Anesthetic Gases and the Developing Nervous System

BACKGROUND

N_2O, first prepared by Joseph Priestley in 1772, is the most durable of all anesthetics having been used clinically for more than 150 years. The noble gas xenon, discovered more than a century ago, has been known to exhibit anesthetic properties since 1946; however, its clinical utility has been limited by relatively high manufacturing costs owing to its rarity in the atmosphere (Cullen and Gross, 1951). The physical and anesthetic properties of these two gases have been well defined (Table 1); although they have many similarities, including activities at the molecular level (Franks. 2006), there are striking difference in their side-effect profile. This has prompted us to explore circumstances in which administration of xenon can provide a clinical benefit over that of N_2O to justify the cost of using the more expensive noble gas.

Table 1. Physical and anaesthetic properties of nitrous oxide and xenon

	Nitrous oxide	Xenon
Molecular weight	44	131
Melting point (°C)	-90.8	-111.9
Boiling point (°C)	-88.5	-108.1
Blood/gas partition coefficient	0.47	0.115
MAC		
Human	1.04	0.71
Dog	1.88	1.19
Rat	2.22	1.61
Mouse	1.54	0.95

Qualitative similarities

Clinical and preclinical

Based upon its widespread use, N_2O has withstood the test of time. More recently, the safety and efficacy of xenon as an inhalational anesthetic has been realized in a variety of clinical settings (Rossaint et al., 2003; Coburn et al., 2005). Xenon anesthesia is associated with remarkable cardiovascular stability, with only a clinically insignificant decrease in heart rate being reported (Boomsma *et al*., 1990; Lachmann *et al*., 1990; Luttropp *et al*., 1993; Dingley *et al*., 2001). Lachmann (1990) suggested that the hemodynamic stability was a result of less stress-induced sympathetic stimulation, a theory supported by the observation of stable epinephrine levels during xenon anesthesia (Boomsma *et al*., 1990). Compared with N_2O anesthesia, less fentanyl was required to maintain cardiovascular stability during xenon anesthesia (Boomsma *et al*., 1990; Lachmann *et a*l., 1990). Perioperatively, plasma cortisol and epinephrine increased in the N_2O group but did not change in the xenon group (Boomsma *et al*., 1990). Similar to N_2O, myocardial function, assessed by transesophageal echocardiography, is unchanged during xenon anesthesia (Luttropp *et al*., 1993). The lack of effect of xenon on cardiac contractility was confirmed in preparations of isolated guinea pig ventricular muscle bundles; contrastingly, an equi-anesthetic concentration of isoflurane was found to decrease myocardial force development by 30% (Stowe *et al*., 2000). Even in the presence of compromised myocardium, xenon anesthesia is remarkably stable; in a study of 20 patients undergoing elective coronary artery bypass grafting, xenon decreased indices of cardiac function significantly less than that noted with N_2O (Ishiguro, 2001).

Molecular

Most general anesthetics act on one or more of the superfamilies of ligand-gated ion channels; these targets include the _-aminobutyric acid type A ($GABA_A$), glycine, 5-hydroxytryptamine type 3A, and neuronal nicotinic acetylcholine (nACh) receptors. In contrast to most general anesthetics that act mainly at $GABA_A$ receptor, N_2O and xenon have been demonstrated to be effective inhibitors of the NMDA subtype of the glutamate receptor (Franks *et al*., 1998; Jevtovic-Todorovic *et al*., 1998). Apart from the NMDA receptor, the two pore domain potassium channel superfamily has been identified as another likely target for the anesthetizing actions of xenon and N_2O (Franks and Honore 2004); these potassium channels are responsible for the background or leak currents that play an important role in modulating neuronal excitability.

Qualitative Differences

Clinical and Preclinical

Analgesic mechanism: N_2O analgesia is dependent on the release of endogenous opioid peptides in the brainstem resulting in the activation of the descending noradrenergic pathway that modulates nociceptive processing in the spinal cord (Ohashi et al., 2003). While the precise mechanism for the antinociceptive effect of xenon remains to be elucidated, Ohara *et al.* (1997) reported that xenon's antinociceptive action is independent of opioidergic and adrenergic receptors. Interestingly, the antinociceptive effects of N_2O require supraspinal to spinal connectivity (Zhang *et al*, 1999) although xenon is capable of exerting analgesia in spinally-transected preparations; unlike N_2O, xenon directly suppresses polysynaptic transmission within the dorsal horn (Miyazagi *et al*., 1999). Based upon their different effects on pain processing, we predicted and confirmed that N_2O has no antinociceptive effect in neonates before the descending inhibitory system has fully developed (Ohashi *et al*., 2002); conversely, xenon is efficacious as an antinociceptive in neonatal rat pups (Ma *et al*., 2004).

