A general mechanism for drug promiscuity: Studies with amiodarone and other antiarrhythmics

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Amiodarone is a widely prescribed antiarrhythmic drug used to treat the most prevalent type of arrhythmia, atrial fibrillation (AF). At therapeutic concentrations, amiodarone alters the function of many diverse membrane proteins, which results in complex therapeutic and toxicity profiles. Other antiarrhythmics, such as dronedarone, similarly alter the function of multiple membrane proteins, suggesting that a multipronged mechanism may be beneficial for treating AF, but raising questions about how these antiarrhythmics regulate a diverse range of membrane proteins at similar concentrations. One possible mechanism is that these molecules regulate membrane protein function by altering the common environment provided by the host lipid bilayer. We took advantage of the gramicidin (gA) channels’ sensitivity to changes in bilayer properties to determine whether commonly used antiarrhythmics—amiodarone, dronedarone, propranolol, and pindolol, whose pharmacological modes of action range from multi-target to specific—perturb lipid bilayer properties at therapeutic concentrations. Using a gA-based fluorescence assay, we found that amiodarone and dronedarone are potent bilayer modifiers at therapeutic concentrations; propranolol alters bilayer properties only at supratherapeutic concentration, and pindolol has little effect. Using single-channel electrophysiology, we found that amiodarone and dronedarone, but not propranolol or pindolol, increase bilayer elasticity. The overlap between therapeutic and bilayer-altering concentrations, which is observed also using plasma membrane–like lipid mixtures, underscores the need to explore the role of the bilayer in therapeutic as well as toxic effects of antiarrhythmic agents.

INTRODUCTION

Cardiac arrhythmias are major causes of morbidity and mortality (Benjamin et al., 1998), with atrial fibrillation, the most prevalent cardiac arrhythmia, being a major risk factor for stroke (Mozaffarian et al., 2015). Amiodarone is the most commonly prescribed antiarrhythmic drug owing to its efficacy and minimal proarrhythmic side effects (Zimetbaum, 2012). Amiodarone acts through multiple mechanisms—prolongation of repolarization, reduction of excitability, and slowing of conduction (Singh, 1983)—exerting these effects by altering the function of diverse membrane proteins: ion channels, ion exchangers, and adrenergic receptors (Heijman et al., 2013b). This multi-target therapeutic mechanism is a feature shared by other antiarrhythmics in current use (Dobrev et al., 2012; Grunnet et al., 2012), suggesting that a multipronged mechanism of action may be a desired feature of antiarrhythmic drugs, although the mechanism for such target promiscuity is unclear.

In the case of membrane proteins, the concurrent regulation of many different proteins could be caused by a common mechanism arising from drug-dependent changes in lipid bilayer properties that alter the energetic coupling between membrane proteins and their host bilayer (Rusinova et al., 2011). This bilayer-mediated mechanism results from hydrophobic coupling between membrane proteins and the surrounding lipid bilayer (Lundbaek et al., 2010b).

The lipid bilayer adaptation to a membrane protein’s hydrophobic domain has an associated energetic cost, the bilayer deformation energy ($\Delta G_{\text{def}}^0$), which varies with changes in protein shape and lipid bilayer properties (Nielsen et al., 1998; Nielsen and Andersen, 2000; Partenskii and Jordan, 2002). Different protein conformations (e.g., Lundbaek et al., 2010a) are thus likely to be associated with different $\Delta G_{\text{def}}^0$ (Fig. 1 A). This concept is important because the free energy cost ($\Delta G_{\text{total}}^{\text{I+II}}$) for a conformational change (between states I and II) is determined by the contributions from the membrane protein ($\Delta G_{\text{protein}}^{\text{I+II}}$) and the bilayer ($\Delta G_{\text{bilayer}}^{\text{I+II}} = \Delta G_{\text{def}}^{\text{II}} - \Delta G_{\text{def}}^{\text{I}}$) (e.g., Lundbaek et al., 2010a):

$$\Delta G_{\text{total}}^{\text{I+II}} = \Delta G_{\text{protein}}^{\text{I+II}} + (\Delta G_{\text{def}}^{\text{II}} - \Delta G_{\text{def}}^{\text{I}}) = \Delta G_{\text{protein}}^{\text{I+II}} + \Delta G_{\text{bilayer}}^{\text{I+II}}$$  (1)

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where $\Delta G_{\text{def}}^{I}$ and $\Delta G_{\text{def}}^{II}$ vary with changes in bilayer elasticity, thickness, and intrinsic lipid curvature (Andersen et al., 2007), which in turn means that $\Delta G_{\text{def}}^{I-II}$ (and therefore $\Delta G_{\text{def}}^{\text{total}}$) will vary with changes in bilayer properties (except when the changes in $\Delta G_{\text{def}}^{I}$ equal the changes in $\Delta G_{\text{def}}^{II}$).

