequent financial cost for society, the development of efficient therapeutic strategies is now clearly needed to minimize the severity of disability and long-term neurological disorders that may result from such brain insults. That xenon is neuroprotective even when given after the insult makes it a possibly attractive neuroprotectant because, in most cases, acute neuronal injury cannot be anticipated. Although closed xenon delivery systems for humans are now being developed to resolve some of the major obstacles to the widespread clinical use of xenon such as its scarcity and excessive production cost, at present the volume of production of this noble gas is quite limited, which limits its availability. However, to reduce the use of xenon, research studies on cost-efficient gas mixtures comprising xenon and other gases with therapeutic action are now being developed. Future studies on the potential neuroprotective action of xenon should investigate whether or not xenon in the range of 40–50 vol% can offer long-term neuroprotection against MCAO-induced brain damage.

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