Local anesthetic inhibition of a bacterial sodium channel

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Recent structural breakthroughs with the voltage-gated sodium channel from *Arcobacter butzleri* suggest that such bacterial channels may provide a structural platform to advance the understanding of eukaryotic sodium channel gating and pharmacology. We therefore set out to determine whether compounds known to interact with eukaryotic NaVs could also inhibit the bacterial channel from *Bacillus halodurans* and NaChBac and whether they did so through similar mechanisms as in their eukaryotic homologues. The data show that the archetypal local anesthetic (LA) lidocaine inhibits resting NaChBac channels with a dissociation constant (KD) of 260 μM, and channels displayed a left-shifted steady-state inactivation gating relationship in the presence of the drug. Extracellular application of QX-314 to expressed NaChBac channels had no effect on sodium current, whereas internal exposure via injection of a bolus of the quaternary derivative rapidly reduced sodium conductance, consistent with a hydrophilic cytoplasmic access pathway to an internal binding site. However, the neutral derivative benzocaine applied externally inhibited NaChBac channels, suggesting that hydrophobic pathways can also provide drug access to inhibit channels. Alternatively, ranolazine, a putative preopen state blocker of eukaryotic NaVs, displayed a KD of 60 μM and left-shifted the NaChBac activation-voltage relationship. In each case, block enhanced entry into the inactivated state of the channel, an effect that is well described by a simple kinetic scheme. The data suggest that although significant differences exist, LA block of eukaryotic NaVs also occurs in bacterial sodium channels and that NaChBac shares pharmacological homology to the resting state of vertebrate NaV homologues.

INTRODUCTION

The initial discovery and characterization of a voltage-gated sodium channel from *Bacillus halodurans* (NaChBac) have held promise that these channels may serve as functional homologues of the eukaryotic sodium channels that control action potential firing in the excitable cells of nerve and muscle (Ren et al., 2001). Such bacterial sodium channels have generated interest for their interrogation via structural approaches that are currently out of reach for the analysis of mammalian NaVs but could aid significantly in closing conceptual gaps that persist in the understanding of the eukaryotic sodium channel gating, selectivity, and pharmacology. This possibility has been partially realized with the structure of the sodium channel from *Arcobacter butzleri* (Payandeh et al., 2011), yet many questions persist regarding the structural and functional conservation between bacterial progenitors and their eukaryotic NaV descendants and whether NaChBac represents a bona fide pharmacological model to advance eukaryotic NaV drug design and understanding.

NaChBac and eukaryotic NaVs selectively allow the transmembrane conductance of sodium ions and share a common fourfold domain arrangement around a central axis that defines the permeation pathway. Each domain contains six segments (S1–S6), with S1–S4 forming a voltage-sensing module, pore S5–S6 segments that comprise the selectivity filter, an inner vestibule, and the S6 segments that gate the passage of ions. In response to membrane depolarization, the S4 segments of NaChBac carry their positively charged Arg and Lys residues in an outward trajectory (Blanchet and Chahine, 2007), similar to mammalian NaV channels (Yang et al., 1996). Despite these intrinsic similarities, considerable differences exist. For one, NaChBac is a homotetramer, whereas eukaryotic NaVs are encoded by a single polypeptide that forms a heterotetramer tethered by cytoplasmic linkers. One obvious outcome of this design posits that eukaryotic NaVs are likely to engage in asymmetrical chemical contacts with ions and cationic blockers that occupy the pore. For instance, four glutamate side chains form the NaChBac Na+ ion–selective filter but can easily be converted to become Ca2+ selective (Yue et al., 2002), suggesting that bacterial sodium channels may as easily serve as models for calcium channel selectivity and permeation. NaChBac channels also lack key cytoplasmic domains that support eukaryotic NaV fast inactivation, namely the cytoplasmic DIII–IV linker and the cytoplasmic carboxyl terminus (Ren et al., 2001), structural absences that likely underlie the significant delayed entry

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into, and recovery from, inactivated states of NaChBac channels. The mechanisms that support NaChBac inactivation are unknown but have been proposed to occur in a manner homologous to the pore rearrangement similar to C-type inactivation in potassium channels (Pavlov et al., 2005), although other mechanisms akin to activation gate “slippage” of the S6 segment remain unexplored (Shin et al., 2004).

