Using Lidocaine and Benzocaine to Link Sodium Channel Molecular Conformations to State-Dependent Antiarrhythmic Drug Affinity

Dorothy A. Hanck, Elena Nikitina, Megan M. McNulty, Harry A. Fozzard, Gregory M. Lipkind, Michael F. Sheets

**Rationale:** Lidocaine and other antiarrhythmic drugs bind in the inner pore of voltage-gated Na channels and affect gating use-dependently. A phenylalanine in domain IV, S6 (Phe1759 in NaV1.5), modeled to face the inner pore just below the selectivity filter, is critical in use-dependent drug block.

**Objective:** Measurement of gating currents and concentration-dependent availability curves to determine the role of Phe1759 in coupling of drug binding to the gating changes.

**Methods and Results:** The measurements showed that replacement of Phe1759 with a nonaromatic residue permits clear separation of action of lidocaine and benzocaine into 2 components that can be related to channel conformations. One component represents the drug acting as a voltage-independent, low-affinity blocker of closed channels (designated as lipophilic block), and the second represents high-affinity, voltage-dependent block of open/inactivated channels linked to stabilization of the S4s in domains III and IV (designated as voltage-sensor inhibition) by Phe1759. A homology model for how lidocaine and benzocaine bind in the closed and open/inactivated channel conformation is proposed.

**Conclusions:** These 2 components, lipophilic block and voltage-sensor inhibition, can explain the differences in estimates between tonic and open-state/inactivated-state affinities, and they identify how differences in affinity for the 2 binding conformations can control use-dependence, the hallmark of successful antiarrhythmic drugs. (Circ Res. 2009;105:00-00.)

**Key Words:** antiarrhythmic drug • voltage clamp • gating currents • lidocaine • benzocaine • local anesthetic

Lidocaine and other local anesthetic (LA) drugs block voltage-gated Na channels. A subset share characteristics that make them effective as antiarrhythmic drugs, ie, they exhibit high affinity, use-dependent block of Na current (I_{Na}) at high heart rates. Despite extensive study, there remains uncertainty regarding how observed block relates to specific drug–channel conformations. Several vocabularies have emerged to describe block, which in general, have their basis in kinetic models of Na channel gating and assume preferential binding to one or more states that produce no1 or altered2 gating. Recent availability of crystal structures in combination with mutagenesis data now allow for linking electrophysiological data, kinetic states, and drug block to specific channel conformations.

It is generally accepted that lidocaine and lidocaine-like drugs bind in the inner pore of voltage-gated Na channels. Scanning mutagenesis studies with various Na channel isoforms and multiple lidocaine-like drugs have identified only one amino acid residue, a phenylalanine (Phe) in domain IV, S6 (DIVS6), which, when mutated, alters use-dependent drug affinity by more than ten-fold. When this Phe (1759 in NaV1.5) is mutated to nonaromatic residues3–8 or to unnatural amino acids with different electron withdrawing capabilities9 the mutated channel shows a marked decrease in high-affinity LA block. Homology modeling with K channels predicts that this Phe faces the pore just below the selectivity filter.10,11 This orientation of Phe is supported by the finding that its cysteine mutant is accessible to MTS reagents applied from inside the pore when the channel is maintained in an open state.12 Furthermore, it has been shown by us13 and others14 that use-dependent block is intimately associated with altered movements of the structurally distant S4 segments in domains III and IV.

Block assayed from negative holding potentials at low rates of stimulation is affected very little by channel mutations in contrast to effects on use-dependent block. This lower affinity block is usually called tonic block, although it has also been called rested-state block (or closed-state block)
when it occurs from holding potentials that bias Na channels to be fully available, ie, they occupy rested/closed states. However, as the membrane potential becomes more depolarized, tonic block also increases, ie, it is voltage-dependent.\textsuperscript{15} In these experiments, we show that drug binding to DIVS\textsubscript{6}-Phe\textsubscript{1759} induces changes in gating currents, which are the hallmark of high-affinity, voltage dependent block.\textsuperscript{16,17} Experiments with Ionic currents and with gating currents allowed separation of block by antiarrhythmic drugs into 2 components. One represents a voltage-independent, low-affinity block that likely results from interaction of drug with channels in the closed conformation, which we term lipophilic block reflecting that it represents a neutral form of the drug interacting with neutral residues in the closed channel pore. The second is one that is associated with modification of gating currents and the open/inactivated conformation. We designate this voltage-sensor inhibition to reflect this important consequence of binding. These 2 forms of block provide a straight-forward method for interpreting ionic current data and for modeling of the drug interaction sites. Part of this work has been published in abstract form.\textsuperscript{14}

