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Biophysical changes induced by xenon on phospholipid bilayers

Ryan D. Booker, Amadeu K. Sum *

Department of Chemical and Biological Engineering, Colorado School of Mines, 1600 Illinois Street, Golden, CO 80401, USA

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ABSTRACT

Structural and dynamic changes in cell membrane properties induced by xenon, a volatile anesthetic molecule, may affect the function of membrane-mediated proteins, providing a hypothesis for the mechanism of general anesthetic action. Here, we use molecular dynamics simulation and differential scanning calorimetry to examine the biophysical and thermodynamic effects of xenon on model lipid membranes. Our results indicate that xenon atoms preferentially localize in the hydrophobic core of the lipid bilayer, inducing substantial increases in the area per lipid and bilayer thickness. Xenon depresses the membrane gel–liquid crystalline phase transition temperature, increasing membrane fluidity and lipid head group spacing, while inducing net local ordering effects in a small region of the lipid carbon tails and modulating the bilayer lateral pressure profile. Our results are consistent with a role for nonspecific, lipid bilayer-mediated mechanisms in producing xenon's general anesthetic action.

1. Introduction

General anesthesia is a physiological state characterized by loss of consciousness, loss of sensation through analgesic effects, immobilization, and temporary amnesia [1]. The physiological effects are known to be produced through depression of nerve function but, despite the fact that general anesthesia has been accepted for clinical use for over a century and is administered to approximately 21 million patients annually in the United States alone [2], the specific molecular mechanisms by which anesthetic agents induce an anesthetic state remain poorly understood.

The correlation between anesthetic efficacy and lipid solubility, known as the Meyer–Overton correlation [3,4], led the majority of research on the molecular mechanisms of general anesthetics to be focused on the interactions between anesthetic agents and the lipid bilayers of neuronal membranes for over 80 years. More recently, however, a number of researchers, most notably Franks and Lieb [5], have shifted the focus to direct protein interactions. Much research, outlined extensively by Barash et al. [6], Urban [7,1], and Franks [8], has focused on a mechanism involving direct interactions with hydrophobic pockets or clefts in transmembrane ion channels and protein targets of general anesthetic agents are well supported by the evidence. However, while significant progress has been made in characterizing the interactions between anesthetics and individual protein and central nervous system (CNS) targets, these investigations have not identified an individual target or comprehensive mechanism of action capable of mediating the complete anesthetic effect [9]. Indeed, it increasingly appears that the clinical effects of general anesthesia are the result of multiple molecular mechanisms occurring at different targets.

Of particular interest recently, both for its lack of undesirable side effects in clinical settings and for its unique molecular interactions, is the noble gas xenon. The clinical viability of xenon as an anesthetic agent has been known for over 60 years [10]. However, its clinical use has been largely curtailed by its high cost until recently [11]. During the last decade, the desire to replace or supplement the use of nitrous oxide due to its environmental impacts [12] and the advantageous pharmacokinetic profile [13], neuroprotective properties [14,15], and potent analgesic effects [16] of xenon have led to renewed interest in xenon in clinical settings [11,17,18].

However, xenon's simple, spherical shape and the fact that it is essentially inert pose an interesting conundrum in regard to the mechanisms of its anesthetic action. Xenon appears to have no significant effect on the most commonly implicated anesthetic target, $GABA_A$ receptors [19–21]. Xenon has been shown to act on glutamatergic receptors, with inhibition of NMDA receptors being the mechanism implicated most often [19,22,20,23,24]. Using molecular dynamics, Liu et al. [25] identify several potential action sites of xenon in the ligand-binding domain of an NMDA receptor. However, results with glutamatergic receptors have been somewhat inconsistent, with other researchers showing that xenon acts on non-NMDA glutamatergic receptors in *Caenorhabditis elegans* [26], although it is possible that general anesthesia occurs by different mechanisms in *C. elegans* versus humans and other mammals. Furthermore, recent research has indicated that NMDA receptors do not mediate the immobilizing effects of xenon and other inhaled anesthetics [27], although this does not preclude the possibility that NMDA receptors mediate the other effects of general anesthesia. It has also been shown that one minimum alveolar concentration (MAC) of xenon

* Corresponding author. Tel.: +1 303 273 3873; fax: +1 303 273 3730.
E-mail address: asum@mines.edu (A.K. Sum).

