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Modeling interactions between blocking and permeant cations in the NavMs channel

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ABSTRACT

Mechanisms of sodium channel block by local anesthetics (LAs) are still a matter of intensive studies. In the absence of high-resolution structures of eukaryotic channels, atomic details of LA-channel interactions are analyzed using homology modeling. LAs are predicted to access the closed channel through a sidewalk (fenestration) between the channel repeats, bind in a horizontal orientation, and leave its aromatic moiety in the interface. Recent X-ray structure of a bacterial sodium channel NavMs with a cationic molecule Pl1, which is structurally similar to LAs, has confirmed this theoretical prediction and demonstrated a reduced selectivity filter occupancy by the permeant ions in the Pl1-bound channel. However, the nature of the antagonism between LAs and permeant ions is still unclear. Here we used the NavMs structure and Monte Carlo energy minimizations to model Pl1 binding. Our computations predict that Pl1 can displace permeant ion(s) from the selectivity filter by both steric and electrostatic mechanisms. We hypothesize that the electrostatic mechanism is more general, because it is applicable to many LAs and related drugs, which lack a moiety capable to enter the selectivity filter and sterically displace the permeant ion. The electrostatic mechanism is also consistent with the data that various cationic blockers of potassium channels bind in the inner pore without entering the selectivity filter.

1. Introduction

Interaction between current-carrying and blocking ions is a fundamental problem in structural pharmacology of ion channels. In simple terms, a pore-blocking particle creates an energy barrier for permeating ions (Catterall, 2002). The antagonism between permeant ions and a charged blocking molecule in the pore is not surprising. Permeant cations can affect the channel blockade either by direct competition with the cationic blocker for the binding site in the inner pore, or by electrostatic repulsion with the blocker (Hille and Schwarz, 1978; Zhorov and Tikhonov, 2004, 2013). The possibility of different types of drug-ion interactions seriously complicates the picture of state- and voltage-dependence of the drug action on ion channels (Bruhova et al., 2008; Hille and Schwarz, 1978; Triggle, 2007; Wulff et al., 2009).

A particularly important problem is the mechanism of sodium channel block by local anesthetics (Catterall, 2000; Clare, 2010; Fozzard et al., 2005; Nau and Wang, 2004; Ruetsch et al., 2001). A widely accepted "modulated-receptor hypothesis" suggests that local anesthetics possess different affinities to different channel states (resting, open and inactivated) and can reach their binding site through hydrophobic and hydrophilic access pathways (Hille, 1977). The hydrophilic access pathway through the open activation gate is obvious, whereas location of the hydrophobic access pathway into the closed channel has long been debated. Using the homology modeling approach we proposed that the pathway involves a "sidewalk" between the pore helix of repeat III and S6 helices of repeats III and IV (Tikhonov et al., 2006). This suggestion resulted in the model, in which a LA molecule such as tetracaine or lidocaine binds in the closed sodium channel in a horizontal orientation with its charged aminogroup entering the inner pore and aromatic moiety remaining in the III-IV repeat interface. In this binding mode, the LA molecule is predicted to displace the resident sodium ion from the central cavity and electrostatically repel a sodium ion in the selectivity filter (Bruhova et al., 2008; Tikhonov and Zhorov, 2007).

High-resolution structures of eukaryotic sodium channels are still unavailable, but X-ray structures of several bacterial sodium channels have confirmed key prediction of the models. Structures of homotetrameric channels NavAb (Payandeh et al., 2011), NavRh (Zhang et al., 2012) and NavMs (McCusker et al., 2012) demonstrate fenestrations between subunits, which are wide enough to provide a sidewalk pathway for organic ligands. More recently, the structure of NavMs channel with a bound LA-like ligand, Pl1, has