### Methods

Experiments used the human heart voltage-gated Na\textsuperscript{+} channel, Na\textsubscript{1.5} (hH\textsubscript{1a}), provided by H. Hartmann (University of Maryland Biotechnology Institute, Baltimore, Md) and A. Brown (Chantest Inc, Cleveland, Ohio).\textsuperscript{18} Channels were expressed transiently in tsA201 cells or stably in HEK293 cell lines. For gating current (\(I_g\)) studies, the background for all channels included C373 years, which increases sensitivity to saxitoxin\textsuperscript{19} and exerts minimal effects on channel kinetics or on local anesthetic action.\textsuperscript{4} Cells were maintained in DMEM (Invitrogen, Carlsbad, Calif) supplemented with 10% FBS, 1% penicillin-streptomycin, and selection antibiotic in 60- to 100-mm culture dishes (Corning, Acton, Mass). For \(I_g\) experiments, multiple tsA201 cells were fused using polyethylene glycol to form large single cells, cultured for several days to allow for membrane remodeling, and then transiently transfected using calcium phosphate (Invitrogen).

For standard \(I_{Na}\) measurements, extracellular Na\textsuperscript{+} was lowered and replaced with Cs\textsuperscript{+} to maintain peak inward currents \(\approx 1 \text{ to } 3 \text{nA}\). Bath solution contained (in mmol/L): 2 to 50 NaCl, 138 to 90 CsCl, 10 HEPES, 2 CaCl\textsubscript{2}, pH 7.4 with CsOH. Pipette solution contained (in mmol/L): 100 CsF, 45 CsCl, 10 EGTA, and 10 HEPES, pH 7.4 with CsOH. For \(I_g\) experiments, the control extracellular solution for \(I_{Na}\) measurements, the \(I_{Na}\) measurements, the

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### Non-standard Abbreviations and Acronyms

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<th>Abbreviation</th>
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<tr>
<td>LA</td>
<td>local anesthetic</td>
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<tr>
<td>MES</td>
<td>2-((N)-morpholino)ethanesulfonic acid</td>
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<tr>
<td>Na-MES</td>
<td>2-((N)-morpholino)ethanesulfonic acid sodium salt</td>
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<tr>
<td>Q-V</td>
<td>gating charge–voltage</td>
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<td>TMA</td>
<td>tetramethyl ammonium</td>
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<td>WT</td>
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1.0 \(\mu\text{mol/L}\) saxitoxin (Calbiochem Corp, San Diego, Calif) and 2 \(\mu\text{mol/L}\) Ca(OH)\textsubscript{2} were added to the extracellular solution. Lidocaine and benzocaine (Sigma-Aldrich, St Louis, Mo) were dissolved in bath solution and applied using either a single chamber bath in which solutions were exchanged using a latching subminiature solenoid valve (The Lee Company, Westbrook, Conn) or a multiple-chamber bath in which channels sealed to the patch pipette were lifted and moved from chamber to chamber to measure currents in the absence and presence of drug.

Whole cell ionic current recordings were made with an Axopatch 200B amplifier and a Digidata 1321A with pClamp 8.2 (Axon Instruments, Union City, Calif) as previously described.\textsuperscript{3} \(I_g\) recordings were made with a large bore, double-barreled glass suction pipette. \(I_g\) data were obtained using a National Instruments PXI-1002 with a PXI-6052 multi-function 16-bit converter using LabView 7.0 (National Instruments Corp, Austin, Tex), filtered by the head stage \((\approx 100 \text{kHz})\), and digitized at 200 kHz. For \(I_g\) measurements, the membrane potential was held at \(-150 \text{ mV}\) and stepped to various test potentials for 26.5 ms at 0.5 Hz at room temperature. All \(I_g\) were leak corrected by the mean of 2 to 4 ms of data usually beginning 8 ms after the change in test potential and capacity corrected using 4 to 8 scaled current responses to steps between \(-150 \text{ and } -180 \text{ mV}\) taken immediately before and after test steps.

