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Effect of the interfacial tension and ionic strength on the thermodynamic barrier associated to the benzocaine insertion into a cell membrane

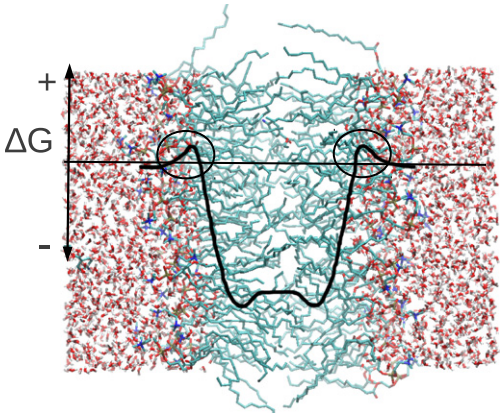
J.J. López Cascales *, S.D. Oliveira Costa

Universidad Politécnica de Cartagena, Grupo de Bioinformática y Macromoléculas (BioMac) Aulario II, Campus de Alfonso XIII, 30203 Cartagena, Murcia, Spain

HIGHLIGHTS

- The insertion of benzocaine into a phospholipid bilayer is a spontaneous process.
- A thermodynamic barrier was evidenced at the bilayer/water interface.
- This barrier diminishes with the fraction of charged lipid in the bilayer.
- The ionic strength diminishes as well this thermodynamic barrier.
- The interfacial tension plays a crucial role in this thermodynamic barrier.

GRAPHICAL ABSTRACT



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ABSTRACT

The insertion of local anaesthetics into a cell membrane is a key aspect for explaining their activity at a molecular level. It has been described how the potency and response time of local anaesthetics is improved (for clinical applications) when they are dissolved in a solution of sodium bicarbonate. With the aim of gaining insight into the physico-chemical principles that govern the action mechanism of these drugs at a molecular level, simulations of benzocaine in binary lipid bilayers formed by DPPC/DPPS were carried out for different ionic strengths of the aqueous solution. From these molecular dynamic simulations, we observed how the thermodynamic barrier associated with benzocaine insertion into the lipid bilayers diminished exponentially as the fraction of DPPS in the bilayer increased, especially when the ionic strength of the aqueous solution increased. In line with these results, we also observed how this thermodynamic barrier diminished exponentially with the phospholipid/water interfacial tension.

1. Introduction

The clinical potency of local anaesthetics (LA) often depends on two facts: their vascular absorption and their distribution in the tissue surrounding the site of deposition. The ability to partition the anaesthetic molecules into various compartments is a crucial aspect related with their activity from a molecular point of view. In this context, local anaesthetics, like many neuroactive drugs, must penetrate into, or pass through the neuronal plasma membrane to be pharmacologically active [1,2]. Furthermore, in clinical local anaesthetic procedures, the pharmacological drugs must pass through the perineum, which is composed of fibrous and cellular barriers before reaching the

* Corresponding author. Tel.: +34 968325567; fax: +34 968325931.
E-mail address: javier.lopez@upct.es (J.J. López Cascales).

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nerve fibres. Hence, membrane permeation and adsorption processes control LA penetration and their resulting concentration at the intended site of action [2].

Certain liposoluble LA in physiological conditions may present a non-protonated (neutral) and protonated (charged) form, where the non-protonated form have been observed to be more membrane-permeant than the protonated one, enhancing it to pass through the hydrophobic barrier of the membrane [3,4]. In this regard, the clinical use of local anaesthetic dissolved in a sodium bicarbonate buffer has been widely used because, it attenuates the pain involved in skin infiltration [5], and together, they produce a more rapid onset and a decrease in the minimum concentration required to achieve a therapeutic dose [6], bearing in mind the high pKa of most such pharmacological species, which range from 7.7 for the mevipacaine to 9.8 for the piperocaine [2,6]. Thus, for example, the clinical effects of bupivacaine and lidocaine are strengthened (and hence, their dosage reduced) when they are administrated dissolved in a sodium bicarbonate buffer [5]. However, the contribution of the ionic strength of the sodium bicarbonate solution (sodium concentration) to the activity of these anaesthetics has, to date, been ignored. In this context, this work focuses on the effect of the ionic strength (sodium concentration) of an aqueous solution on the insertion process of benzocaine into a lipid bilayer composed of different fractions of charged lipids. In this regard, the cell membrane was modelled by symmetric binary lipid bilayers formed of dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylserine (DPPS), where the DPPC is a neutral phospholipid, the DPPS is a phospholipid which bears a negative charge in physiological conditions, and benzocaine is a non-charged liposoluble local anaesthetic in physiological conditions due to its low pKa of 3.5 (in this regard, the effect associated with the hydrolysis of the anaesthetic can be discarded) [4,7,8], and where it was ignored the presence of other important molecules in our model of cell membrane, such as the presence of cholesterol. Molecular dynamics simulation was used to carry out the present study.