Drug-induced changes in lipid bilayer properties thus will shift a protein’s conformational distribution by changing $\Delta G_{\text{bilayer}}^{I-II}$, thereby producing changes in protein function (Fig. 1 B). Such bilayer-mediated regulation is exemplified, for example, in the effects of amphiphiles on voltage-gated sodium (NaV) channels (Lundbæk et al., 2004; Rusinova et al., 2011; Ingólfsson et al., 2014), mechanosensitive MscL, voltage-gated potassium (KV2.1) channels (Ingólfsson et al., 2014), and the proton-gated prokaryotic potassium channel KcsA (Rusinova et al., 2014). Importantly, because $\Delta G_{\text{bilayer}}^{I-II} = \Delta G_{\text{bilayer}}^{\text{def}} - \Delta G_{\text{def}}^{II}$, the relative changes in function will vary among membrane proteins.

Figure 1. A schematic illustration of how amphiphilic drugs can modulate membrane protein function by a bilayer-mediated mechanism and structures of the antiarrhythmics. (A) Schematic representation of the bilayer-mediated regulation of membrane protein function, which arises because the reversible partitioning of the amphiphiles between the aqueous solution and the bilayer–solution interface alters lipid bilayer properties, including the elasticity (Evans et al., 1995; Zhelev, 1998; Bruno et al., 2013) and thus $\Delta G_{\text{def}}$ (and therefore $\Delta G_{\text{bilayer}}^{I-II}$). In the figure, conformations I and II are denoted as “closed” and “open,” respectively. (B) Molecular structures of the antiarrhythmics amiodarone, dronedarone, propranolol, and pindolol.
We used gramicidin (gA) channels to quantify how amiodarone, dronedarone, propranolol, and pindolol (Fig. 1 C) alter $\Delta\Delta G_{\text{bilayer}}^{\text{G}}$. The bilayer deformation resulting from channel formation makes gA channels powerful probes because changes in the gA monomer+dimer equilibrium, as reflected in changes in channel activity, can be directly related to changes in bilayer properties, making it possible to quantify $\Delta\Delta G_{\text{bilayer}}^{\text{G}} = \Delta G_{\text{bilayer}}^{\text{G,drug}} - \Delta G_{\text{bilayer}}^{\text{G, no drug}}$ (Lundbæk et al., 2010a).

Using a gA-based fluorescence assay (GBFA), we determined the bilayer-modifying potency of each drug (Ingólfsson and Andersen, 2010) and used gA single-channel electrophysiology (Lundbæk et al., 2010a; Rusinova et al., 2011) to obtain detailed information about channel electrophysiology (Lundbæk et al., 2010a; Ingólfsson and Andersen, 2010) and used gA single-channel experiments was the naturally occurring mixture, which is 80–85% gA plus gA B and C (Abo-Riziq et al., 2006). (The mixture is often called gA D [gD] after R. Dubos, who discovered the cell membrane outer leaflet. All the antiarrhythmics tested alter lipid bilayer properties, with amiodarone and dronedarone doing so at clinically relevant concentrations. Our results show that, in addition to direct effects on specific targets, amiodarone and dronedarone may alter the function of diverse membrane proteins by a general bilayer-mediated mechanism.

**MATERIALS AND METHODS**

Materials

DC18:1PC, 1,2-dioleoyl-sn-glycero-3-phosphocholine (DC22:1PC), hSM, and plant cholesterol were from Avanti Polar Lipids, Inc. Dronedarone, amiodarone, propranolol, pindolol (all ≥98% pure), and sodium thiosulfate (Na2S2O3; 99.999% pure) were from Sigma-Aldrich. Iodine (I2; 99.8% pure) was from VWR International. 8-Aminonaphthalene-1,3,6-trisulfonate (ANTS) was from Invitrogen. The gA analogues [Ala1]gA and gA(15), and the sequence-shortened, left-handed des-d-Val-Gly-gA(13), and gA(13), used for the single-channel experiments, were used in bilayer properties (Ingólfsson and Andersen, 2010). Each measurement consisted of (four to eight) individual mixing reactions, and the rates for each mixing reaction were averaged and normalized to the control rate in the absence of drug.