Data were analyzed using Matlab (The Mathworks Inc, Natick, Mass) and Origin (OriginLab Corp, Northampton, Mass). There were no differences in parameter estimation for fitting using these programs even though the nonlinear regression technique differs between them. Voltage-dependent availability curves were fit with a Boltzmann relationship: 

\[
\frac{I_{Na}}{I_{Na}} = \frac{1}{1 + \exp\left(V - V_{1/2}\right)},
\]

where \(I_{Na}\) is the \(I_{Na}\) during a step depolarization to the test potential, \(V_{1/2}\) that typically was \(-10 \text{ to } -30 \text{ mV}\) after conditioning for 1000 ms at various potentials (mV) with 4 or 5 seconds between trials. The fitted parameters were: the maximal current (\(I_{Na}\)), the half-point of the relationship (\(V_{1/2}\)), and the slope factor of the relationship (\(dx\)). The same relationship was used for charge; slope factors had opposite sign. For concentration–response analyses, data were normalized to peak \(I_{Na}\) in control, and these were fit with a single-site binding equation: fraction remaining = \(1/(1 + [\text{drug}] / ED_{50})\), where \(ED_{50}\) represents the drug concentration at which one-half \(I_{Na}\) is blocked. \(ED_{50}\) values are reported as the estimate and standard error of the mean (SEM) from the fits. For analysis of populations, parameters from individual fits were meaned and grouped data are reported as means\(\pm\)SEM for each parameter. Differences between parameters were assessed using paired \(t\) tests. Parameters were considered significant when \(P<0.05\).

### Results

#### Use-Dependent Lidocaine Block Is Associated With Voltage Sensors

Mutations of Phe\textsubscript{1759} in Na\textsubscript{1.5} have been shown to eliminate use-dependent block of \(I_{Na}\) by lidocaine.\textsuperscript{5,9} In addition, we have previously shown that high-affinity block of Na channels by lidocaine is associated with a characteristic set of changes in the gating charge–voltage (Q-V) relationship.\textsuperscript{17} In the presence of high concentrations of lidocaine (Figure 1A), which ensures that nearly all Na channels are bound to drug, Q-V relationships demonstrate a smaller maximal gating charge \((Q_{max})\), less dependence of charge on potential, a negative shift in the half-point \((V_{1/2})\), and the presence of additional charge at more negative potentials. These signature changes result from stabilization of the DIII\textsubscript{4} in a depolarized position and partial inhibition of movement of DIVS\textsubscript{4} with an alteration of its voltage dependence.\textsuperscript{13,14,16,17} We term this complex set of changes voltage-sensor inhibition. The magnitudes of these changes with lidocaine are concentration-dependent and proportional to the magnitude of use-dependent block of
I_{Na} as expected if both effects resulted from the same binding event with an ED_{50} in the 20 μmol/L range, comparable to that found for high-affinity lidocaine block of I_{Na}.^{15}

If use-dependent block of I_{Na} by lidocaine is obligatorily linked to these signature changes in the Q-V relationship, then the absence of use-dependent block by lidocaine in the F1759K channel^{3} should be associated with the loss of these signature changes. Figure 1B shows this to be the case. In the presence of 10 mM lidocaine, a concentration that tonically blocked ~80% of I_{Na} (data not shown), neither Q_{max} nor the slope factor was reduced although V_{1/2} was slightly, and reversibly, shifted leftward. These results suggest that drug interaction with Phe1759 is required to produce the signature changes in the Q-V relationship associated with use-dependent block.

**Lidocaine Block Not Involving the Voltage Sensors**

Even though 10 mM lidocaine did not produce voltage-sensor inhibition in the F1759K channel, it did reduce the magnitude of ionic current. To investigate this voltage sensor-dependent block.

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**Figure 1.** Q-V relationships for WT (A) and F1759K (B) channels before and after exposure to 10 mM lidocaine. The insets show I_{p} for representative cells in step depolarizations to 0 mV in the absence and presence of 10 mM lidocaine. The Q_{max} and F1759K channels (n=4 cells) in lidocaine from the Boltzmann fits to the Q-V relationships of the individual cell in control solution was used to normalize the Q_{max} in lidocaine (A) and after wash (B). The protocols were performed from a holding potential (V_{hold}) of -150 mV. A, For WT (n=2 cells), the Q_{max} after lidocaine was 0.67±0.02 of control, V_{1/2} shifted from -67±4 mV to -80±5 mV, and the slope factor flattened (-12±3 mV to -20±3 mV). All changes were significant at P<0.05. The inset shows that I_{p} was decreased in drug. B, For F1759K channels (n=4 cells) in lidocaine Q_{max} was 0.99±0.01 of control, and the slope factor was unchanged after lidocaine (13±1 mV in both groups). V_{1/2} was significantly shifted from -72±2 mV to -89±1 mV. In the inset, both I_{p} traces appear superimposed.