Therapeutic sodium channel inhibition by local anesthetic (LA) drugs is used to calm membrane excitability that is manifest in epilepsy, cardiac arrhythmia, and painful syndromes. These amphiphilic drugs contain titratable amine moieties with pKas in the physiological range, and it has been proposed that the neutral species of the drug can readily diffuse into closed channels, producing a resting or “tonic” inhibition of channel activity (Hille, 1977). Alternatively, after traversing the span of the membrane in the neutral form, the reprotonated drug can act as a charged, rapid open channel blocker from the cytoplasmic face (Kimborough and Gingrich, 2000). Such divergent hydrophobic and hydrophilic access routes to active sites, in combination with differential drug interactions with resting and active channel conformational states, give rise to the clinically important phenomena of tonic and use-dependent channel inhibition (Schwarz et al., 1977). In the latter case, channel (over)use results in up to 10-fold higher affinity caused by enhanced drug interactions with open/inactivated channel conformations.

NaChBac shares pharmacological similarities with Cav channels in that both are inhibited by cadmium and dihydropyridine compounds (Ren et al., 2001), yet very little is known of the possible interactions between NaChBac and LA compounds known to inhibit eu-karyotic Na$_{\text{v}}$8. It has been suggested that etidocaine preferably blocks open NaChBac channels, but the supporting data are sparse on the details of such mechanisms (Zhao et al., 2004). Structural cues provided by Arcobacter butzleri suggest that large cationic drugs may occupy the channels, but it is not known whether LA drugs are capable of inhibiting the NaChBac channels once they reach the inner vestibule.

## Materials and Methods

### Two-electrode voltage clamp

Voltage-clamped sodium currents were recorded with two microelectrodes using an OC-725C voltage clamp (Warner) in a standard Ringer’s solution (in mM): 116 NaCl, 2 KCl, 1 MgCl$_2$, 0.5 CaCl$_2$, and 5 HEPES, pH 7.4. All recordings were performed at 20–22°C. Glass microelectrodes had resistances of 0.1–1 MΩ and were backfilled with 3 M KCl. The holding potential was −120 mV in all cases. G-V relationships were derived by plotting the isochronal tail current amplitudes (the current amplitude measured after stepping from the test potential back to a holding potential of −120 mV) as a function of the depolarizing pulse potential. All displayed current traces show the full active voltage range of G-Vs. All data are means ± SEM. The T220A mutant was transiently expressed via calcium phosphate transfection in HEK-293 cells for patch-clamp recordings. The electrode resistance was in the range of 1.0 to 1.5 MΩ, and the voltage errors resulting from series resistance was always <3 mV after compensation. Liquid junction potentials between the bath and the pipette solution were corrected. All experiments were performed at room temperature. The patch pipette contained (in mmol/L): 60 CsCl$_2$, 13 NaCl, 80 Kaspatic acid, 11 EGTA, 1 MgCl$_2$, 1 CaCl$_2$, and 10 HEPES, pH 7.2. The bath contained (in mmol/L) 150 NaCl, 2 KCl, 1.5 CaCl$_2$, 1 MgCl$_2$, and 10 HEPES, pH 7.4.

All drug stock solutions were kept at −20°C. Ranolazine and lidocaine working solutions were prepared by diluting from stock solutions, 50 mM and 200 mM, respectively, in Ringer’s at room temperature. 500 mM benzocaine stock solution was made in DMSO, and it was diluted in Ringer’s for appropriate working concentrations.

### Homology modeling

The sequences of the pore (S5–S6 region) of NaChBac were aligned using ClustalW2 with that of the S3–S6 region of the NavAb structure (Protein Data Bank accession no. 3RVY; Payandeh et al., 2011). Two homology models of NaChBac subunits based on this alignment were generated using 3RVY chain A and B as template structures using the Swiss-Model server. The modeled subunits representing the symmetric and asymmetric subunits of the NavAb structure were then assembled to form a NaChBac tetramer by fitting the modeled subunits onto the biologically relevant NavAb tetramer structure. All structures and electrostatic surfaces are displayed using PyMol.

