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Anesthetics mechanism on a DMPC lipid membrane model: Insights from molecular dynamics simulations

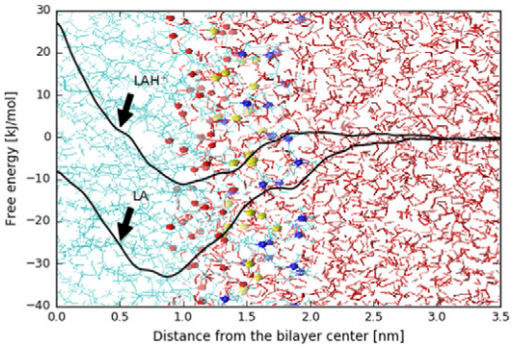
Marzieh Saeedi[a], Alexander P. Lyubartsev[b,*], Seifollah Jalili[a]

[a] Department of Chemistry, K. N. Toosi University of Technology, Tehran 15875-4416, Iran
[b] Division of Physical Chemistry, Department of Materials and Environmental Chemistry, Stockholm University, SE-10691 Stockholm, Sweden

HIGHLIGHTS

- Molecular dynamics and metadynamics simulations of local anesthetics lidocaine and articaine in a lipid bilayer have been carried out.
- Localization and orientation of both charged and neutral forms of the drug molecules in the lipid membrane were investigated.
- Binding free energies and partitioning properties of the drug molecules were determined.

GRAPHICAL ABSTRACT



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ABSTRACT

To provide insight into the molecular mechanisms of local anesthetic action, we have carried out an extensive investigation of two amide type local anesthetics, lidocaine and articaine in both charged and uncharged forms, interacting with DMPC lipid membrane. We have applied both standard molecular dynamics simulations and metadynamics simulations to provide a detailed description of the free energy landscape of anesthetics embedded in the lipid bilayer. The global minimum of the free energy surface (equilibrium position of anesthetics in the lipid membrane) occurred around 1nm of the bilayer center. The uncharged anesthetics show more affinity to bind to this region compared to the charged drugs. The binding free energy of uncharged lidocaine in the membrane (−30.3kJ/mol) is higher than uncharged articaine (−24.0kJ/mol), which is in good agreement with higher lipid solubility of lidocaine relative to the articaine. The octanol/water partition coefficient of uncharged drugs was also investigated using expanded ensemble simulations. In addition, complementary standard MD simulations were carried out to study the partitioning behavior of multiple anesthetics inside the lipid bilayer. The results obtained here are in line with previously reported simulations and suggest that the different forms of anesthetics induce different structural modifications in the lipid bilayer, which can provide new insights into their complex membrane translocation phenomena.

1. Introduction

Investigation of local anesthetics (LAs) has been the subject of extensive studies to determine their pharmacological mechanism of action. Local anesthetics prevent or relieve pain by interrupting nerve conduction via two different mechanisms: binding to the trans-membrane proteins like ion channels and blocking the influx of ions directly [1] or perturbing the lipid membrane matrix and altering the channel activity indirectly [2]. Despite the fact that local anesthetics have been used for a long time in medical and dental applications, the contribution of each mechanism to their nerve blocking action is still unclear. In addition, anesthetic's physicochem-

* Corresponding author.
E-mail address: alexander.lyubartsev@mmk.su.se (A.P. Lyubartsev).

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ical properties (stability, solubility, pKa) and partitioning behavior between non-polar (lipid) and polar (aqueous) phase can strongly affect the strength and the way of anesthetic action [3,4]. It was shown that the potency of LA increases roughly in proportion to the lipid/water partition coefficient which correlates to its binding ability to the lipid membrane [5]. Thus, a detailed understanding of the interaction between LAs and lipid structures is vitally important, which many authors have been looking for.

Anesthetics could partition to the cell membranes by passive diffusion. The rate of passive diffusion across a membrane is proportional to the partition coefficient of drug between the membrane and external medium [6]. The partition coefficient is a measure of the difference in solubility of a compound between two solvents. Among many alternative non-polar solvents e.g. hexanol, cyclohexane, dodecane, chloroform, n-octanol, the closest analogue to the phospholipid of membrane is n-octanol [7]. The partition coefficient of a solute between n-octanol and water ($\log P_{OW}$), which corresponds to the negative logarithm of the ratio of the concentration of the substance in the hydrophobic and aqueous phases, was first introduced by Hansch and Fujita [8]. Numerous experimental methods have also been developed for determination of $\log P_{OW}$ [9], which is a key parameter in the prediction of pharmacological and environmental properties of solutes.