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**Figure 2.** Steady-state voltage-dependent Na channel availability protocol (A, bottom) showing data for a representative cell expressing F1759K channels in control (left) and after exposure to 10 mM lidocaine. Summary data for cells expressing WT (B) and F1759K (C) channels exposed to lidocaine. Means±SEM in control solution are shown as open symbols, and in the presence of various concentrations of lidocaine as filled symbols. B, Data for 0.5 mM lidocaine (○; n=5) and 1 mM lidocaine (●; n=3) lidocaine are shown. V_{1/2} was -150 mV. Means±SEM for parameters to fits with individual datasets: half-point: -93±1 mV (control), -122±2.8 mV (0.5 mM lidocaine), and -126±4.3 mV (1 mM lidocaine); slope factor: 5.3±0.3 mV (control), 6.0±0.8 mV (0.5 mM lidocaine), and 6.2±1.2 mV (1 mM lidocaine). The inset shows summary of shifts in V_{1/2} of availability for cells exposed to four concentrations of lidocaine including cells exposed to 5 μmol/L (n=7) and 50 μmol/L (n=9), concentrations at which little reduction in I_{Na} was evident (data not shown). C, Data for F1759K exposed to 1 mM lidocaine (●; n=8), 3 mM lidocaine (●; n=4), and 10 mM lidocaine (●; n=3) lidocaine. Means±SEM of parameters for fits to individual datasets were for half-point: -91±1 mV (control), -104±1 mV (1 mM lidocaine), -103±2 mV (3 mM lidocaine), and -102±1 mV (10 mM lidocaine), and for slope factors: 6.2±0.3 mV (control), 6.4±0.6 mV (1 mM lidocaine), 7.3±0.8 mV (3 mM lidocaine), and 9.3±1.7 mV (10 mM lidocaine). The inset shows concentration response relationship of tonic block, estimated from the fitted asymptotes of the availability relationships. ED_{50} values were 0.78±0.2 mM for WT, and 2.2±0.2 mM for F1759K.
independent block by lidocaine, we constructed steady-state voltage-dependent Na channel availability as a function of lidocaine concentration. It has long been appreciated that the \( V_{1/2} \) of Na channel availability in wild-type (WT) channels shifts leftward in the presence of lidocaine\(^2\) in a concentration-dependent manner (Figure 2B). Although \( I_{Na} \) in F1759K was reduced in a concentration-dependent manner, ie, asymptotes were dose-dependently reduced (Figure 2C, inset), there was only a very small concentration-dependent shift in availability half-point (Figure 2C). Note that all 3 scaled lines have nearly identical \( V_{1/2} \) values. Similar to what was found for the Q-V relationships for F1759K in lidocaine (Figure 1C), there was a small leftward shift in \( V_{1/2} \). To distinguish this essentially voltage-independent block from use-dependent block associated with voltage-sensor inhibition, we introduce the term lipophilic block. We choose this term to emphasize its voltage-independent behavior, although it likely represents the same affinity observed in the first depolarization after exposing cells to drug while holding at a very negative potential (ie, first pulse block, see Discussion). The greater block of \( I_{Na} \) in WT compared to F1759K (Figure 2), when the holding potential is \(-150 \text{ mV} \) (a fully available potential in the absence of drug) suggests that tonic block in WT channels arises from a combination of both voltage-sensor inhibition and lipophilic block, whereas F1759K demonstrates only lipophilic block.

