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The interaction of local anesthetics with lipid membranes

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ABSTRACT

Molecular Dynamic Simulations are performed to evaluate the interaction of lidocaine, procaine and tetracaine with a lipid membrane. The main interest is to evaluate the structural changes produced by these local anesthetics in the bilayers. Penetration trajectories, interaction energies, entropy changes and an order parameter are calculated to quantify the destabilization of the lipid configurations. We show that such structural parameters give important information to understand how anesthetic agents influence the structure of plasma membranes. Graphic processing units (GPUs) are used in our simulations.

1. Introduction

Anesthetics are drugs that block the nervous impulse causing temporal disruption of sensation in specific parts of the body [1–3]. Although there are several theories to explain their action, there is a still an unfinished debate. The main approach suggests the existence of specific binding sites on the ion channels that regulate the membrane hyperpolarization [4–10]. Yet, important concerns remain due to the fact that this approach does not take into consideration how anesthetic molecules affect the properties of the lipids surrounding such interaction sites [11–15]. A theory that addresses the anesthetic effect in a more general fashion is based on the old work of Meyer and Overton [16,17]. Succinctly, it teaches us that the oil/water partition coefficient can be seen as a measure of the anesthetic potency: if the drug is soluble in oil, it diffuses into the hydrophobic region of the membrane. The nature of this interaction is non-polar, where van der Waals forces are present [18]. This phenomenon is reduced into a constant induction of dipoles of both anesthetic and the hydrophobic region of the bilayer (hydrocarbonated tails), causing, in general, a destabilization and an increase of the membrane fluidity [19]. Indeed, there are recent studies that demonstrate explicitly [20] and implicitly [21,22], the importance of the polarizability in the drug–lipid interaction. Related to such findings, other researchers have reported that in order to produce anesthesia, local anesthetics must have a large affinity to water [23].

Though local and general anesthetics have different sites of action, they share the same type of electrostatic interaction in proteins or lipids: mostly van der Waals. An exquisite survey about how inhaled anesthetics work has been furnished by Eckenhoff [24]. The author observes that the mechanism of drug action is poorly represented in the pharmacology literature, which remains entrenched in the single-target model for drugs. He proposes, instead, that anesthesia is due to small effects at many biological sites. Though his small-effects-at-many-sites model may be considered inelegant (because it will be difficult to validate) he affirms that this multiple target hypothesis is most consistent with clinical observation, the available data, and the remarkable resistance that this 160 year-old dilemma has offered to solution. Motivated by such rationale, Molecular Dynamic Simulations have been carried out to investigate the molecular mechanism of drug attachment in membranes [25] or in protein cavities [26].

The lipid-centered mechanism cannot be fully discarded in the light of a recent work proposed by Heimburg and Jackson [27,28]. The authors suggest that a mechanical perturbation, a soliton, accompanies the electrical impulse. Looking neural excitability through this novel prism, it is a tantalizing idea to consider local anesthesia as a simple blockage of such putative soliton (the strength of it depends on the compressibility of the medium through which it travels, so if the medium is fluidized the solitary wave weakens). If this interesting idea is right, we need to know how anesthetics cause membrane disruption and therefore fluidization.

Local anesthetics are amphiphilic (i.e. they have polar and non-polar regions) and, clinically, the most commonly used are of the amide-type. However, although they have a polar region,

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anesthetics exhibit oil/water partition coefficients larger than one [29] and therefore are prone to interact with the hydrophobic region of the lipid membrane. We use Molecular Dynamic Simulations (MDS) to explore how the structural details of a lipid membrane changes during the interaction of three cationic amphiphilic anesthetic drugs. Needless to say that MDS is a well established tool to study the thermodynamic properties of pure lipid [30–35], and doped [36–39] membranes.

In this work we study the effects of lidocaine ($C_{14}H_{22}N_2O$), procaine ($C_{13}H_{20}N_2O_2$), and tetracaine ($C_{15}H_{24}N_2O_2$) on a DPPC ($C_{40}H_{80}NO_8P$) bilayer membrane. For the first time, interaction energies (Coulomb and Lennard-Jones), configurational entropies and order parameters are calculated all together in order to understand the effect of anesthetic drugs on the membrane structure.

2. Methods

2.1. Molecular Dynamic Simulations

Since choline phospholipids are the most abundant species in plasma membranes [40], the membrane we study is formed by 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) lipids. 193 of them were grouped in a 10 nm × 9 nm × 10 nm box to form a bilayer. The superficial extension of the membrane corresponds to the *XY* plane while *Z* represents the height. 10 molecules of each drug were studied separately; homogeneously positioned 2 nm above the membrane, at time zero. The 3D structures, force fields, and partial charges of the molecules, were obtained from the following sources: DPPC was obtained from Krüeger and Fischer [41], lidocaine from Högberg et al. [42], procaine and tetracaine were built using the online server PRODRG (available at http://davapc1.bioch.dundee.ac.uk/prodrg/) and swissParam (http://swissparam.ch/) following the indications of diverse references [43–46]. The parameters of the GROMOS 53a6 force field library were used to obtain the information of bonds, dihedral angles and the Lennard-Jones interactions (for more information see Oostenbrink [47]). Once we have the structures and their parameters, the minimum energy states for the drug molecules, prior to the build-up of the systems, are found.