Since the water-membrane partitioning of solutes is often difficult to monitor experimentally [10], computer simulations can be used as an alternative tool. Molecular dynamics (MD) simulations enable to explore the motions of biomolecules on a picosecond to multinanosecond time scale [11]. In the last years, simulations have been extensively used for studying the fully solvated lipid bilayers [12] and lipid bilayers containing drug molecules [13–18]. Despite this, fully understanding the passive diffusion of a solute from water phase to the center of the membrane requires a detailed examination of the underlying free energy landscape via the free energy calculation. The free energy changes along the selected degrees of freedom can be calculated from MD simulations by a variety of techniques [19–22]. The umbrella sampling (US) [19] is one of the most employed method to evaluate the translocation free energy profile of compounds such as amino acids [23], peptides [24], and drugs [25,26], through the membrane. Another powerful technique to explore the multidimensional free energy surface of complex polyatomic systems is metadynamics which has been tested and used in the variety of biophysical applications [27]. Granata et al. [28] have shown that the metadynamics simulations enhance the sampling efficiency of proteins by two or more orders of magnitude compared to the standard MD simulations. Minozzi et al. [29] applied this method to calculate the permeation of a boron-based β-lactamase inhibitor through the cell membrane. Jämbeck et al. [30] obtained the free energy profiles of aspirin, diclofenac, and ibuprofen embedded in lipid bilayer using metadynamics simulations. Recently, Bochicchio et al. [31] showed that both metadynamics and umbrella sampling methods yield the same estimates for the water-membrane free energy profile, but metadynamics can provide lower statistical uncertainties.

Studies of the interaction of local anesthetics and lipid membranes have provided valuable information at molecular level on the perturbing effects of anesthetics on the lipid membrane, which can be extrapolated into their mechanism of action [32]. Högberg et al. [13] and Mojumdar et al. [33] investigated the dynamical and structural properties of lidocaine and articaine in the dimyristoylphosphatidylcholine (DMPC) lipid membrane model at clinical concentrations. They have reported that the charged and uncharged forms of anesthetics show different properties in the lipid membrane. The charged lidocaine resides in the lipid headgroup region with the preferential orientation along the bilayer normal, while uncharged one is located further down in the upper part of the lipid tails and perpendicular to the bilayer normal. Different behavior of the charged and uncharged forms of anesthetics in the lipid membrane may be related to their different mechanisms of action. To study the diffusive nature of anesthetics through the lipid membrane quantitatively, Jalili et al. [26] investigated the hexadecane/water partition coefficient of drugs using expanded ensemble simulation. They have shown that the uncharged form of procaine and tetracaine anesthetics have greater partition coefficients compared to the charged ones. Because the complex nature of the potential energy surface with high energy barriers, the standard MD simulations could not sample the whole parts of phase space in the short time scale. To shed further light on the anesthetics partitioning from water to the membrane interior, particular care needs to be devoted into the sampling of high-energy states, which may not be sampled within the attainable simulation time. In order to enhance the sampling provided by regular MD simulations, we have used metadynamics to investigate the free energy landscape of anesthetics in the lipid bilayer, which will be discussed later. The use of the free energy landscape rationalizes the anesthetics translocation behavior by providing a clear illustration of this interaction in different parts of lipid membrane.

In this work we employ metadynamics simulations to predict the translocation energy barriers of two amide type local anesthetics, lidocaine and articaine, across the lipid membrane model. Both of these molecules exist in neutral and protonated (positively charged) forms. The pKa values are evaluated as 7.8 for lidocaine and 7.9 for articaine [34], that is why in neutral aqueous solution (pH = 7) charged forms of the molecules are predominant, while inside membranes the balance between the charged and neutral forms is expected to be shifted in favor of neutral forms. Since in classical molecular dynamics it is not possible to model protonation directly, we, similarly to other previous works, considered both charged and uncharged forms of the molecules separately. To our knowledge, this is the first attempt in computing the free energy profile for the partitioning of the local anesthetics as they percolate through the lipid membrane. We have analyzed the dynamical and structural properties of drugs during this process. We have also studied the dynamical behavior of multiple drug molecules, which were randomly distributed in the water-membrane interface at the beginning of the simulation, into the DMPC bilayer using standard MD simulations. The previous articles [13,33] have been focused on the simulation of lidocaine and articaine in the DMPC lipid membrane using united atom (UA) force field parameters, in which all nonpolar hydrogens have been included in the heavier atoms. Because the presence of hydrogen bond network around the choline headgroup of lipid molecules, using an accurate all-atomistic (AA) force filed is crucial [35]. In our study, all simulations have been performed using a new AA lipid force field (Slipids force filed) developed by Jämbeck et al. [36], which provides a well-balanced description between the hydrophilic and hydrophobic forces in the lipid bilayer. By coupling the conventional MD simulation of anesthetics in the lipid bilayer with the computed free energy profiles, we can gain full insight into the interaction between local anesthetics, water, and membrane to interpret their mechanism of action. We also measured the octanol/water partition coefficient for uncharged drugs using expanded ensemble simulation to compare with experimental data. The results discussed here will be valuable for revealing the molecular mechanism of LAs action.

2. Methods

2.1. Metadynamics

Free energy calculations are one of the most important techniques in theoretical and computational chemistry and have been proven to be a powerful tool in studying the biological systems [37]. Since the regular molecular dynamics simulations sample the free