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actually depresses metabolic activity in the human brain [28,29], whereas two anesthetics that have also been shown to inhibit NMDA receptors, ketamine and nitrous oxide, increase cerebral metabolism [27]. This inconsistency with anesthetic agents known to interact with NMDA receptors may indicate that glutamatergic receptors play little or no role in the anesthetic effects of xenon in vivo [28]. Xenon has also been shown to exert effects on nicotinic acetylcholine receptors [22] and certain potassium channels [30], some of which have recently been implicated as playing a role in the anesthetic effect [31,32], but the significance of these results remains unclear.

Recently, there has been substantial renewed interest in the role lipid bilayers play in modulating the function of membrane proteins [33–36]. It has been shown that the open probability of voltage-dependent potassium channels is affected by the mechanical state of the surrounding lipid membrane [37] and that small changes in bilayer thickness can reverse the response polarity of gramicidin A channels [38]. Furthermore, amphiphiles have been shown to regulate voltage-gated ion channel function through a lipid bilayer-mediated mechanism [39,40] and $GABA_A$ receptor function is regulated by lipid bilayer elasticity [41]. Cantor [42] proposed that an inhomogeneous modulation of the lateral pressure (more accurately called the stress virial) profiles in the cellular membrane by anesthetic molecules exerts lateral forces on transmembrane gated ion channels, inducing effects on channel current by increasing the energy needed to change the channel between open and closed conformations. Other recent studies have since shown that a change in the lateral pressure profile could influence the conformation of ion channels [43,44] and molecular dynamics simulations with ethanol and 1-alkanols have indicated that this mechanism is consistent with the action of general anesthetics [45,46]. Furthermore, this hypothesis predicts the alkanol cutoff effect and anomalously low anesthetic potencies of strongly hydrophobic molecules such as perfluorocarbons.

It is generally accepted that cellular membranes in vivo can be described by a fluid mosaic model, proposed by Singer and Nicolson [50], in which proteins are embedded within a lipid bilayer and all components can diffuse laterally within the plane of the membrane [51]. The accumulation of a variety of small molecules in a lipid bilayer is known to cause changes in the bilayer biophysical and thermodynamic properties and these interactions between small molecules and lipid bilayers have commonly been studied through computer simulation. Molecular dynamics studies have primarily focused on small hydrocarbons and organic acids [52–55], sugars [56–59], alcohols [60,54,61,45,62], and some small biological molecules [63,64].

Molecular dynamics studies of interactions between general anesthetic agents and lipid membranes have been more limited in scope. Older simulations were generally restricted to coarse-grained simulations [65], short timescales [60,66–68] or studies solely of alcohols [60,61,45]. However, more recently, a number of atomistic simulation studies conducted over longer timescales have indicated substantial effects of anesthetic agents on a number of lipid bilayer properties. Stimson et al. [69] performed 100 ns, atomistic molecular dynamics simulations investigating the interactions between xenon and dioleoylphosphatidylcholine (DOPC) bilayers, with initial results indicating that partitioning of xenon into the bilayer causes small increases in the area per lipid and orientational order of the lipid acyl chains. More recently, Yamamoto et al. [70] reported an MD study of POPE bilayer exposed to xenon at different pressures to probe the pressure reversal effect in general anesthesia. The results from their simulations showed that pressure had little effect on the properties of the lipid bilayer, but suggested that xenon accumulated in the middle of the lipid bilayer and had its diffusivity substantially decreased. Simulation studies conducted by Tu et al. [71], studying halothane, and Darvas et al. [72], studying halothane, chloroform, diethyl ether, and enflurane, show reversal of the effects of general anesthetic agents on membrane properties at high pressures.

