Nitrous Oxide and Xenon Prevent Amphetamine-Induced Carrier-Mediated Dopamine Release in a Memantine-Like Fashion and Protect Against Behavioral Sensitization

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Background: *Amphetamine administration induces stimulation-independent dopamine release in the nucleus accumbens (NAcc) through reverse dopamine transport, a critical neurochemical event involved in its psychostimulant action, and furthermore decreases stimulation-dependent vesicular dopamine release. These effects may involve possible indirect glutamatergic mechanisms.*

Methods: *We investigated the effects of nitrous oxide and xenon, which possess antagonistic action at the N-methyl-D-aspartate (NMDA) receptor, on brain slices ex vivo on amphetamine-induced changes in carrier-mediated and KCl-evoked dopamine release in the NAcc, and in vivo on amphetamine-induced locomotor sensitization.*

Results: *Like the low-affinity NMDA receptor antagonist memantine, but not the prototypical compound MK-801, nitrous oxide and xenon at appropriate concentrations blocked both the increase in carrier-mediated dopamine release and locomotor sensitization produced by amphetamine.*

Conclusions: *In contrast to what has generally been found using prototypical NMDA receptor antagonists, these data regarding the effect of memantine, nitrous oxide, and xenon support the hypothesis that activation of certain NMDA receptors (possibly those containing the NR1a/NR2D subunit) in the NAcc is involved in the amphetamine-induced increase in carrier-mediated dopamine release and the development of behavioral sensitization to amphetamine. Nitrous oxide, xenon, and memantine may be of therapeutic interest for treating drug dependence.*

Key Words: Amphetamine, dopamine release, locomotor sensitization, memantine, MK-801, nitrous oxide, xenon

The effects of psychostimulant drugs are thought to result from an increase in extracellular dopamine concentrations in limbic brain regions, such as the nucleus accumbens (NAcc; Koob and Bloom 1988; Self and Nestler 1995; Wise 1996). Amphetamine crosses plasma membranes by lipophilic diffusion (Liang and Rutledge 1982a; Mack and Bonisch 1979; Zaczek et al 1991a, 1991b), and it is also a substrate for the dopamine transporter (Liang and Rutledge 1982a, 1982b; Seiden et al 1993; Zaczek et al 1991a, 1991b). Amphetamine inhibits dopamine reuptake by the plasma membrane monoamine transporter (Jones et al 1999; Parker and Cubeddu 1988; Wieczorek and Kruk 1994) and, once inside the neuronal cell, promotes the redistribution of dopamine from synaptic vesicles to the neuronal cytoplasm (Floor et al 1995; Floor and Meng 1996; Sulzer and Rayport 1990) from which dopamine is released by reversing transport through the monoamine transporter (Fischer and Cho 1979; Heikkila et al 1975; Jones et al 1998; Raiteri et al 1979; Seiden et al 1993; Sulzer et al 1993, 1995). In contrast, amphetamine reduces depolarization-dependent dopamine efflux that is attributable to synaptic vesicle exocytosis (Jones et al 1998; Kuhr et al 1985; Wieczorek and Kruk 1994). Reverse transport of dopamine release, which has little calcium dependence and is impulse-independent, but not depletion of vesicular dopamine release, which is calcium- and impulse-dependent, has been identified to be necessary for the facilitating action of amphetamine on striatal dopamine release, although the latter is the rate-limiting factor in the effects of amphetamine (Jones et al 1998).

The key role played by the NAcc in sensitization to amphetamine (Everitt and Wolf 2002; Hyman 1996) and the well-known functional interactions between dopaminergic and glutamatergic neurotransmissions in the NAcc (David et al 2004; Morari et al 1998; West et al 2003) support the possibility that alterations in the glutamatergic neurotransmission could participate in extracellular dopamine overflow and behavioral sensitization produced by amphetamine. Despite the beneficial effect of blocking the N-methyl-D-aspartate (NMDA) receptor in animal models of brain disease and dysfunction such as drug addiction or schizophrenia (for review, see Parsons et al 1998), pharmacologic studies using prototypical NMDA receptor antagonists have met limited clinical success because these compounds produce adverse side effects and possess their own neurotoxicity (Olney et al 1989, 1991; Pulvirenti and Koob 2002), that is possibly due to the prolonged nonphysiologic channel block induced by MK-801-like agents (Rogawski 1993). To avoid or at least to reduce such adverse side effects, the potentially therapeutic interest of low-affinity use-dependent NMDA receptor antagonists, which often possess additional mild to moderate action at other neurotransmitter receptors, has been suggested (for review: Palmer and Widzowski 2000; Pulvirenti and Koob 2002).