### Results

A NaChBac homology model was constructed from the recent crystal structure of the voltage-gated sodium channel from Arcobacter butzleri (NavAb) and is shown in Fig. 1. An alignment of the S5 and S6 segments of the two bacterial sodium channels shows strong conservation of regions of known importance for LA block, including the selectivity filter and S5 and S6 transmembrane segments. Notably, however, the NavAb “fenestrations” that are possibly guarded by an aromatic residue, Phe203, are poorly conserved in sodium channels, and this site is Leu in NaChBac channels. Nonetheless, the model suggests that both channels have well-defined cytoplasmic and alternative access routes within the transmembrane region and large internal vestibules that could accommodate blocking molecules. We first examined the effects of the amphiphatic classical LA lidocaine on two-electrode voltage-clamped currents recorded from Xenopus laevis oocytes injected with NaChBac cRNA. NaChBac channels in this expression system displayed stable expression after 24–48 h and had similar kinetic properties and activation and inactivation steady-state behavior to channels expressed in mammalian cultures (Table 1; Ren et al., 2001). Most striking in each case for both oocyte and cultured cell expression is the apparent stability of a long-lived inactivated state of the channel once the channel had been fully inactivated. However, this issue can be partially overcome with negative holding potentials, −120 mV in the
peak current-voltage relationships for NaChBac cur-
tents where channels do not spend significant amounts
time visiting inactivated states. Fig. 2 A shows that
tentials where channels do not spend significant amounts
present case, and by using infrequent or brief test po-
response curve was generated, and the mean data were
representative family of sodium currents shown in the inset. To as-
consistent with a sodium selective pore, with a represen-
tated current traces from
drug-free conditions (not depicted). A family of normal-
tions, very little current loss was seen under control
pulses to
application of lidocaine during repeated depolarizing
steadystate inhibition of currents produced by the
slope of
Statistical significance was set at P < 0.05.
Mean ± SEM is given.

<table>
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<th>Drug</th>
<th>lidocaine (1 mM)</th>
<th>benzocaine (1 mM)</th>
</tr>
</thead>
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<tr>
<td>V1/2 (mV)</td>
<td>dx (mV)</td>
<td>V1/2 (mV)</td>
</tr>
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<td>Control</td>
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<tr>
<td>Drug</td>
<td>−32.8 ± 2.1</td>
<td>−30.5 ± 2.6</td>
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Mean ± SEM is given.
*Statistical significance was set at P < 0.05.

Figure 1. Homology model of NaChBac. (A) Sequence alignment of the pore region (S5–selectivity filter–S6 segments) of NaChBac aligned with NavAb. SF, selectivity filter. Stars indicate conservation, double dots show strong conservation, and single dots indicate weak conservation. (B) A homology model of the pore region of NaChBac generated based on the crystal structure of NavAb. The structure is shown with a top view (left) and a side view (right), with one monomer removed for clarity.

<table>
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<th>Drug</th>
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<th>benzocaine (1 mM)</th>
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<td>V1/2 (mV)</td>
<td>dx (mV)</td>
<td>V1/2 (mV)</td>
<td>dx (mV)</td>
</tr>
<tr>
<td>Control</td>
<td>−57.9 ± 0.4</td>
<td>6.8 ± 0.6</td>
<td>−62.1 ± 1.1</td>
</tr>
<tr>
<td>Drug</td>
<td>−57.9 ± 0.4</td>
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Effects on activation of NaChBac