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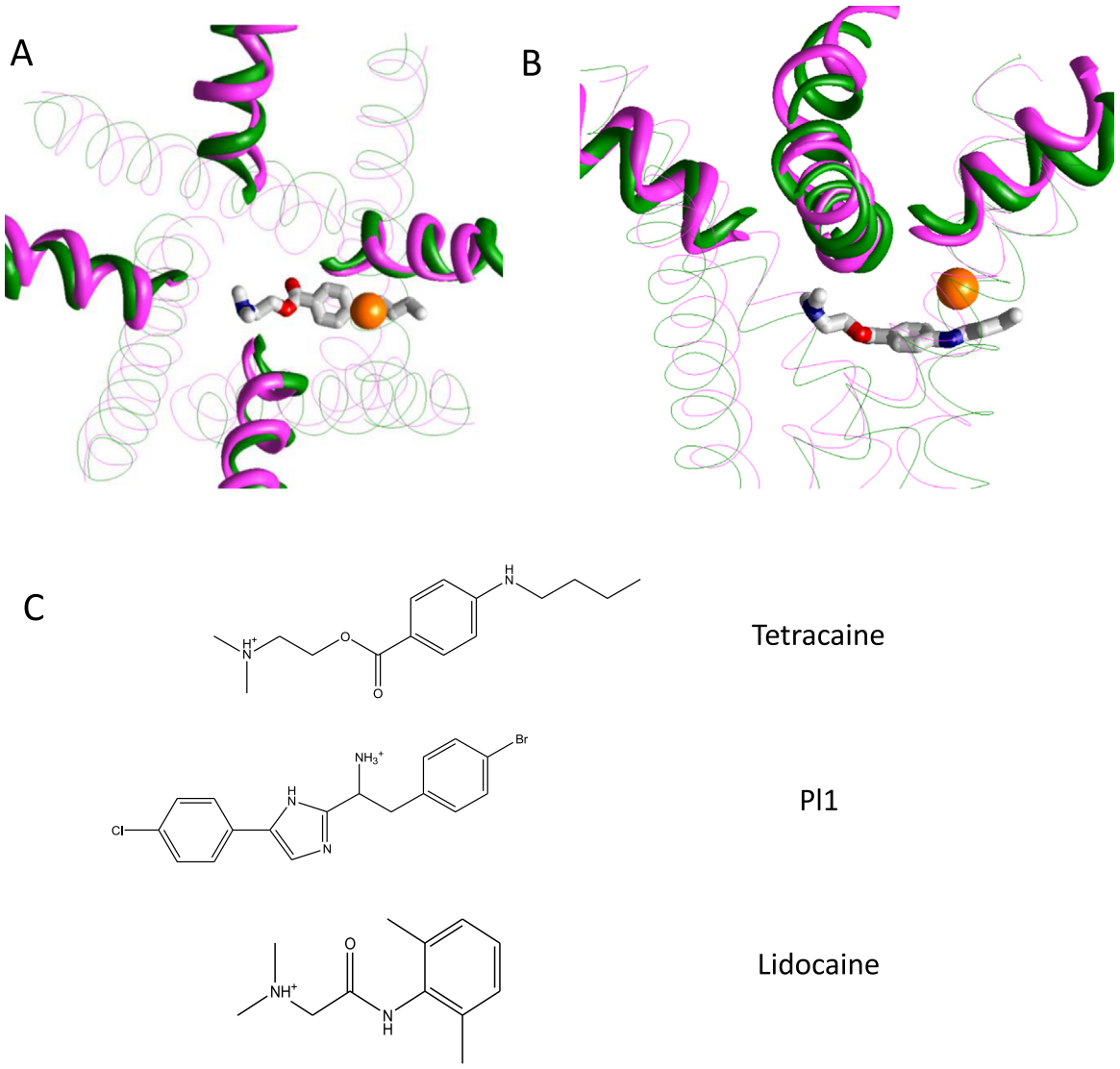

Fig. 1. Local anesthetics in the closed sodium channels. A and B, Superimposition of the tetracaine-Nav1.4 model (Bruhova et al., 2008) with the x-ray structure of NavMs (Bagneris et al., 2014). The Nav1.4 backbone is magenta, NavMs backbone is green. The orange sphere represents position of the bromine atom of Pl1 in the x-ray structure. Tetracaine molecule is shown by sticks. C, chemical structures of local anesthetics. The protonated aminogroup is in the middle of Pl1, while tetracaine and lidocaine have rather large moieties only at one side of the aminogroup. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

been published (Bagneris et al., 2014). Despite limited resolution of the structure of the bound ligand, two important features are seen. First, a bromine atom in the para-position of a terminal aromatic group is located in the subunit interface. Second, ion occupation of the selectivity filter above the central cavity is significantly reduced. Based on these findings, a model is proposed, in which the bromine-substituted aromatic moiety of Pl1 is located in the subunit interface, the aminogroup binds in the inner pore, and the chlorine-substituted aromatic ring at the opposite end of Pl1 enters the selectivity filter and sterically displaces the resident ion (Bagneris et al., 2014).

Some key features of this model confirm our earlier suggestions (Bruhova et al., 2008), which are based on modeling interpretation of indirect experimental data (Fig. 1A, B). The only significant difference is that in our models LA molecules like tetracaine or lidocaine, which lack long substituents at the amino group (Fig. 1C), are unable to sterically displace the permeant ion from the selectivity filter (Bruhova et al., 2008). Therefore, the experimentally observed interactions between LAs and sodium ion (Cahalan and Almers, 1979) is explained in our models by electrostatic repulsion.

Applicability of the Pl1-NavMs model (Bagneris et al., 2014) to explain interactions of eukaryotic sodium channels with different LAs remains unclear. It is also unclear whether or not Pl1 can displace the permeant ions from the NavMs selectivity filter due to electrostatic repulsion. To address this question, here we used Monte Carlo energy minimizations to dock Pl1 in NavMs in the presence of sodium ions in the selectivity filter. Our calculations predict that Pl1 can displace the permeant ions from the selectivity filter by both steric and electrostatic mechanisms. We suggest that the latter mechanism is more general because it applies to different channels and different channel blockers many of which are too bulky to enter the outer pore where selectivity filter is located.

2. Materials and methods

All calculations were performed by using the ZMM program (ZMM Software, Inc.). Nonbonded interactions were calculated