Parallel to these studies, nitrous oxide and xenon are anesthetic gases shown to be effective inhibitors of the NMDA receptor, to have remarkably safe clinical properties (Franks et al 1998; Jevtovic-Todorovic et al 1998; Yamakura and Harris 2000), and to possess moderate antagonistic action at the nicotinic cholinergic receptor (Yamakura and Harris 2000), as does the low-affinity use-dependent NMDA receptor antagonist memantine (Palmer and Widzowski 2000). Both nitrous oxide and

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xenon readily cross the blood–brain barrier; have low blood-gas solubility, which is advantageous in terms of rapid inflow and washout (Goto et al 1998), conditions that could favor their use in treatment and reduce risk of adverse side effects; and have been recently shown to possess potentially therapeutic properties (for review, see Abraini et al 2005). Possible neuroprotection by nitrous oxide and xenon was first approached by demonstrating that these agents prevent excitotoxic neuronal death in cultured neurons (Wilhelm et al 2002) and reduce in vivo neuronal degeneration (Jevtovic-Todorovic et al 1998; Wilhelm et al 2002). In addition, other investigations have shown that nitrous oxide and xenon reduce ischemia-induced cerebral infarct volume (David et al 2003; Homi et al 2003), as well as NMDA-induced Ca2+ influxes in cultured neurons (David et al 2003), a critical event involved in excitotoxic neuronal death. Moreover, clinical studies from a single group have suggested that nitrous oxide at analgesic, but not at anesthetic, concentrations may be used for the treatment of addiction to alcohol and other drugs (Daynes and Gillman 1994; Gillman and Lichtigfeld 2004), although this has been contested (Alho et al 2003) and remains to be shown through experimental evidence.

We examined whether nitrous oxide or xenon at subanesthetic concentrations could alter the increase in carrier-mediated dopamine release or the concomitant reduction in evoked dopamine release induced by amphetamine in the NAcc (or both). We demonstrate in rat brain slices that nitrous oxide and xenon reduce the amphetamine-induced increase in carrier-mediated dopamine release, as does the low-affinity NMDA receptor antagonist memantine but not the prototypical NMDA receptor antagonist MK-801. Given these effects, we performed additional behavioral studies; we showed that posttreatment with either gas prevents the development of locomotor sensitization to amphetamine.

Methods and Materials

Animals

All animal-use procedures were in accordance with the Declaration of Helsinki and were within the framework of the French legislation for the use of animals in biomedical experimentation. Male adult Sprague–Dawley rats (Janvier, Le Genest Saint-Isle, France) weighing 250 to 300 g were used. They were housed socially in groups of 3–4 at 21 ± .5°C, in perspex home cages with free access to food and water. Light was maintained on a reverse light–dark cycle, with lights on from 8 PM to 8 AM.

Neurochemical Studies

Preparation and Incubation of Brain Slices. Rats were killed by decapitation and the brains were carefully removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) containing in mM: 4.9 KCl, 118 NaCl, 1.18 MgCl2, 1.25 NaH2PO4, 1.25 CaCl2, 3.6 NaHCO3, 10 d-glucose, 30 HEPES. Coronal brain slices (400 μm thickness) including the NAcc (anteriority: + 1.2 to + 2 mm from the bregma) were cut using a chopper (Mickie Laboratory Engineering, Gomshall, United Kingdom). Before being used, brain slices (n = 4–5 per condition) were transferred to an isolated brain slice chamber containing freshly prepared oxygenated aCSF and allowed to recover at room temperature for at least 1 hour. Slices were then placed in a recording chamber (1 mL volume) at 34.5 ± .5°C and superfused at a flow rate of 1 mL/min with aCSF saturated with medical air (75 vol% nitrogen 25 vol% oxygen). Following a 20-min period of control, amphetamine (10 μM) was added for 30 min to aCSF in the presence of air, nitrous oxide or xenon of medical grade at 50 vol% or 75 vol% (with the remainder being oxygen), memantine, or MK-801 (in the presence of air). Control slices were given saline and air.