Effects on inactivation of NaChBac
that the blocking site is on the intracellular side of the channel, out of reach of QX-314. Consistent with this possibility, Fig. 3 B shows that when QX-314 is injected intracellularly into oocytes under voltage clamp at −120 mV, sodium current was rapidly blocked upon depolarization, suggesting access to the intracellular binding site is cytoplasmic and hydrophilic in nature. To further test this possibility, we examined the effects of extracellular benzocaine, an uncharged molecule at pH 7.4. Fig. 4 A shows that NaChBac channels could be blocked, albeit with a reduced affinity of IC50 of 650 µM and a Hill slope of 1.0. The ability of benzocaine to inhibit NaChBac currents when applied extracellularly supports a role for hydrophobic pathways for LA block in NaChBac that the blocking site is on the intracellular side of the channel, out of reach of QX-314. Consistent with this possibility, Fig. 3 B shows that when QX-314 is injected intracellularly into oocytes under voltage clamp at −120 mV, sodium current was rapidly blocked upon depolarization, suggesting access to the intracellular binding site is cytoplasmic and hydrophilic in nature. To further test
channels. Similar to lidocaine effects on gating properties, benzocaine induced a statistically significant hyperpolarizing shift in SSI curves (Table 1). Lastly, we explored the possibility that a conventional drug with a divergent structure from lidocaine, such as ranolazine, also retained the ability to block NaChBac. The data clearly show that external perfusion of ranolazine potently inhibited sodium currents compared with lidocaine, with an IC50 of 60 µM (Fig. 5). Similar to lidocaine and benzocaine, inhibition by ranolazine is illustrated in the diary plot in Fig. 5 C. Current inhibition was reversed after 5 min of washout with Ringer’s solution at a holding potential of −120 mV. To confirm that our results were not specific to the Xenopus oocyte expression system, ranolazine was applied to NaChBac channels transiently transfected in HEK-293 cells under patch-clamp conditions. Here, similar to the oocyte, 200 µM ranolazine potently and reversibly inhibited channels (not depicted). In the oocyte, ranolazine induced a significant left shift in both G-V and SSI curves (Fig. 5 D). The V1/2 of G-V curves after ranolazine exposure was −32.8 ± 2.1 mV compared with a V1/2 of control of −19.8 ± 1.4 mV (n = 4, P < 0.05). The hyperpolarizing left shift in the SSI curve is slightly less prominent with −8 mV, started with a V1/2 of control of −50.81 ± 0.60 mV to −57.91 ± 0.43 mV after ranolazine exposure (n = 4, P < 0.05).

As the concentration of each of the compounds was increased, there were two effects on NaChBac current: an instantaneous decrease in the peak current and an apparent speeding in the inactivation rate of the current. The concentration-dependent increase in the apparent inactivation rate of the current could be explained by an on-rate of LA block that should increase linearly with the concentration of LA for a bimolecular reaction scheme. Alternatively, the increase in apparent inactivation rate could actually be a result of the blocked state of the channel having an increased probability of inactivating. In support of the latter possibility, the rate of inactivation did not increase linearly with concentration but saturated with a similar concentration to the maximal block concentration (Fig. 6). The rates of inactivation were well fit for lidocaine and benzocaine with a three-state model in which inactivation into an absorbing inactivated state was more rapid from the blocked state (Fig. 6 D). In this model, K represents the experimentally determined equilibrium block equal to the IC50, kOI represents the rate of inactivation from the LA-bound state. The concentration dependence of the inactivation rate is then described by 

\[ \rho = k_{oi} \times (1 - P_b) + k_{bi} \times P_b \]

where \( P_b = 1/(1 + K/[\text{LA}]) \) is the equilibrium probability that the channel is blocked. In this model, \( k_{oi} \) and \( P_b \) are determined experimentally. The model was fit to the mean data, giving fitted parameters (±SEM of the fitting) of \( k_{oi} = 0.0046 ± 0.0005 \) s−1 for lidocaine (where \( k_{oi} = 0.0015 \) s−1) and 0.0062 ± 0.0006 s−1 for ranolazine (where \( k_{oi} = 0.0015 \) s−1), indicating that the increase of inactivation rate can be described by the LA block promoting inactivation, similar to a “spring in the door” model proposed previously for mutant Shaker channels (Ahern et al., 2009). For benzocaine, which showed the most dramatic speeding of

Figure 4. Blockade by the neutral drug benzocaine. (A) A benzocaine concentration response curve was generated with various benzocaine concentrations (30 µM, 100 µM, 300 µM, 1 mM, and 3 mM). The potency of benzocaine is weaker than lidocaine with an IC50 of 650 µM. Representative traces were obtained from 30 µM, 100 µM, 300 µM, 1 mM, and 3 mM benzocaine applications and are shown in the bottom inset. The dashed line indicates the zero current level. The top inset is a chemical structure of benzocaine. Bar, 500 ms. (B) 3 mM benzocaine was applied extracellularly for 5 min while holding the cells at −120 mV. Test pulse delivery and washout were performed as described in Fig. 2 C. Representative current traces are shown with the zero current lines (dotted lines). Bar, 500 ms. (C) Conductance-voltage relationship curves were generated for control (○) and for after 3 mM benzocaine (□). Similarly, SSI curves were constructed before (■) and after the exposure to 3 mM benzocaine (□). (A–C) n = 4. Error bars represent SEM.
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inactivation (Fig. 6 C), the three-state model failed to fit the data well at low concentrations, as indicated by the dashed line. To account for this deviation, we considered previous work on LA block that has suggested there may be two interaction sites in the channel: a rapidly accessible low affinity site that catalyzes the entry to a higher affinity site (Gingrich et al., 1993). In our model, we conceived that the IC50 we measured from peak currents corresponded to the fast blocking site (BS1) that is accessed before a second site that, when occupied, promotes inactivation (BS2). This then leads to a four-state model, depicted in Fig. 6 D, in which the inactivation rate will be described by 

$$ p_i = k_{oi} \times [(1 - P_{BS2}) - P_{BS1} + k_{oi} \times P_{BS1} \times P_{BS2}] $$