**Single-channel gA current measurements**

gA single-channel recordings were done at 25°C using the bilayer punch method (Andersen, 1983; Rusinova et al., 2011). In brief, planar bilayers were formed from DC18:1PC in w-dodecane or a DC18:1PC/hSM/Chol in w-dodecane across a hole in a Teflon partition that separated two 1.0-M NaCl (buffered to pH 7.0 with HEPES), as described previously (Greathouse et al., 1999; Rusinova et al., 2011). The membranes were doped with gA(15) and gA(13), and control channel activity was recorded after a 30-min equilibration. Aliquots of 10–100-nM DMSO stock solutions of dronedarone, amiodarone (±Na2S2O3), propranolol, pindolol, and I2 (in H2O) were then added to both sides of the bilayer; the solutions were equilibrated with the membrane for 10 min before recording. DMSO did not exceed 1% (vol/vol), a concentration that has no effect on gA channel function (Ingólfsson and Andersen, 2010). Appearance frequencies ($f$) were determined only if the bilayer remained intact for the duration of the entire experiment (before and after drug addition). The number of gA channel events from one to two recordings before drug addition was determined from the number of channel appearances obtained from the analysis of single-channel lifetimes and divided by the total recording time (≥5 min) to give the control $f_{\text{basal}}$. If the bilayer remained intact, in a similar procedure to the one above, $f_{\text{drug}}$ was determined from one to two recordings immediately after a 10-min equilibration with the drug. Single-channel lifetimes ($\tau$) were determined by fitting survivor histograms with a single-exponential distribution ($N(t)/N(0) = \exp(-t/\tau)$, where $N(t)$ is the number of channels with lifetime longer than time $t$) using Origin 6.1 (OriginLab). The results represent mean ± SD ($n = 2–4$) or mean ± range (for $n = 2$). Relative changes in bilayer deformation energy were calculated as (Artigas et al., 2006; Rusinova et al., 2011):

$$\Delta\Delta G_{\text{bilayer}}^{\text{G}} = -kT \cdot \ln \left( f_{\text{drug}} \cdot f_{\text{drug}} / f_{\text{basal}} \cdot f_{\text{basal}} \right) \right)$$

where $f_{\text{drug}}$ and $f_{\text{basal}}$ denote the gA channel appearance frequency and lifetime in the presence of an antiarrhythmic.

**Online supplemental material**

Fig. S1 plots the relative changes in gA(13) and gA(15) $\tau$ and $f$ with increasing concentrations of amiodarone, dronedarone, propranolol, and pindolol.
have acute effects on cardiac excitability (Kodama et al., 1997; Heijman et al., 2013b) and alter sarcolemmal enzyme activities (Chatelain et al., 1989), and dronedarone at its clinical concentrations (Table 1) using the GBFA.

The GBFA takes advantage of gA channels’ permeability to Tl+, a quencher of the water-soluble fluorophore ANTS, where the rate of influx is a function of the time-averaged number of gA channels in the LUV membrane (Ingólfsson and Andersen, 2010). In the absence of gA (top horizontal traces in Fig. 2 A), the drugs have no effect on the rate of fluorescence quenching, meaning that the compounds did not compromise lipid bilayer stability at the concentrations tested. In the presence of the drugs, the gA-dependent fluorescence quench rate increases (bottom traces in Fig. 2 A), demonstrating that the antiarrhythmics increase the rate of propranolol, and pindolol. Fig. S2 shows effects of antiarrhythmics on gA(13) and gA(15) current transition amplitude. Fig. S3 (A and B) shows changes in ΔΔG_{bilayer} as a function of changes in gA(13) and gA(15) current transition amplitudes as a function of antiarrhythmic mole fraction in the bilayer. Fig. S3 C plots changes in ΔΔG_{bilayer} as a function of changes in gA(13) and gA(15) current transition amplitudes. Fig. S4 shows time dependence of changes in gA(13) and gA(15) τ by amiodarone in the absence and presence of Na2S2O3, which reduces I2 to I−. Fig. S5 shows absence of amiodarone-effect time dependence in GBFA experiments. Table S1 lists the sequence and channel hydrophobic length of gA analogues used in this study. The online supplemental material is available at http://www.jgp.org/cgi/content/full/jgp.201511470/DC1.