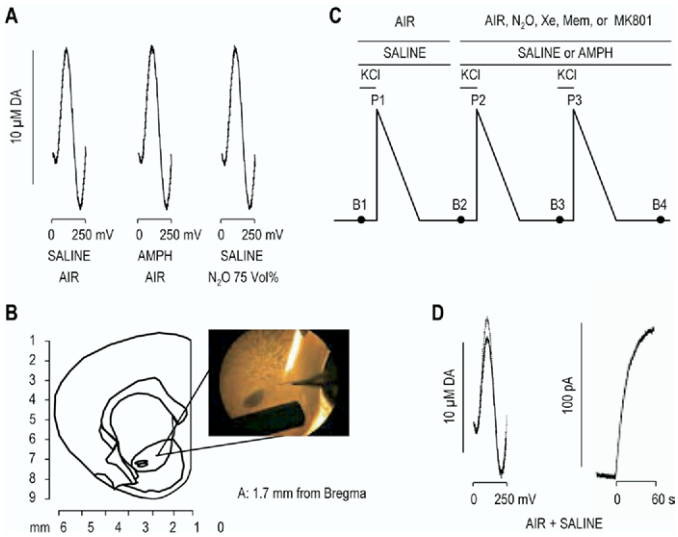

Figure 1. (A) Typical prexperimental calibration voltammograms obtained successively (4-min intervals) from a single electrode in response to 10 μM dopamine in artificial cerebrospinal fluid (aCSF) saturated with air (left) or nitrous oxide at 75 vol% (right) or in the presence of 10 μM amphetamine (middle); all solutions were made from a same primary dopamine solution of 10 μM. Dopamine oxidation peak potentials were similar, at approximately 100 mV as measured by differential normal pulse voltammetry. **(B)** Microphotography and schema showing a typical placement of the recording carbon fiber microelectrode in the core of the NAcc midway from the anterior commissure and the lateral ventricle at anteriority 1.2 to 2 mm from bregma; The Ag/AgCl reference electrode can be seen at the bottom of the microphotography. **(C)** Schema illustrating the typical experimental protocol used: following saline, drug, air, or gas treatment. Carrier-mediated and KCl-evoked (100 mM, 1 min) dopamine release was measured by differential pulse amperometry set at the dopamine oxidation peak potential. Changes in carrier-mediated dopamine release were estimated as follows: changes in carrier-mediated dopamine release were calculated as [B4-B1], [B3-B1], and [B2-B1]; Peak 2 (P2) and Peak 3 (P3) KCl-evoked dopamine responses were compared to Peak 1 (P1) KCl-evoked dopamine release taken as a 100 % value, using each slice as its own control. Brain slices were treated with amphetamine in the presence of air, nitrous oxide (N2O), xenon (Xe), memantine (Mem), or MK-801; memantine and MK-801 were given with air. Control slices were exposed to saline and air. **(D)** Typical postexperimental carbon fiber microelectrode calibration by differential pulse voltammetry and amperometry in 10 μM dopamine in aCSF: comparison of the current waveforms and dopamine oxidation peak potentials obtained preexperimentally (dotted line) and postexperimentally shows no major change or shift. The sensitivity of amperometic responses in .1–10 μM dopamine was linear.

Measurement of Dopamine Release. Experimental design and procedure are illustrated in Figure 1. Carrier-mediated and depolarization-dependent (KCl: 100 mM, 1 min, applied every 15 min) dopamine release in the NAcc were monitored using a Biopulse polarograph (Radiometer, Villeurbanne, France) and standard glass-encased nafion-precoated recording carbon fiber electrodes 10 μm in diameter and 50 μm long (World Precision Instruments, Aston-Stevenage, Hertfordshire, United Kingdom). The carbon fiber microelectrode sensitivity for dopamine was improved by applying an electrochemical treatment, which involved placing a working carbon fiber microelectrode, a platinum auxiliary electrode, and an Ag/AgCl reference electrode in a beaker of phosphate-buffered saline solution (pH 7.4) and applying a 70-Hz triangular wave form of 0–2.6 V for 20 sec