Cardiac preconditioning: Administration of N_2O before coronary artery occlusion-reperfusion injury does not protect against myocardial infarction in rats (Weber *et al*., 2005) and dogs (Siker *et al*., 1992). Contrastingly, prior exposure to xenon can induce subsequent cardioprotection *via* the preconditioning mechanism (Weber *et al*., 2005). Xenon also exerts

cardioprotective effects when given during reperfusion as evidenced by reduced infarct size after regional myocardial ischemia in rabbits *in vivo* (Preckel *et al*., 2000).

Neuroprotection: Activation of *N*-methyl-D-aspartate receptors plays a pivotal role in the propagation of acute neuronal injury (Choi *et al*., 1988); hence, many have advocated the use of NMDA antagonists to interrupt the pathogenesis of acute neuronal injury. While both N_2O and xenon are capable of antagonizing the NMDA receptor, only xenon appears to exert neuroprotection (Ma *et al*., 2006); crucially, xenon provides marked protection against injury well below anesthetic concentrations, with IC_{50} concentrations in some models as low as 0.1 MAC (Ma *et al*., 2005). Xenon decreases acute neuronal injury in response to both the exogenous administration of excitotoxins or through deprivation of oxygen and glucose in mice neuronal-glial co-culture system (Wilhelm *et al*., 2002). *In vivo*, xenon prevents the morphologic and functional consequences of acute neuronal injury provoked by ischemia (middle cerebral artery occlusion) in adult mice (Homi *et al*., 2003), cardiopulmonary bypass in adult rats (Ma *et al*., 2003), and excitotoxins in adult rats (Ma *et al*., 2002).

Neurotoxicity: Even though NMDA receptor antagonists reduce neuronal damage after cerebral ischemia, concomitantly, this class of compounds also produce psychotomimetic side-effects (Olney *et al*, 2002); although psychotomimetic effects have been observed after administration of some NMDA antagonist anesthetics, such as ketamine and N_2O , this has not been seen after xenon (Ma *et al*., 2002). A reliable morphological marker of neuronal toxicity of NMDA antagonists is c-Fos expression in the posterior cingulate and retrosplenial cortices (Ma *et al*., 2002); xenon, in contrast to both N_2O and ketamine, does not induce c-Fos expression in the in rats. Combination of NMDA receptor antagonists may exacerbate neurotoxicity; thus, Nagata *et al.* (2001) demonstrated that N_2O alone produced a small amount of c-Fos expression but significantly *enhanced* ketamine-induced neurotoxicity. In contrast, xenon alone exhibited no neurotoxicity and concentration-dependently *reduced* the ketamine-induced c-Fos expression in rat posterior cingulate and retrosplenial cortices (Nagata *et al*., 2001). Our recent *in vivo* microdialysis studies suggest that the mechanism for these qualitative difference may be due to xenon's ability to inhibit dopamine release, which contrasts with both ketamine and N_2O in which dopamine release is enhanced (Sakamoto *et al*., 2006); furthermore, ketamine-induced dopamine release is further enhanced by N_2O but blocked by xenon (Sakamoto *et al*., 2006).

Molecular

Xenon activates protein kinase C (PKC)-ε, the isoform of an enzyme that is crucial in the signal transduction pathway for myocardial preconditioning (Weber *et al*., 2005); neither preconditioning nor activation of PKC occurs with N_2O (Fukura *et al*., 2000). Downstream of PKC, xenon exposure increases pCREB in brain; again, N_2O lacks a property that may be pivotal to the neuroprotective signaling pathway (Ma *et al*., 2006).

PRECLINICAL STUDIES Neurotoxicity in neonates

Anesthetics which block the NMDA receptor subtype of the glutamate and/or positively modulate or gate the $GABA_A$ receptor have been associated with apoptotic neurodegeneration (AAN) in the developing neonate (Jevtovic-Todorovic *et al*., 2003). Using cleaved caspase 3 as a tissue marker of ANN, we investigated the effects of equi-anesthetic concentrations of isoflurane, N_2O, xenon or combinations of isoflurane with either N_2O or xenon (both NMDA receptor antagonists) in 7 day old neonatal rat pups. Following 6h of isoflurane administration, we observed the expected ANN injury (see Fig. 1). A striking *enhancement* in the injury was noted when N_2O was combined with isoflurane; remarkably, when xenon was added to isoflurane, ANN was significantly *reduced*. We have now confirmed the exacerbating effect of N_2O and mitigating effect of xenon on isoflurane-induced toxicity in an *in vitro* organotypic hippocampal slice culture system (see Fig. 2) setting the stage for an exploration of the mechanisms involved (Ma *et al*., 2007). Next, we propose to establish whether these contrasting effects on ANN result in functional neurological consequences later in life.