RESULTS

Effects of antiarrhythmics on lipid bilayer properties

We tested the bilayer-modulating effects of amiodarone, propranolol, and pindolol at concentrations where they have acute effects on cardiac excitability (Kodama et al., 1997; Heijman et al., 2013b) and alter sarcolemmal enzyme activities (Chatelain et al., 1989), and dronedarone at its clinical concentrations (Table 1) using the GBFA.

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### Figure 2

Antiarrhythmics alter lipid bilayer properties. (A, left) Fluorescence quench traces showing Tl+ quenching of ANTS fluorescence in DC22:1PC LUVs without gA (−gA; the top two traces) and with gA (+gA; the bottom five traces) in the absence of drug (black, control) or with dronedarone (green), amiodarone (orange), propranolol (cyan), and pindolol (purple). Amiodarone, dronedarone, and propranolol increase the fluorescence signal up to 12% depending on the concentration, but the flux rate measurements were not affected. The results for each drug represent five to eight repeats (dots) and their averages (solid lines). (Right) Single repeats (dots) with stretched exponential fit (solid line). (B) Normalized quench rates determined from the stretched exponential fits at varying antiarrhythmic concentrations. Error bars represent mean ± SD (n = 3–5).

### Table 1

<table>
<thead>
<tr>
<th>Antiarrhythmic</th>
<th>Clinical plasma concentrations (µM)</th>
<th>Experimental concentrations (µM) Nominal</th>
<th>Free</th>
<th>LogP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>m&lt;sub&gt;n&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dronedarone</td>
<td>0.15–0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10</td>
<td>7 × 10&lt;sup&gt;−5&lt;/sup&gt;</td>
<td>8.75</td>
<td>4.0 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30</td>
<td>1 × 10&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>7.8</td>
<td>10&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.01–1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>200</td>
<td>90</td>
<td>5.48</td>
<td>2.5 × 10&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pindolol</td>
<td>0.04&lt;sup&gt;f&lt;/sup&gt;</td>
<td>300</td>
<td>300</td>
<td>1.75</td>
<td>1.5 × 10&lt;sup&gt;−2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Benet et al. (2011).
<sup>b</sup>Estimated following Bruno et al. (2007), Ingólfsson et al. (2007), and Rusinova et al. (2011).
<sup>c</sup>Heijman et al. (2013a).
<sup>d</sup>Haffajee et al. (1983), Latini et al. (1984), and Seydel (2003).
<sup>e</sup>Woosley et al. (1979), Wilson et al. (1982), and Seydel (2003).
<sup>f</sup>Channer et al. (1994) and Mehvar and Brocks (2001).
to the rate in the absence of drug) as a function of drug concentration (Fig. 2 B).

The antiarrhythmics decreased $\Delta G_{\text{bilayer}}^{MD\rightarrow}$ with rank order: dronedarone $\geq$ amiodarone $>$ propranolol $>$ pindolol. The concentrations used in Fig. 2 are the nominal concentrations, which are not corrected for drug distribution between the aqueous and membrane phases. We estimated the aqueous concentrations following Bruno et al. (2007) using measured partition coefficients into lipid bilayers, LogP, and compared those to the free plasma concentrations at therapeutic doses (Table 1).

$Tl^+$ influx into the LUVs. This increase is caused by a shift in the gA monomer$\leftrightarrow$dimer equilibrium toward the conducting dimer state as antiarrhythmics decrease $\Delta G_{\text{bilayer}}^{MD\rightarrow}$ and thereby the free energy of dimerization (Andersen et al., 2007):

$$
\frac{[D]}{[M]} = K^{MD\rightarrow} = \exp \left( \frac{\Delta G^{MD\rightarrow}_{\text{protein}} + \Delta G^{MD\rightarrow}_{\text{bilayer}}}{k_B T} \right)
$$

(5)

The relative potency of each antiarrhythmic was quantified by plotting the change in quench rate (normalized to the rate in the absence of drug) as a function of drug concentration (Fig. 2 B).