In previous investigations carried out by our group [9,10], it was determined how the insertion of benzocaine into lipid bilayers is a spontaneous process from a thermodynamic point of view, in which entropy is the driving thermodynamic force responsible of this spontaneous process [10]. Furthermore, the existence of a thermodynamic barrier during the benzocaine insertion into the cell membrane was determined. This barrier, located at the lipid/water interface, diminished as the fraction of charged lipids in the lipid bilayer increased [10]. Keeping in mind the results mentioned above, this work looks at how the ionic strength of the aqueous solution affects the insertion of benzocaine into a lipid bilayer, concentrating on two main aspects:

1 The effect of the ionic strength on the thermodynamic barrier associated with benzocaine insertion into a lipid bilayer.
2 How the interfacial tension between the phospholipid bilayer and the aqueous solution plays a crucial role in diminishing this thermodynamic barrier.

2. Model and methods

2.1. Model

Five different lipid bilayers were generated with the goal of analysing the full range of lipid compositions of a binary bilayer composed of DPPC and DPPS. The starting system was formed by a bilayer composed of 72 DPPC molecules (36 DPPC per leaflet) and 5042 water molecules of the SPC water model [11]. A precise description of how the three dimension periodical box was generated is given elsewhere [12–14,9]. Once this bilayer of DPPC was generated, four additional bilayers were constructed by substituting 12, 24, 48 and 72 DPPC molecules with 12, 24, 48 and 72 DPPS molecules. To balance the negative charge associated to each DPPS molecule under physiological conditions, 12, 24, 48 and 72 sodium ions (Na^+) were introduced into the system by substituting water molecules with sodium ions. In summary, five different binary bilayer of DPPC:DPPS were generated with the following compositions 72:0, 60:12, 48:24, 24:48 and 0:72.

The molecular fraction of DPPS that forms the lipid bilayer, χ, was defined as follows:

$$\chi = \frac{n_{DPPS}}{n_{DPPC} + n_{DPPS}} \quad (1)$$

where χ represents the molecular fraction of DPPS (n_{DPPS}) with respect to the total number of lipids in the bilayer ($n_{DPPC} + n_{DPPS}$).

Finally, to simulate a 0.5 N NaCl concentration in the aqueous solution, a water molecule was randomly substituted by one sodium and one chloride ion, respectively, every 111 water molecules in each of the systems generated above (after considering the following approximation: 0.5 N (NaCl) = 0.5 M (NaCl) = 0.5 moles (NaCl)/1 (litre of solution) ≈ 0.5 moles (NaCl)/1 kg (H_2O) = 0.5 moles (NaCl)/55.6 moles H_2O, i.e., we obtain a ratio of 1(Na^+):111(H_2O), and the same for the Cl^-).

2.2. Simulation parameters

The GROMACS 3.3.3 package [16,17] was used to perform the molecular dynamic simulations, with a constant integration time step of 2 fs. The electrostatic contribution was calculated using a long range electrostatic interaction by the Particle Mesh Ewald method [18,19], in which all the coordinates of the simulated trajectories were recorded every 5 ps of simulation time. Bond lengths were constrained using the LINCS algorithm [20]. All the simulation boxes were coupled to an external pressure and temperature bath, using the Berendsen algorithm [21], with temperature and pressure coupling constants of 0.1 and 1 ps, respectively. Due to the anisotropy of the membranes along the *Z*-axis, all the simulations were carried out using a semi-isotropic pressure algorithm coupling bath. The simulated trajectory lengths were of 100 ns, where the first 10 ns of each simulation were discarded for analysis because this was the time required by the systems to achieve an equilibrated state. All the simulations were carried out at 350 K, a temperature that was chosen because it is above the transition temperature of 314 K [22] and 326 K [23] for pure bilayers of DPPC and DPPS, and also because this temperature is above the transition temperature of all the binary bilayers formed by DPPC/DPPS, as deduced from the corresponding experimental phase diagram [24]. In short, the temperature of 350 K ensures that all these binary bilayers are in liquid crystalline state, regardless of the fraction of DPPS in the lipid bilayer. The molecules of DPPC and DPPS were simulated using the force field employed in previous simulations of lipid bilayers formed exclusively of DPPC [25] and DPPS [12]. As in previous articles in which DPPS was involved in our simulations [13,26,27], the charge distribution of DPPS and of the rest of species with net charge were reduced by a factor of two to compensate, in part, the absence of polarizability in our models. This reduction that has been effective in the study of soap/alcohol/water interfaces, biological membranes and micelles [25,28] is based, in short, to the fact that coulombic interactions in these systems are exaggerated due to the insufficient screening performance of the SPC [11] water model used in our simulations.

Benzocaine was modelled as in previous simulations [9,10], where Fig. 1 shows the charge distribution used to simulate this molecule. The bond distances, angles, and LJ parameters were taken from the standard GROMOS-87 force field [29].

Since calculation of the lateral pressure profile needs much computing power, the trajectories generated using the Particle Mesh Ewald method were employed to recalculate the pressure tensor of Eq. (10) using the cut-off method rather than the Particle Mesh Ewald method mentioned above. In the process of calculating the lateral pressure profile, short and long spherical cutoffs of 1.4 and