This model fit the data well by accounting for the deviation at low concentrations (Fig. 6 C, solid line) and gave fitted parameters for $k_{oi}$ of $0.0184 \pm 0.0017$ s$^{-1}$ and an equilibrium constant for the second site, BS2, of $K_2 = 320.1 \pm 142.7$ µM. These data suggest that LA binding to NaChBac, if we assume open channel block is primarily a fast block that reaches equilibrium faster than channel opening, has the added effect of stabilizing the inactivated state when blocking.

Next, in an attempt to further understand lidocaine block of NaChBac, we performed an extensive alanine scan of the selectivity filter and S6 segments. However, many mutations displayed nonexpressing phenotypes, including L182A, Q186A, Q186N, Q186E, V187A, T189A, L190A, E191A, W193A, W193Y, W193F, S195A, V197A, R199A, L217A, L217F, and N225A, whereas S180A, L181A, T183A, L184A, S192A, and F227A showed expression but had modest effects on channel gating. This strategy ultimately revealed an S6 mutation, T220A, shown in the homology model in Fig. 7 A, which nearly abolished inactivation in NaChBac (Fig. 7 B). This mutant displays little inactivation over a wide range of voltages, as shown in Fig. 7 C, and has a hyperpolarizing shift in the G-V relationship. Importantly for use in the study of blockers, this mutation had little impact on the overall channel affinity for lidocaine, with an IC50 from 260 µM to 765 µM for wild-type and T220A channels, respectively (Fig. 8 B). We aimed to use this mutant to investigate the kinetics of drug block in a channel without inactivation to more cleanly characterize the blocking kinetics in a manner akin to the domain III–IV linker IFM to QQQ mutation in eukaryotic NaV channels. Surprisingly, application of lidocaine to the mutant produced robust inhibition with little evidence of blocking kinetics (Fig. 8 A), suggesting lidocaine acts as a fast cytoplasmic blocker of NaChBac.
The initial cloning, subsequent characterization, and recent structural advances with bacterial sodium channels have begun to shed new understanding on these channels and how they might relate to the basic principles of sodium channel gating and pharmacology (Ren et al., 2001; Pavlov et al., 2005; Blanchet and Chahine, 2007; Blanchet et al., 2007; Irie et al., 2010; Payandeh et al., 2011; Yarov-Yarovoy et al., 2012). Up to 12 voltage-gated bacterial sodium channel genes have been discovered (Koishi et al., 2004), but it remains to be shown which aspects of NaChBac channel gating are specific to eukaryotic sodium channels and which inform on the generic features of NaChBac channel gating.

**Figure 6.** LA block enhances the rate of NaChBac inactivation. (A–C) Rates of NaChBac inactivation (1/τ) were plotted against drug concentration for lidocaine (A), ranolazine (B), and benzocaine (C). The insets in each plot are normalized traces illustrating the increased inactivation rates when the channel is blocked. n = 4. (D) The three- and four-state models used to fit the increase in inactivation rate where O is the open channel, B is the channel when occupied by the blocker, and I is the inactivated state. The three-state model adequately fit the data for lidocaine and ranolazine, whereas for benzocaine the addition of an extra blocking state, BS2, from which inactivation was promoted (solid line fit in C), improved the fit at lower concentrations when compared with the three-state model (dashed line shown for comparison in C). Error bars represent SEM.