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<table>
<thead>
<tr>
<th>gA single-channel properties</th>
<th>Amiodarone</th>
<th>Dronedarone</th>
<th>Propranolol</th>
<th>Pindolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{13}/f_{13\text{ctrl}}$</td>
<td>2.5 ± 0.7</td>
<td>6.5 ± 0.3</td>
<td>2.1 ± 0.1</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>$f_{15}/f_{15\text{ctrl}}$</td>
<td>1.6 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>$\tau_{13}/\tau_{13\text{ctrl}}$</td>
<td>1.5 ± 0.3</td>
<td>2.3 ± 0.04</td>
<td>1.2 ± 0.1</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>

Figure 3. Antiarrhythmics increase gA single-channel activity and decrease the bilayer deformation energy ($\Delta G_{\text{bilayer}}^{MD\rightarrow}$). (A) gA single-channel traces without (top row) and with (bottom row) the antiarrhythmics at the indicated concentrations; red and blue dashed lines indicate the average gA$^{(13)}$ and gA$^{(15)}$ single-channel current amplitudes. (B) Changes in $\Delta G_{\text{bilayer}}^{MD\rightarrow}$, which were estimated from the ratio of the time-averaged number of gA channels in the presence ($\tau_{\text{drug}} \cdot f_{\text{drug}}$) and absence ($\tau \cdot f$) of the antiarrhythm (compare Eq. 4). Blue symbols denote results for gA$^{(15)}$ channels, and red symbols denote results for gA$^{(13)}$ channels. Error bars represent mean ± SD, if $n \geq 3$; mean ± range/2, if $n = 2$. 

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The free concentrations of amiodarone and dronedarone, the most hydrophobic compounds, are comparable to the clinical concentrations, whereas the free concentrations of the less hydrophobic propranolol and pindolol are orders of magnitude higher than the clinical concentrations (Anavekar et al., 1975; Woosley et al., 1979), although there is overlap between the concentration range where propranolol alters lipid bilayer properties and the concentration range where it alters the function of voltage-dependent sodium channels (Wang et al., 2010).

The lipid bilayer–modifying potency of the tested antiarrhythmics need not scale with the compounds’ bilayer-partitioning coefficient (Bruno et al., 2007; Rusinova et al., 2011). At the given partitioning coefficient and concentration, antiarrhythmics reach varying mole fraction in the membrane (Table 1, \(m_m\)) to achieve similar magnitudes of bilayer-modifying effects (Fig. 2 B), where dronedarone requires approximately an order of magnitude lower \(m_m\) than propranolol for comparable effects; to achieve a comparable effect with pindolol would require a much higher \(m_m\) than for the other compounds.

Antiarrhythmics increase bilayer elasticity, which contributes to the decrease in \(\Delta G_{\text{bilayer}}\)

To determine what bilayer properties the antiarrhythmics alter, we determined the effects on the average single-channel lifetimes (\(\tau\)), appearance frequencies (\(f\)), and current transition amplitudes (compare Andersen et al., 2007; Lundbæk et al., 2010a; Rusinova et al., 2011). The gA single-channel \(\tau\) is the inverse of the gA dimer dissociation rate constant, and \(f\) is proportional to monomer association rate constant. Changes in \(\tau\) and \(f\) in the presence of a bilayer-modifying amphiphile reflect the amphiphile’s effect on the bilayer deformation energy, and the relative changes in \(\tau\) and \(f\) allow us to estimate the amphiphile-induced change in the bilayer deformation energy, \(\Delta G_{\text{bilayer}}\) (Eq. 4). Because the experiments were done with gA analogues of different lengths, the 15-amino acid gA(15) and the enantiomeric, sequence-shortened analogue gA−(13), respectively, which form channels of different lengths (Table S1) and thus different bilayer-channel hydrophobic mismatches. Consequently, if a drug alters bilayer elasticity, the magnitude of the resulting changes in \(\tau\) and \(f\) will be larger for the channels with the larger hydrophobic mismatch (in this case those formed by the gA−(13) subunits). Conversely, if the changes in bilayer deformation are similar for channels of different length, the drugs have minimal effects on bilayer elasticity.