**Studies With the Neutral Drug Benzocaine**

At the physiological pH of 7.4, lidocaine molecules are mostly charged, and the positively charged tertiary amine is thought to interact strongly with Phe1759, 3,5, 6,9 In contrast, benzocaine is neutral and produces little or no use-dependent block,\(^2,20,21\) However, benzocaine has been shown to shift the \( V_{1/2} \) of the steady-state availability curve leftward\(^2\) and to reduce gating charge \(^{16,22,23}\) suggesting that benzocaine actions are similar to lidocaine even though it shows no use-dependence. Although exposure to 2 mmol/L benzocaine (close to the maximal amount soluble in water at room temperature) did not completely block all \( I_{Na} \), it was able to produce signature changes in gating currents in WT (Figure 3A) similar in kind, but lesser in magnitude, to those in lidocaine. In contrast, 2 mmol/L benzocaine had minimal effects on the Q-V relationship of F1759K except for a small leftward shift in the \( V_{1/2} \) of 7 mV (also seen with lidocaine), which was reversible on washout of the drug (Figure 3B).

If benzocaine can produce voltage-sensor inhibition in WT then it should also cause large leftward shifts in the \( V_{1/2} \) of the steady-state availability curves. Figure 4A shows this was the case for WT channels where benzocaine produced a large, concentration-dependent leftward shift of \( V_{1/2} \) (Figure 4A) as well as a reduction in \( I_{max} \) with an \( ED_{50} \) of 0.32 mmol/L (Figure 4A, inset). In contrast, benzocaine had minimal effects on \( V_{1/2} \) of availability of F1759K; it produced only a small leftward shift (Figure 4B), which was similar in magnitude to that seen for its Q-V relationship in lidocaine (see Figure 3B). The \( ED_{50} \) calculated from the availability asymptotes in F1759K was 0.66 mmol/L (Figure 4B, inset). If this represents only lipophilic block, then the 2-fold smaller \( ED_{50} \) of 0.32 mmol/L for benzocaine in WT reflects components from both lipophilic block and voltage-sensor inhibition. It should be recognized that the shift in the \( V_{1/2} \) of voltage-dependent Na channel availability in WT results from greater voltage-sensor inhibition as the membrane potential becomes depolarized.

**Homology Model of Inner-Pore Na Channel Interactions With Drugs That Produce Lipophilic Block**

To consider how voltage sensor-independent block might be produced by lidocaine and benzocaine binding in the closed channel, we used the KcsA channel structure\(^24\) as a structural template.\(^11\) The four S6 \( \alpha \)-helices at their C-ends at the level of Tyr1766 in DIV5S6 form a so called “S6 crossing,” producing an inner cavity below the selectivity filter with a restricted space for only 40 to 50 molecules of water.\(^24\) If lidocaine is placed in this volume, it displaces 20 to 25 molecules of water (ie, approximately half of the water). This would organize the remaining water, making it “structured” around lidocaine, likely producing an environment with a lower dielectric constant in the range of 4 to 10\(^2\) than bulk water (\( \approx 80 \)). As a consequence, the lower dielectric constant would be expected to bias occupancy of the closed channel with neutral forms of lidocaine and other local anesthetic.
In this model, neutral lidocaine and benzocaine would be predicted to show comparable block in the closed state, as the tertiary amine of lidocaine and the primary amine of benzocaine are predicted to be oriented similarly in the open/inactivated channel where the drugs adopt a vertical orientation. The aromatic ring of benzocaine fits into the interface between IIS6 and IVS6. E and F, Lidocaine and benzocaine are shown aligned as they are oriented in the closed channel and in the open/inactivated channel. In the closed channel both are oriented more horizontally (E) with their aromatic rings aligned with Phe1759.

Figure 5. Proposed location for lidocaine (A and B) and benzocaine (C and D) in the closed (A and C) and open/inactivated Na channel (B and D). Lidocaine and benzocaine are shown as space-filled docking in the interface of IIS6-IVS6 (green ribbons). Green color represents C atoms, blue represents N atoms, and red represents O atoms. Amino acid residues making contacts with drugs are shown by blue (IIS6) and red (IVS6) colors. The optimized structures of the complexes with the inner pore were calculated with the Discover module of Insight II. It should be noted that because of the differences in sizes between residues in Na1.5 when compared to those in KcsA, construction of the homology model in Na channel with its bulky residues required additional optimization of the structure at the S6 crossing to avoid nonbonded repulsion between side chains. The tertiary amine of lidocaine and the primary amine of benzocaine are predicted to be oriented similarly in the open/inactivated channel where the drugs adopt a vertical orientation. The aromatic ring of benzocaine fits into the interface between IIS6 and IVS6. E and F, Lidocaine and benzocaine are shown aligned as they are oriented in the closed channel and in the open/inactivated channel. In the closed channel both are oriented more horizontally (E) with their aromatic rings aligned with Phe1759.