**Figure 7.** Characterization of a noninactivating mutant, T220A. (A) A side view of the NaChBac homology model highlighting the location of T220 of the S6 segment (left). The structure is also shown with a top view (right) that is sliced approximately through the middle of the pore from the side parallel to the membrane, then rotated 90° horizontally to highlight the orientation of T220. (B) Mutation of T220A of NaChBac has resulted in a noninactivating channel. Normalized traces of wild-type NaChBac and T220A mutant are shown. Cells were held at a holding potential of −120 mV, and channels were activated by 500 ms of depolarization pulse to −20 mV. The dashed line indicates the zero current level. Bar, 250 ms. (C) Representative current traces of T220A mutant. HEK-293 cells were held at a holding potential of −120 mV with patch-clamp configuration. NaChBac channels expressing T220A were activated by 1 s of depolarizing pulses from −100 to 0 mV in 10-mV increments. Bar: horizontal, 250 ms; vertical, 4 nA. (D) Current-voltage relationship between wild-type NaChBac and T220A mutant. A significant 20-mV left shift was observed with T220A (V1/2 = −65.765 ± 2.89 mV; mean ± SEM, n = 5) as compared with wild type (V1/2 = −45.52 ± 0.90 mV; mean ± SEM, n = 5), with P < 0.01. Error bars represent SEM.
The application of increasing concentrations of lidocaine to the control trace (bottom) significantly reduced the peak currents. Lidocaine was applied by a fast perfusion while applying depolarizing pulses from $-120$ to $-20$ mV for $300$ ms with $3$-s intervals. The dashed line indicates the zero current level. Bar, $100$ ms. (B) The representative lidocaine concentration response curve describing the effects of lidocaine on T220A was generated with the mean of $I_{\text{drug}}/I_{\text{control}}$ obtained from the peak currents after exposing cells to $100$ µM, $300$ µM, $1$ mM, and $3$ mM lidocaine. The mean IC$_{50}$ for lidocaine is $765 \pm 76.3$ µM, with a mean slope of $0.92 \pm 0.1$ ($n=4$). Error bars represent SEM.
observation that when QX-314 is injected directly into oocytes expressing NaChBac, currents are reduced instantaneously upon opening. What, then, could account for the apparent increase in the inactivation kinetics observed in wild-type channels? We suggest that blocker occupancy of the inner vestibule enhances entry into the inactivated state, a possibility that can be accounted for with the model proposed in Fig. 6. The inactivation mechanism of bacterial sodium channels is not yet known, but one possibility is that the blocker promotes reclosure or slippage of the S6 gate similar to HCN (hyperpolarization activated cyclic nucleotide gated) channels (Shin et al., 2004). Alternatively, the blocker could effect ion occupation in the selectivity filter, which, in turn, could promote conformational changes that reduce conductance (Baukrowitz and Yellen, 1996).

Regardless of the mechanism, the data and modeling support a mechanism in which LA s act as fast open channel blockers that enhance channel inactivation. The results from NaChBac interactions with lidocaine derivatives support the working model shown in Fig. 9. The data show that external application of QX-314 has no effect on current levels, whereas internal injection results in rapid blockade, suggesting the existence of an internal blocking site and that the hydrophobic membranous pathways bar the passage of charged molecules. Once bound, the blocker effects inactivation through an unknown mechanism. However, interactions between neutral species of LAs could also effect channel gating through interactions in the membrane with other channel domains, such as the voltage sensor (Hanck et al., 2009; Munro and Chanda, 2009).

Lidocaine has been shown to block a variety of channel types, including KATP, K2P, and nicotinic acetylcholine receptors, which show IC50 estimates of 43 µM, 1 mM, and 73 µM, respectively (Olschewski et al., 1996; Kindler et al., 2003; Alberola-Die et al., 2011). However, NaChBac affinity for lidocaine also approximates the resting block of cardiac sodium channels with an IC50 of 226 µM (Chahine et al., 1992). At present, it is not known which aspects of the block of NaChBac described here will shed new light on the mechanism of eukaryotic NaV interactions with LAs. In regard to the possibility of functional fenestrations that might serve as drug access routes, the data suggest that NaChBac is quite different from eukaryotic NaV s. In particular, it has been shown that QX-314 applied externally can enter cardiac, but not neuronal, sodium channels, and introduced mutations to residues homologous to side chains that abut the bacterial fenestrations can impact this entry. However, our experiments show that QX-314 applied externally has no effect on current levels, suggesting that if such fenestrations are present in NaChBac, they are functionally divergent from cardiac NaV s. Nonetheless, in both bacterial and eukaryotic NaV s, many key mechanistic details remain unresolved on the effects of blockers on the NaV S6 gates and of drug modulation of inactivation. Thus, future determination of the details of NaChBac inactivation is a prerequisite for the comparison to eukaryotic NaV s.

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