All the antiarrhythmics increased \(\tau\) and \(f\) (Figs. 3 A and S1, and Table 2). The initial experiments with amiodarone showed a time-dependent increase in channel activity (Fig. S4), which we surmised might be caused by the iodine in amiodarone. We therefore did experiments in which we added Na2S2O3, which reduces I2 to I− (Finkelstein and Cass, 1968), together with the amiodarone. Na2S2O3 did indeed abolish the time-dependent increase in channel activity (lifetime), but the effect of amiodarone could not be mimicked by adding I2 (Fig. S4, legend). In any case, all the experiments with amiodarone were done in the presence of 50 µM Na2S2O3. Dronedarone and amiodarone produced larger changes in \(\tau\) and \(f\) for the shorter gA−(13) channels, as compared with the longer gA(15) channels; within experimental error, propranolol and pindolol produced similar changes in \(\tau\) and \(f\) for both channels (Table 2 and Fig. S1).

Knowing the relative changes in \(\tau\) and \(f\) (Table 2), we can estimate the changes in \(\Delta G_{\text{bilayer}}\) following Artigas et al. (2006), Andersen et al. (2007), Lundbæk et al. (2010a), Rusinova et al. (2011), Eq. 4, and Fig. 3 B. All the antiarrhythmics decreased \(\Delta G_{\text{bilayer}}\) with dronedarone being the most, and pindolol the least, potent, whether they increased or decreased membrane fluidity (Chatelain et al., 1985).

Confirming previous studies (Lundbæk et al., 2010b; Rusinova et al., 2011), structurally diverse compounds produce remarkably similar relative increases in the lifetimes of the short gA−(13) channels relative to the long gA(15) channels. Fig. 4 A shows the linear relation between the natural logarithms of the changes in \(\tau\) for the short gA−(13) channels ln[\(\tau_{13/cntrl}\)] versus the changes for the long gA(15) channels ln[\(\tau_{15/cntrl}\)], where we included earlier results from Rusinova et al. (2011) and Ingólfsson et al. (2014).

The overall similarity among the ln[\(\tau_{13/cntrl}\)] versus ln[\(\tau_{15/cntrl}\)] relations for different compounds, together with the similar effects on left- and right-handed channels (the channels formed by gA−(13) and gA(15) have opposite chirality), shows that the changes in gA activity primarily are caused by changes in general bilayer properties that are insensitive to the structural characteristics of the compounds, as opposed to direct interactions with gA itself. The slope of the ln[\(\tau_{13/cntrl}\)] versus ln[\(\tau_{15/cntrl}\)] relation is 1.20 ± 0.03 (lower and upper confidence limits: 1.14 and 1.25), regardless of the particular compound’s bilayer-modifying potency (Fig. 4 A). Focusing on the individual antiarrhythmics, the slopes of the ln[\(\tau_{13/cntrl}\)] versus ln[\(\tau_{15/cntrl}\)] relationships are: 1.3 ± 0.2 for dronedarone, 1.5 ± 0.1 for amiodarone, 1.5 ± 0.3 for propranolol (Fig. 4 B, inset), and 1.1 ± 0.3 for pindolol. In the case of propranolol, however, given the miniscule changes in \(\tau\) (Table 2 and Fig. S1), slope of the ln[\(\tau_{13/cntrl}\)] versus ln[\(\tau_{15/cntrl}\)] relation does not differ from 1. A histogram of individual slopes can be fit with a Gaussian with a mean of 1.30 ± 0.02, \(\sigma = 0.3\) (Fig. 4 B), suggesting that there may be additional compound structure–dependent effects on the lipid bilayer, because of their varying effects on the acyl chain packing and dynamics in the bilayer core, in
addition to the increase in bilayer elasticity that arises for thermodynamic reasons (Evans et al., 1995; Needham et al., 1998; Bruno et al., 2013).