Here, we investigate the interactions of xenon with model phospholipid membranes using molecular dynamics (MD) simulations of pure DOPC bilayers in two initial configurations with xenon at a range of concentrations and pressures and over timescales of 150–300 ns. Differential scanning calorimetry (DSC) measurements are presented to characterize the effects of xenon on lipid membrane phase transition temperatures and fluidity. Xenon is shown to exert broad biophysical effects on membrane fluidity, lateral pressure profiles near the lipid head groups, and bilayer structure. These interactions are consistent with a lipid bilayer-mediated mechanism of general anesthetic action.

2. Materials and methods

2.1. Molecular dynamics simulations

Molecular dynamics simulations were performed on two different types of systems: 1) single bilayer systems containing 288 DOPC molecules arranged in a bilayer structure (144 molecules in each leaflet); and 2) double bilayer systems containing 576 DOPC molecules arranged in two bilayers, separated by aqueous phases. A DOPC molecule was initially constructed and then arranged in a bilayer configuration (Fig. S1 in the Supporting material is a line diagram of a DOPC molecule). A total of six xenon concentrations were simulated: 0, 1, 1.5, 2, 2.5, and 3 xenon atoms per lipid. The minimum alveolar concentration (MAC) for xenon is 71% [73]. Stimson et al. [69] point out that the specific concentration, in a tissue comparable to those simulated here, required to induce an anesthetic state is unknown and this remains true. The concentrations chosen here are comparable to those used in previous studies [69,70].

Bilayers were fully hydrated, with 40 water molecules per lipid in the systems containing xenon concentrations of 0 to 2 xenon atoms per lipid and 50 water molecules per lipid in the systems containing 2.5 and 3 xenon atoms per lipid. Prior to insertion of xenon atoms, the bilayers were heated to 450 K to erase memory effects from the initial configuration. Bilayers were then allowed to equilibrate for 20 ns at 310 K. Initial configurations were generated by adding a number of excess water molecules equal to the intended number of xenon atoms, then randomly replacing the appropriate number of water molecules with xenon atoms. Fig. 1 shows snapshots of the initial (A) and final (B) configurations of a double bilayer system. Fig. 3B shows a snapshot of a single bilayer system and Fig. S3 shows snapshots of the initial (A) and final (B) configurations of a single bilayer system. For the results below, data were analyzed over the last 20 ns for single bilayer simulations and over the last 40 ns for double bilayer systems, when the systems had reached dynamic equilibrium. The systems reached dynamic equilibrium when net diffusion of xenon between the bilayer and aqueous phases ceased and when changes in bilayer surface area over time stabilized around a mean value (this was true over the final 20 ns for all single bilayer systems and over the final 40 ns for all double bilayer systems). An example of the distribution of xenon between the aqueous phase and lipid bilayer in a double bilayer is shown in Fig. S4.

The forcefield employed for the lipids used the Berger lipid parameters [74] with the GROMOS forcefield. Water was represented by the single point charge (SPC) model [75] and the xenon atoms were represented as simple Lennard–Jones sites with $\varepsilon = 1.837$ kJ/mol and $\sigma = 0.410$ nm [76]. This approach is consistent with previous MD simulations of lipid bilayers and with simulations of bilayers with xenon specifically [69,70]. Simulations were performed using single precision GROMACS 4 [77] with the Berendsen thermostat and barostat [78]. Simulations were held at a constant temperature of 310 K, above the $L_{\beta\alpha}$ (gel–liquid crystalline) phase transition temperature for DOPC (−20 °C [79]), and four different pressures were used: 1, 50, 100, and 200 bar. Anisotropic pressure coupling was applied. A short range cut-off of 1.0 nm was used for nonbonded interactions (Coulombic and Lennard-Jones) and long-range electrostatic interactions were calculated by the particle-mesh Ewald method