Figure 4. Steady-state voltage-dependent Na channel availability of WT (A) and F1759K (B) channels exposed to benzocaine. Protocol as described in Methods. A, WT in control ( ), 0.3 mmol/L ( ), n = 3, 0.5 mmol/L ( ), n = 7, 1 mmol/L ( ), n = 6, and 2 mmol/L ( ) benzocaine. V_half was -135 ± 150 mV. The dashed lines represent the solid lines fitted to mean data but scaled to a value of 1 and adjusted for the background shift in kinetics based on the difference in time between recordings. Means ± SEM of parameters to fits to individual datasets are for half-point: -88 ± 1 mV (control), -101 ± 1 mV (0.3 mmol/L), -106 ± 2 mV (0.5 mmol/L), 117 ± 2 mV (1 mmol/L), and -122 ± 4 mV (2 mmol/L); and slope factor: 6.7 ± 0.2 mV (control), 6.8 ± 0.5 mV (0.3 mmol/L), 9.0 ± 0.8 mV (0.5 mmol/L), 8.8 ± 0.3 mV (1 mmol/L), and 8.5 ± 0.5 mV (2 mmol/L). The inset shows the concentration response for tonic block calculated from the means ± SEM of the I_max from the fits of the individual datasets by a Boltzmann (see Methods) normalized to the I_max in control. Single-site concentration–response fit (see Methods) estimated the ED_{50} to be 0.32 ± 0.02 mM/B, F1759K channels in control ( ), 1 mmol/L ( ), n = 5 and 2 mmol/L ( ) benzocaine. The dashed lines represent data scaled to a value of one and adjusted as described above. V_half shifted -8.3 ± 0.7 mV (1 mmol/L) and -9.7 ± 1.1 mV (2 mmol/L). Means ± SEM of parameters from fits to individual datasets were for half-point: -91 ± 1 mV (control), -100 ± 2 mV (1 mmol/L), and -99 ± 2 mV (2 mmol/L); and for slope factor: 6.2 ± 0.2 mV (control), 5.4 ± 0.3 mV (1 mmol/L), and 7.1 ± 0.3 mV (2 mmol/L). The inset shows the concentration–response relationship calculated from the means ± SEM of the I_max from the fits of the individual datasets by a Boltzmann (see Methods) normalized to the I_max in control. Solid line shows the single-site concentration–response relationship; ED_{50} was 0.66 ± 0.10 mmol/L.

their aromatic rings located close to the aromatic ring of Phe1759 (Figure 5A, 5C, and 5E). In this drug orientation (across the inner cavity), the other flexible end of each molecule would extend to just above the narrow part of the cavity close to the S6 crossing. This model is consistent with mutational data in which alanine or even charged residue substitutions of multiple S6 residues in different domains produce little or no change in tonic block, suggesting that size rather than charge of the amino acid side chain is important. Such low affinity interactions would likely be nonbonded van der Waals drug/protein interactions that do not require the presence of water or charge. In support of this
idea, the energies of interactions, recalculated with lidocaine interacting with our closed channel model with mutations of Phe1759, did not appreciably change.

Homology Model of Inner-Pore Na Channel Interactions With Drugs That Produce Voltage-Sensor Inhibition

Crystal structures for bacterial K channels in the open/inactivated conformation\(^{28}\) can also be used to model the inner pore conformation that permits voltage-sensor inhibition,\(^{11}\) although the open/inactivated conformation of bacterial K channels is expected to differ in its details from that of the voltage-gated Na channel. In the open conformation, the carboxyl ends of the S6 \(\alpha\)-helices in domains I-IV are predicted to form a wide opening filled by bulk water (Figure 5B and 5D), with 2 important consequences. First, it favors protonation of the amino group of lidocaine, thereby permitting the positively-charged amine to participate in \(\pi\)-cation interactions with Phe1759. Second, the open pore favors hydrophobic interactions of the nonpolar part of lidocaine (its alkyl chains and aromatic ring) with the nonpolar residues of the S6 helices that form the sides of the channel, thereby achieving dense packing of lidocaine against the interface of DIII and DIV (Figure 5B). The highest affinity interaction between drugs and the open/inactivated channels is correlated with drugs that have a positively charged amine such as lidocaine, the permanently charged analogs QX-222 and QX-314, and flecaïnide (with its pKa of 9.3). All of these drugs are expected to have high affinity via an interaction of the charged amine with the aromatic ring of Phe1759.\(^{4,11}\)

