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Preferential location of prilocaine and etidocaine in phospholipid bilayers: A molecular dynamics study

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ABSTRACT

In this work, we report a 20-ns constant pressure molecular dynamics simulation of the uncharged form of two amino–amide local anesthetics (LA), etidocaine and prilocaine, present at 1:3 LA:lipid, molar ratio inside the membrane, in the hydrated liquid crystal bilayer phase of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC). Both LAs induced lateral expansion and a concomitant contraction in the bilayer thickness. A decrease in the acyl chain segment order parameter, $-S_{CD}$, compared to neat bilayers, was also observed. Besides, both LA molecules got preferentially located in the hydrophobic acyl chains region, with a maximum probability at ~12 and ~10 Å from the center of the bilayer for prilocaine and etidocaine, respectively.

1. Introduction

The amphiphilic character of phospholipids allows them to spontaneously form organized structures, like thin lipid films and liposomes. These organized structures can be used to create novel structures, materials, and devices for use in nanotechnology (like biosensors, drug carriers, etc.) [1]. Drug insertion into these structures can change their physical properties and could be used to functionalize them.

Back at the end of the nineteen century, the Meyer–Overton rule disclosed the relationship between hydrophobicity of anesthetic compounds and their potency. Since the majority of the local anesthetic (LA) molecules are amphiphile amines in which pK_as lie around physiologic pH, the importance of the uncharged LA species became evident [2].

In this work, we carried out a series of simulations where the uncharged form of two aminoamide LAs, etidocaine (EDC) and prilocaine (PLC), were introduced into a POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine) phospholipid bilayer up to a LA:lipid molar ratio of 1:3 (Fig. 1).

2. Methodology

Simulations were performed using the NAMD2 program [3] with the CHARMM27 parameter set for POPC; the water molecules were described by the TIP3P model. The simulated system consisted in a lipid bilayer containing 120 fully hydrated POPC molecules (60 in each monolayer), 3600 water molecules and 40 neutral LA (EDC or PLC) molecules, periodically replicated. In addition, a plain and fully hydrated POPC bilayer was simulated, as reference.

Classical MD simulations were performed within the NPT ensemble. The temperature was set at 310 K and the pressure at 1 atm, in order to POPC bilayers to be found in the fluid lamellar phase. Langevin dynamics and Langevin piston methods were used to keep temperature and pressure constant. The shortest time step was 2 fs. The short-range forces were computed using a cutoff of 10 Å and the long-range forces were taken into account by means of the particle mesh Ewald (PME) technique.

The simulations consisted of an equilibration period of about 5 ns, within each of the LA molecules migrated to their preferential location relatively to the membrane, followed by a 20 ns production run.

3. Results

Both LA were initially placed randomly inside the lipid bilayer. The presence of the LAs inside the lipid bilayer disturbs its properties. For example, the area per lipid, defined as the average projected in-plane area divided by the number of lipid molecules per monolayer, expands from 58.14(1) for plain bilayer to 73.88(4) and 70.85(3) Å^2 for EDC-bilayer and PLC-bilayer, respectively. On the other hand, the bilayer thickness, L_z, decreased in the presence of both anesthetics, from 71.72(1) to 62.29(3) and 64.14(2), respectively, suggesting a disorganization of the membrane in the

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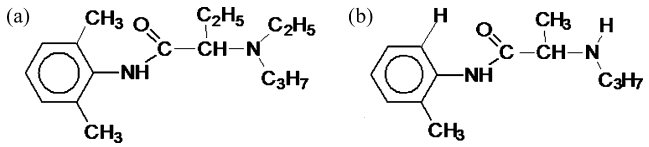

Fig. 1. Etidocaine (a) and prilocaine (b) molecules.

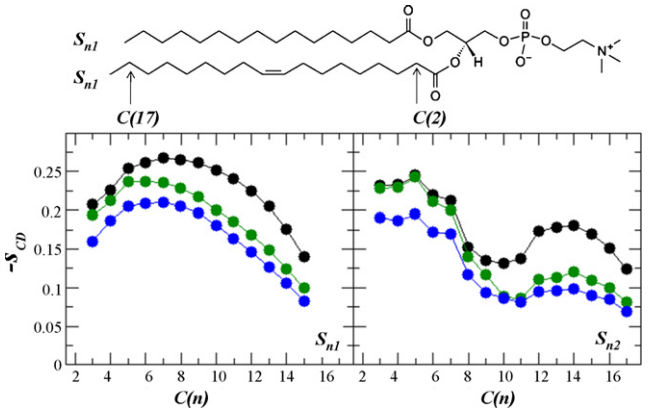

Fig. 2. Changes in the order parameter, $-S_{CD}$, for plain bilayers (black); EDC (blue) and PLC (green)-containing bilayers along the hydrocarbon chain (see scheme on top of the figure) for (i) saturated and (ii) unsaturated chains of POPC. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