After obtaining the initial output of the system, 15,151 single point charge (SPC) water molecules were added to cover the entire simulation space. 153 of these were substituted by NaCl to emulate a physiological ionic concentration of 0.14 M. A pertinent observation is needed: the drugs studied here are normally (clinically) administrated in a substance with low pH (pH 4–5). Since the pK's of the drugs are around 8, they enter the organism in a protonated state. In this work, the pH of the medium was 7, so they are also protonated.

As a previous step to the MDS, the solvated systems were put through short minimization runs to reach values of 1×10^3 kJ/mol. The parameters employed for the dynamics were as follows: PME (Particle Mesh Ewald) conditions were employed to calculate the electrostatic interaction, then a cut-off of 1.2 nm was assigned to Lennard-Jones and Coulomb interactions. A leap-frog algorithm was employed with time steps of 2 fs. The overall system center of mass translation and rotation was removed at every step. The bond lengths were constrained using the LINCS algorithm [48] with a relative geometric tolerance of 10^{-4}. The system was kept at 37 °C and of 1 bar of pressure. The thermostat we used was the Nose–Hoover [49] and the barostat is based on the work of Parrinello–Rahman [50]. Since earlier work has shown that simulations of membranes and small solutes can been drastically underestimated due to the presence of hidden sampling barriers involving the slow reorganization of the lipid–water interface in response to solute insertion [51,52], we run our MD simulations up to 100 ns with 100 ns to pre-equilibrate the system. It is worthwhile to sate here that similar or lesser time lengths are used in related articles [44,45]. We used the GROMACS v.4.6.3 simulation package [53] which run in a GPUs platform. The entire time periods were used for analysis.

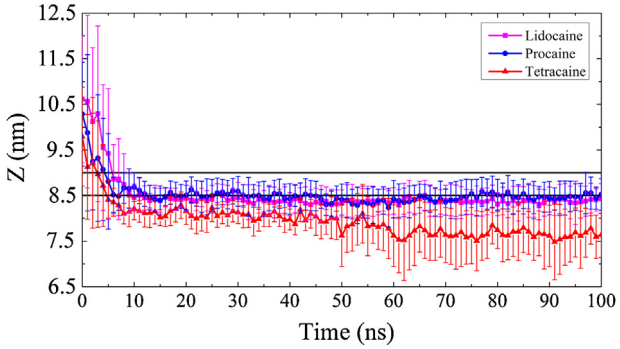

Fig. 1. The distances from the center of the bilayer to the center of mass of a drug, as a function of time. Lidocaine (squares); procaine (circles); tetracaine (triangles). The black lines identify the upper polar region of the membrane, and the center of the bilayer is at *Z* = 7 nm.

It was possible to reduce the degrees of freedom of the system applying the united atom models methodology, following the indications of various articles [37,42,44]. By adding 10 drug molecules (which correspond to a normal dose in anesthesia: around 20 mM) with different initial angles and positions, we avoid the requirement of repeating the simulation a number of times. Then, averaged results are gathered and presented. All simulated drugs reach their final position at around 30–50 ns. The main objective of this article is to differentiate the perturbation each drug causes on the membrane, rather than the time it takes to the system to reach its final equilibrium.

We employed the data obtained from the corresponding Coulomb and Lennard-Jones energies of the 193 lipids that form the membrane interacting with each one of the 10 molecules. In total, we register 3860 interactions per case during the last 80 ns of

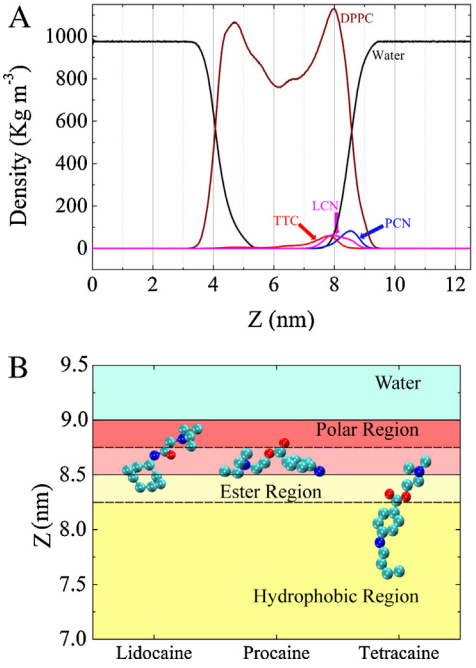

Fig. 2. (A) Mass density profiles for water, lipids, lidocaine, procaine, and tetracaine. (B) Schematic final positions of each drug to illustrate their final orientation. The background shows different regions of the upper lipid bilayer. The center of the bilayer is at *Z* = 7 nm.