How does neutral benzocaine interact with Phe1759 and produce voltage-sensor inhibition? Although benzocaine (Figure 5F) has an overall neutral charge, it does have a polarized structure. The aromatic part of benzocaine is an aniline, which has a dipole moment of 1.5 Debye attributable to the lone electron pair of nitrogen delocalizing into the aromatic ring. The other side of the molecule has an aromatic ring of the ester group further increasing the dipole moment of benzocaine to 3.9 Debye.\(^{29}\) Benzocaine is an arylamine, and for this class of compounds, the partial positive charges on the hydrogen atoms of the primary amino group are approximately +0.22 electronic charge, and the charge on N is approximately −0.42.\(^{30}\) This same charge distribution is found in the amine of the side chains of Asn and Gln,\(^{25}\) which have a propensity to interact with aromatic side chains (amino-aromatic interactions) in proteins.\(^{31}\) Therefore, it is reasonable to suggest that benzocaine could participate in such an amino-aromatic interaction. If the primary amine of benzocaine behaves like the tertiary amine of lidocaine, then the amine of benzocaine would be expected to adopt a position at right angles to the ring of Phe1759, as shown in Figure 5D. The combination of an absence of charge, the smaller size of benzocaine, and the fact that it cannot make an aromatic-aromatic interaction with Tyr1766 would then most likely make it lower affinity.

It should be noted that in the restricted volume of the closed channel, both lidocaine and benzocaine adopt a somewhat horizontal position (Figure 5E), where their aromatic rings interact weakly with Phe1759 (Figure 5A, 5C, and 5E). In contrast, in the open/inactivated channel both lidocaine and benzocaine reorient vertically (Figure 5F) with their tertiary and primary amines directed upward for stronger interaction with \(\pi\)-clouds of Phe1759 (Figure 5B and 5D).

Discussion

These experiments characterize 2 distinct components to antiarrhythmic drug block of voltage-gated Na channels, which can be related to structural channel conformations and can be used to interpret experimental data that may reflect contributions from both types of block. The first type is essentially voltage-independent. We term it lipophilic block to emphasize our proposal that it represents neutral forms of drugs interacting with neutral residues in the closed channel pore. The closest analog in the literature would be first pulse block, that block observed in the first depolarization after exposure to drug while holding at a very negative potential. The other is voltage-dependent, and we term it voltage-sensor inhibition to emphasize that it results from stabilization of the S4s in domains III and IV. Voltage-sensor inhibition depends on interaction of drug with Phe1759 (in DIVS6 just below the selectivity filter), and it is responsible for high-affinity block in the tens of micromolar range for lidocaine. Lipophilic block, on the other hand, has a lower affinity (in the millimolar range) and involves the closed pore, but without a specific interaction with Phe1759 or the voltage sensors. Tonic block, as frequently reported in the literature, can have contributions from both types, and it may have a great variability depending on the Na channel isoform, the specific affinity of the drug and the effects of the membrane potential on intrinsic channel kinetics.

Modeling suggests that LA interaction with the pore walls is lipophilic, consistent with the finding that benzocaine and lidocaine have similar voltage-independent affinities for closed channels. In contrast, their affinities differ markedly between open/inactivated or closed/inactivated channels, a reflection of difference in energy of interaction with the \(\pi\)-cation property of Phe1759.\(^{9}\)