presence of both LAs. LAs are believed to create inter-lipid spaces that would permit an increase in the probability of trans-gauche conformations, decreasing the acyl chain order. In this way, we calculated the order parameters of each CH_n ($n = 1, 2$) groups as shown in Fig. 2. The CH_n groups are numbered consecutively (see scheme in Fig. 2). We have separated the *sn*-1 (a) palmitoyl and *sn*-2 (b) oleoyl tails. As a reference we show in black the order parameter for plain POPC. The overall effect of both LAs (at 1:3 LA:lipid molar ratio inside the membrane) is to disorganize the membrane (decrease the order parameters). The effect is seen for both LAs, however it is more pronounced for EDC than for PLC. The main difference between EDC and PLC effect upon the order parameters can be seen for carbons numbers 3–7 of the oleoyl lipid tails, where the $-S_{CD}$ values for EDC are considerable lower. The changes in S_{CD} for the palmitoyl tail are more uniform and smaller. This difference suggests that EDC and PLC are found in different regions of the lipid bilayer. To verify that, we have calculated the electron density profile (EDP) [4].

We show, in Fig. 3a, the EDP of each of the CH_n groups of the oleoyl tail of EDC-bilayer. The order of the CH_n groups corresponds to the numbering in Fig. 2a. $z = 0$ corresponds to the bilayer center. Just subtle differences were found in respect to the same tail of the PLC-bilayer. The tail densities differences were more evident when compared with the plain bilayer, as expected from the order parameter results.

In the same scale, we show in Fig. 3(b) the electron density profile corresponding to the EDC (blue) and PLC (green) molecules. We can see from this figure that both uncharged LAs are found in the lipid tail region. The maxima are located around C(4) and C(5) for PLC, and around C(7) for EDC. Besides, EDC molecules enter deeply in the hydrophobic core, as one can see comparing the density in the membrane center. In this way, we show that the penetration in the membrane is strongly related to the LA hydrophobicity. Besides, neither PLC nor EDC shows preferential orientation inside the bilayer.

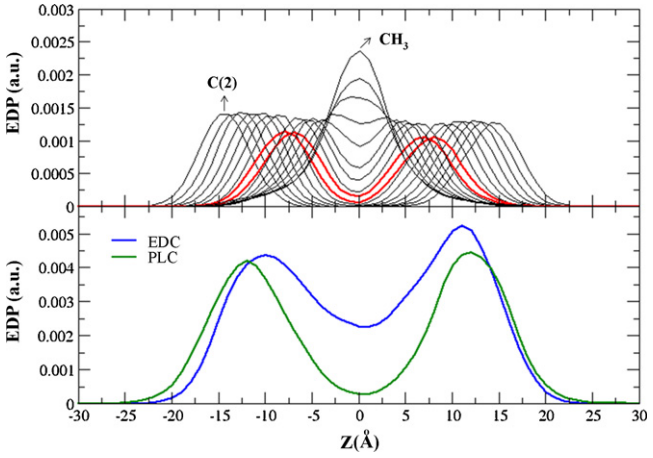

Fig. 3. (a) Contributions to the to EDPs of CH_n groups from the oleoyl tail of the EDC-containing lipid bilayer. The number sequence for the lipid CH_n groups ($n = 1$, 2) was given in Fig. 2. Unsaturated groups are shown in red. (b) EDP of etidocaine (blue) and prilocaine (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

4. Discussion

In the present work, we have investigated the interaction of the uncharged form of two local anesthetics, PLC and EDC, with lipid membranes through molecular dynamics simulations. The partition of both LAs induced lateral expansion and a concomitant contraction in the bilayer thickness.

Through the analysis of the acyl chain order parameters, we found a decreasing of the overall lipid tail organization, both for saturated and unsaturated tails. This effect was more pronounced for the EDC case. Moreover, our results are in good agreement with de Paula et al. [5], who found that PLC decreased the segmental order parameters of perdeuterated DPMC-*d*54 enriched egg phosphatidylcholine liposomes acyl chain as a whole, but mainly at carbons deeper than C(6), probably due to its preferential positioning up to this position, at the same LA:lipid molar ratio (1:3) than ours. On the other hand, it is not possible, experimentally, to reach 1:3 EDC:lipid molar ratio inside the bilayer due to the low solubility of the uncharged EDC species that induces membrane "saturation" [6]. We will discuss these results in more detail elsewhere.

PLC and EDC are essentially found in the hydrophobic acyl chains region, with a maximum probability between C(4)–C(5) and C(7) methylene groups for PLC and EDC, respectively. In this way, we detected a direct correlation between LA preferential location and hydrophobicity and therefore with potency, following the Meyer–Overton rule.

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