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Molecular dynamics simulation of the partitioning of benzocaine and phenytoin into a lipid bilayer

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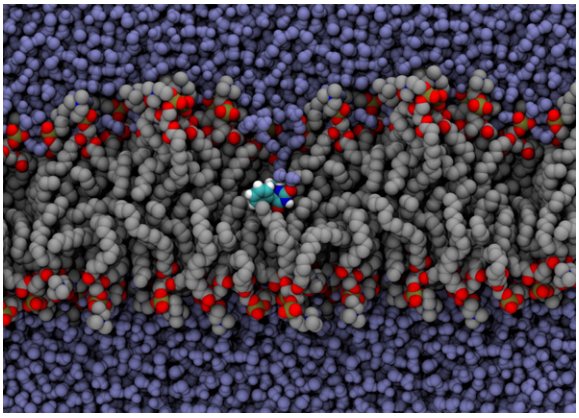
HIGHLIGHTS

- The local anaesthetics benzocaine and phenytoin partition into a lipid membrane.
- Both drugs face a barrier to cross the bilayer centre.
- Both drugs can pull water into the membrane and create extended water chains.
- Once in the membrane they can alter bilayer properties or reach target proteins.
- Results depend on atomic charges showing importance of validating new drug parameters.

GRAPHICAL ABSTRACT



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ABSTRACT

Molecular dynamics simulations were used to examine the partitioning behaviour of the local anaesthetic benzocaine and the anti-epileptic phenytoin into lipid bilayers, a factor that is critical to their mode of action. Free energy methods are used to quantify the thermodynamics of drug movement between water and octanol as well as for permeation across a POPC membrane. Both drugs are shown to favourably partition into the lipid bilayer from water and are likely to accumulate just inside the lipid headgroups where they may alter bilayer properties or interact with target proteins. Phenytoin experiences a large barrier to cross the centre of the bilayer due to less favourable energetic interactions in this less dense region of the bilayer. Remarkably, in our simulations both drugs are able to pull water into the bilayer, creating water chains that extend back to bulk, and which may modify the local bilayer properties. We find that the choice of atomic partial charges can have a significant impact on the quantitative results, meaning that careful validation of parameters for new drugs, such as performed here, should be performed prior to their use in biomolecular simulations.

1. Introduction

Local anaesthetic, anti-epileptic and anti-arrhythmic drugs are known to target voltage-gated sodium channels residing in cell membranes [1]. The potency of these compounds is positively correlated with lipophilicity, [2–5] indicating that their ability to enter or cross membranes is critical to their mechanism of action. It is likely that the efficacy of these compounds is directly related to their ability to reach their protein target, where they act to block currents or alter channel kinetics [6]. Electrophysiological studies have shown that these compounds can reach their binding site in the centre of the sodium

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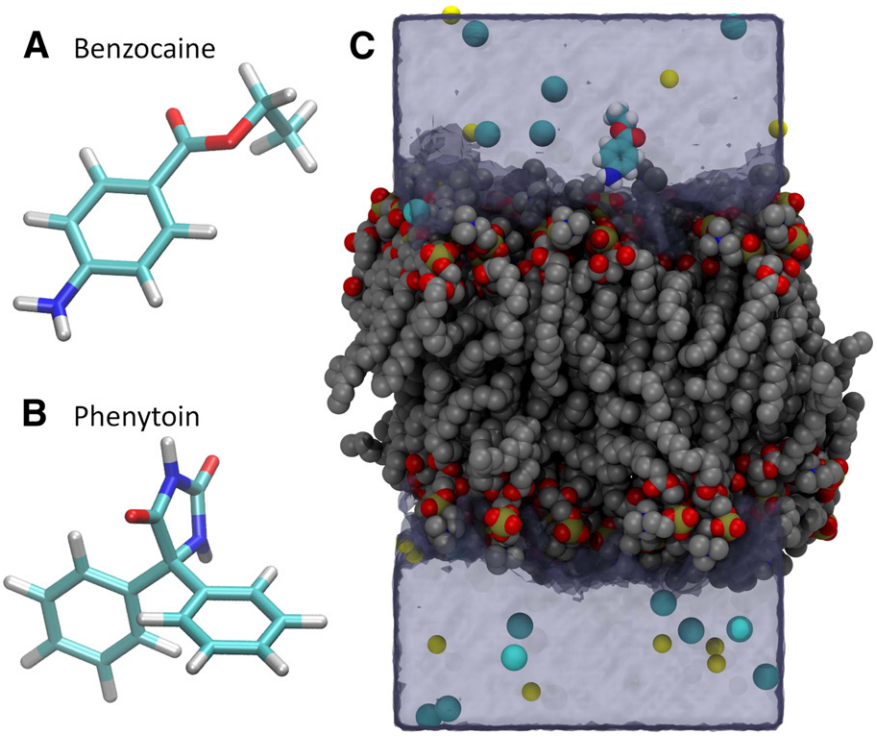