Link Between Phe1759 and High-Affinity Voltage-Sensor Inhibition

Phe1759 is a critical residue connecting antiarrhythmic drug binding in the pore with the effects on gating charge causing voltage-sensor inhibition. Because LA drugs modify the gating charge movements of the S4s in both domains III and IV,\(^{13,14}\) it is unlikely that drug interaction solely with Phe1759 in DIVS6 would be sufficient to affect the DIIIIS4. In our homology model of the open channel, LA is predicted to sit in a cleft between the side chains of Leu1461 (DIIIIS6) and Phe1759 (DIVS6) (Figure 5). Supporting evidence is also available from mutagenesis studies in which mutations at Leu1461 also affect use-dependent block.\(^{32}\) Close interaction of lidocaine and benzocaine with the interface between both DIIIIS6 and DIVS6 may constrain the movement of both S6s. Based on K channel structures,\(^{33}\) the constrained S6s may be allosterically linked to their respective S4 voltage sensors by interactions of the S4-S5 linkers with the intracellular tails of the S6 helices. LA interaction with Phe1759 appears to be the key to the complex structural changes that underlie the
Both lidocaine and QX drugs are low-affinity blockers when binding. Moreover, when the domain III and domain IV lipophilic block of closed/rested channels. This is probably at very negative membrane potentials would correspond to voltage-sensor inhibition. Under ideal conditions, tonic block results from both lipophilic block and voltage-dependent Na channel availability curves observed components allows for understanding the classical changes in the separation of LA block into lipophilic and voltage-sensor voltage-dependent Na channel availability and antiarrhythmic drugs. The voltage-dependent Na channel availability curves observed for antiarrhythmic drugs. At any given holding membrane potential, tonic block results from both lipophilic block and voltage-sensor inhibition. Under ideal conditions, tonic block at very negative membrane potentials would correspond to lipophilic block of closed/rested channels. This is probably never achieved in practice after cells have been repeatedly depolarized, but the block evident in the first depolarization after exposure to drug from a very negative holding potential may best approximate it. In contrast, as the membrane potential becomes more positive (even at potentials where all channels are fully available to open in the absence of drug; see Figure 2A) or as the concentration of drug is increased, the probability of Na channels becoming drug-bound in the voltage-sensor–inhibited conformation increases. Furthermore, voltage-sensor inhibition by LA occurs at voltages negative to channel opening, suggesting that preopen, closed states, in addition to open/inactivated states, may increase interaction with Phe1759. In summary, the experiments reported here differentiate between 2 types of block that lidocaine and lidocaine-like drugs achieve in clinical use. One type, which we term voltage-sensor inhibition, depends on drug interactions with DIV–S6 Phe1759 and its consequent effects on gating charge. The other type occurs in the closed channel without specific interaction with Phe1759, and it appears to reflect distributed interactions of the uncharged drug with the channel walls of the closed pore with an affinity approximating 1 mmol/L. In order for a drug to be a local anesthetic, either interaction would be sufficient. However, voltage-sensor inhibition is the more important drug–channel interaction for an antiarrhythmic drug. Moreover, the LA affinity for voltage-sensor inhibition needs to be much greater than its affinity for closed, hyperpolarized channels, so that block becomes more prominent in depolarized than in normal polarized tissue. In addition to producing voltage-sensor inhibition, kinetic rates of drug–channel interaction must match kinetics of channel transitions for use-dependence to occur. Benzoicaine is not useful as an antiarrhythmic not because it lacks the ability to produce voltage-sensor inhibition, but because its affinity for that conformation is too low. Its involvement with the voltage sensors is indirectly evident in ionic current measurements, but it can be directly observed in gating current recordings. At 2 mmol/L, it produced only about half of the maximum effect on gating charge compared to lidocaine, which was estimated to have an ED_{50} of _= 15 μmol/L_. lidocaine, on the other hand, with its >50-fold differential between lipophilic block and voltage-sensor inhibition, is a commonly used antiarrhythmic drug.

Acknowledgments

We thank Wen Qing Yu (molecular biology) and Tiehua Chen at the University of Utah and Jack Kyle (molecular biology) and Constance Mlecko (cell preparation and electrophysiology) at the University of Chicago for their excellent technical contributions.

Sources of Funding

This work was supported by NIH grants HL-R01-044630 (to M.F.S. and D.A.H.), HL-065661 (to D.A.H. and H.A.F.), and T32 HL072742 (to M.M.M.).

Disclosures

None.

References


Disclosures

None.

References


Using Lidocaine and Benzocaine to Link Sodium Channel Molecular Conformations to State-Dependent Antiarrhythmic Drug Affinity
Dorothy A. Hanck, Elena Nikitina, Megan M. McNulty, Harry A. Fozzard, Gregory M. Lipkind and Michael F. Sheets

_Circ Res._ published online August 6, 2009; _Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/early/2009/08/06/CIRCRESAHA.109.198572.citation

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