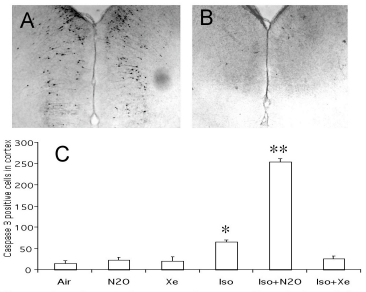

Figure 1. Apoptotic neurodegeneration induced by anaesthetics (75%N_2O, 75%Xe, 0.75%Isoflurane (Iso), 0.75%Iso+75%N_2O or 0.75%Iso+60%Xe) as measured with caspase-3 immunostaining in the cortex of seven day old neonatal rats. A: 0.75%Iso+75%N_2O. B: 0.75%Iso+ 60%Xe. C: Mean data (mean ± SEM, n = 6). * $p < 0.01$ *vs* Air; ** $p < 0.001$ *vs* Air.

In order to elucidate whether the intrinsic and/or extrinsic apoptotic pathways are involved in isoflurane-induced neuronal apoptosis in an in vivo rat model 7 day Sprague-Dawley rat pups were exposed to one of the following gas regimens: air, 60% xenon, 70% N_2O, 0.75% isoflurane, 60% xenon+0.75% isoflurane, 70% N_2O+0.75% isoflurane. Gas delivery occurred for 6 hours after which the animals were immediately sacrificed and their cortices removed for western blotting of caspase-3, -8, -9, cytochrome C, OX-42 and GFAP (glial fibrillary acidic protein). Results were normalised to the control and the housekeeping protein _-tubulin with densitometric analysis.

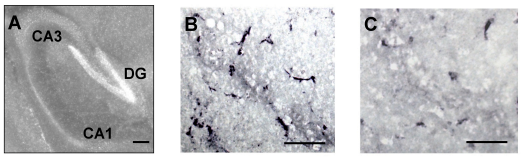

Figure 2. Cleaved caspase-3 expression in the hippocampus of organotypic hippcampal slice culture derived from C57BL6 neonatal mice pups following anesthetic gas treatment. A: A representative living culture was killed by exposure to glutamate (50 μM) and image was enhanced with propidium iodide staining, acting as a guide to outline of CA area of hippocampus in Fig B and C. B: A representative photomicrograph of hippocampal culture treated with isoflurane (Iso) 0.75% + N_2O 75% followed by caspase-3 immunostaining. C: A representative photomicrograph of hippocampal culture treated with Iso 0.75% + xenon 60% followed by caspase-3 immunostaining.

Although exposure to N_2O alone and in combination with isoflurane increases caspase-8 expression, there is no change of protein expression among the other groups. Xenon does not increase the activation of caspase-3, -9, or cytochrome C ($p>0.05$ vs air). However, caspase-3 and -9 are increased by isoflurane and N_2O respectively ($p<0.05$ vs air), and cytochrome C is significantly increased by N_2O alone ($p<0.05$ vs air). Furthermore, when combined with isoflurane, N_2O significantly enhances isoflurane-induced expression of caspase-3 ($p<0.05$ vs air) whereas xenon does not ($p>0.05$ vs air). GFAP and OX-42 expression are enhanced with N_2O and isoflurane in combination, and is mitigated during exposure to xenon and isoflurane ($p>0.05$ vs air). None of the other treatment groups seem to be affected by either glial marker ($p>0.05$ vs air).

Conclusion: N_2O enhances while xenon mitigates isoflurane-induced apoptotic neurodegeneration (Ma *et al*., 2007). This occurs via the intrinsic pathway, and most likely, via the extrinsic pathway, which is activated at a later time point. Identifying the mechanisms underlying the above-mentioned effects may provide novel and more specific anti-apoptotic treatment strategies.

Neuroprotection

The neuroprotective property of xenon has been reported by our research group and others in both *in vitro* and *in vivo* models of acute neuronal injury (Wilhelm *et al*., 2002; Ma *et al*., 2003; Homi *et al*., 2003; Petzelt *et al*., 2003; Petzelt *et al*., 2004). In *in vitro* studies we showed that xenon reduced injury in a mouse neuronal-glial cell culture induced by either NMDA, glutamate, oxygen deprivation or oxygen-glucose deprivation (OGD) (Wilhelm *et al*., 2002). In a series of preclinical studies involving three different species, we and others have shown neuroprotective effects of xenon in a variety of *in vivo* models of acute neuronal injury involving administration of excitotoxins to rats (Ma *et al*., 2003), cardiopulmonary bypass in rats (Ma *et al*., 2003), middle cerebral artery occlusion in mice (Homi *et al*., 2003), cardiac arrest in pigs (Schmidt et al., 2005), and hypoxic-ischemia in neonatal rats (Ma *et al*., 2005). Recently, we have shown that xenon prevents damage when provided up to 24 h prior to ischemic neurological injury in 7 day old neonatal rats; complementary *in vitro* studies revealed that this effect is abolished by cycloheximide, implying that this property of xenon requires new protein synthesis (Ma *et al*., 2006); contrastingly, equi-anesthetic concentration of N_2O did not exert preconditioning against neurological injury.

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