Fig. 1. Images of (A) benzocaine, (B) phenytoin and (C) the simulation system. The drugs are coloured according to atom type (carbon cyan, oxygen red, nitrogen blue and hydrogen white). In (C) Na^+ is shown in yellow, Cl^- in cyan, phosphorus in brown and the lipid carbons in grey. The transparent surface indicates the volume sampled by water.

channel pore by one of two routes [7]. The first involves passing through the intracellular activation gate from the cytoplasm when the channel is open. The second involves passing through an alternative hydrophobic access route directly from the lipid [7] to yield tonic block of resting channels. The hydrophobic pathway through the protein was given clarity in recent studies of bacterial channels, [8–11] and the ability of a drug to find this passage is critically dependent upon its apportionment into the lipid bilayer as well as its ability to fit through the gap in the protein. In each case the compounds have to be able to enter or cross the membrane and so understanding the partitioning of these compounds between water and lipid is of great importance to understanding their mode of action.

In addition to direct interaction between local anaesthetics and sodium channels, the cumulative effect of drugs on the lipid bilayer is also implicated in anaesthetic action [12]. Even before the nature of cell membranes was understood, a correlation between the hydrophobicity of an anaesthetic agent and its potency was discovered [13,14]. More recently anaesthetic action has been tied to changes in the lateral pressure of a lipid bilayer, a property that can vary along the membrane normal and possibly affect channel activity by altering the conformational landscape [15–17]. The partitioning of anaesthetics into membranes could also alter other local properties of the bilayer and thus modify the behaviour of a range of membrane bound proteins.

As the ability of a huge number of drugs to passively cross the bilayer is essential to their activity, a great many simulation and experimental investigations of lipid permeability and water/lipid partitioning have been conducted (see [18] and references therein). More specifically a number of simulation studies have examined the interaction between sodium channel targeting drugs and membranes at atomic detail. These have included investigations into the thermodynamics of insertion of benzocaine into DPPC and mixed DPPC/DPPS lipid bilayers, [19,20] and the likely positioning and influence of a DMPC bilayer of charged and uncharged lidocaine [21,22] and articaine [23] relative to a DMPC bilayer. Indeed, the free energy of solute transfer from the water phase into the membrane has been calculated for various anaesthetic compounds and it was found that the work to create a cavity able to locate a permeant solute is lower inside the membrane than in water, whereas the electrostatic contribution to the solute transfer increases monotonically going from water into the membrane interior. A balance between these two opposite effects causes dipolar compounds to accumulate at the water/membrane interface, whereas apolar compounds resided predominantly in the membrane core [18]. This behaviour was qualitatively related to the anaesthetic power of these compounds, with the most polar compounds that concentrate at the interface being the most powerful [24]. Polar anaesthetics thus tend to experience a barrier to cross the centre of the bilayer due either to the removal of interactions with the polar components of the bilayer or the compound reducing the lipid mobility when placed in the membrane core. There appear to be specific relationships between solute size on mobility and partitioning, although there is still some doubt as to which property is most strongly altered by solute size [18].

The choice of force field parameters can have a large influence on the results of molecular dynamics simulations, but this aspect has not been thoroughly explored in the context of local anaesthetic partitioning. The effect of properties such as partial atomic charge on the spontaneity of lipid partition can be tested in simulation and measured against values determined by experiment to determine their accuracy. The specific studies of local anaesthetics described above have employed united atom force fields and it is possible that this can alter the effective polarity and size of the compounds. In addition, these parameters were not verified for their ability to reproduce measured partition coefficients.

Here we examine the behaviour of the local anaesthetic benzocaine (Fig. 1A) and the anti-epileptic phenytoin (Fig. 1B) in a POPC membrane and calculate the free energies of drug partitioning and bilayer permeation. The polar surface areas of benzocaine and phenytoin are 54.3 $Å^2$ (24.0% of total) and 69.5 $Å^2$ (23.4% of total) respectively, meaning that benzocaine would be expected to cross membranes more easily. We calculate the results using a range of atomic partial charges to examine how the choice of these parameters alters the thermodynamics of partitioning. The comparison between linear benzocaine and the