In addition to the changes in $\tau$ and $f$, the antiarrhythmics reduced the current transition amplitudes of both $gA(15)$ and $gA^- (13)$ channels (Fig. S2). The changes in current transition amplitudes most likely arise because charged amphiphiles that adsorb to the bilayer–water interface in the vicinity of a $gA$ channel will impart a surface potential that will alter the local ion concentrations and thus the single-channel current amplitude transitions (Apell et al., 1979; Lundbæk et al., 1997; Bruno et al., 2007, 2013; Rusinova et al., 2011). The antiarrhythmics are secondary and tertiary amines with pKs of $\sim 9$, and will thus impart a positive surface charge when they partition into the membrane–solution interface at pH 7.0 (Froud et al., 1986). The positive charge will reduce the cation concentration near the pore entrance, which would account for the reduction in current transition amplitudes, which is opposite to the increases that were observed with the negatively charged polyunsaturated fatty acid, docosahexaenoic acid, which increases the current transition amplitudes (Bruno et al., 2013). We cannot completely rule out the possibility that the antiarrhythmics interact directly with $gA$ channels, but the similar effects on left- and right-handed channels, the correlation between the magnitude of the current transition amplitude reduction and the antiarrhythmic’s LogP (Table 1), as well as the correlation between antiarrhythmic mole fractions at which the current transition amplitude shift occurs (Fig. S3 A) and at which they decrease $\Delta G_{\text{bilayer}}$ (Fig. S3 B) all suggest that accumulation of surface charge is a primary determinant of the decreased current transition amplitudes. Plotting the changes in $\Delta G_{\text{bilayer}}$ as a function of the changes in current transition amplitude, $(i_{\text{ctrl}} - i) / i_{\text{ctrl}}$ (Fig. S3 C), show that all the antiarrhythmics produce similar slopes in the $\Delta G_{\text{bilayer}}$ versus $(i_{\text{ctrl}} - i) / i_{\text{ctrl}}$ relations for the $gA(15)$ channels, indicating that the two parameters correlate and depend in a similar manner on the mole fraction of the compound in the bilayer. For the $gA^- (13)$ channels, the slopes of the $\Delta G_{\text{bilayer}}$ versus $(i_{\text{ctrl}} - i) / i_{\text{ctrl}}$ relations for pindolol and propranolol are similar to those for $gA(15)$, whereas the slopes for dronedarone and amiodarone are larger (as would be expected because dronedarone and amiodarone increase bilayer elasticity).

**Figure 4.** The antiarrhythmic-induced changes in the single-channel lifetimes of $gA^- (13)$ channels versus the changes in the lifetimes of $gA(15)$ channels. (A) Natural logarithm of relative changes in $\tau_{13}$ (ln($\tau_{13}/\tau_{13\text{ctrl}}$)) versus the natural logarithm of relative changes in $\tau_{15}$ (ln($\tau_{15}/\tau_{15\text{ctrl}}$)) observed for dronedarone (green), amiodarone (orange circle), propranolol (blue triangle), and pindolol (black square) plotted together with results from Lundbæk et al. (2010b) and Rusinova et al. (2011). The points cluster around a straight line with slope 1.2 ± 0.03 (error bars represent mean ± SE). (B) Distribution of the slopes for the ln$\tau_{13}$ versus ln$\tau_{15}$ relations for the individual compounds in A. The distribution is fit by Gaussian function with a mean ± SD ($\mu$ calculated from the fit) of 1.3 ± 0.2, $\sigma = 0.3$. Changes in the histogram bin size result in the median slope ranging between 1.2 and 1.3. Inset illustrates an individual linear fit to the (ln($\tau_{13}/\tau_{13\text{ctrl}}$)) versus ln($\tau_{15}/\tau_{15\text{ctrl}}$) in the presence of dronedarone (green symbols). Slopes of the linear fits, such as that in the inset, obtained for each compound were used to construct the distribution in B.

**Experiments with ternary lipid bilayer mixtures**

Experiments in DC14:1PC bilayers yield unambiguous information that can be used to calculate changes in the bilayer deformation energy caused by reversible partitioning (Artigas et al., 2006; Andersen et al., 2007; Lundbæk et al., 2010a; Rusinova et al., 2011) of drugs without the complications of domain reorganization and heterogeneous phospholipid mixing that may confound the quantification of effects on more complex bilayers. The bilayers of cell membranes, however, have complex lipid compositions (Wenk, 2005; van Meer et al., 2008). To explore whether the antiarrhythmics alter bilayer properties in bilayers with heterogenous lipid
composition, where their effects could be dampened or obscured (or, maybe, enhanced) by lipid redistribution and changes in domain organization (e.g., Heerklotz, 2002; Heerklotz et al., 2003), we did experiments using DC_{18:1}PC/bSM/Chol (1:1:1) mixtures, where there is coexistence of liquid-ordered and liquid-disordered domains (Veatch and Keller, 2005; Baumgart et al., 2007; Petruzielo et al., 2013). If gA forms conducting dimers in both the liquid-ordered and liquid-disordered domains that have different properties (i.e., stiffness), we would expect this to differentially alter gA single-channel properties, which would be observed as two populations of channels with different average lifetimes (τ). We observe only a single, kinetically homogeneous channel population, however (Fig. 5). The parsimonious interpretation of these results is that ΔG_{bilayer} in the stiffer liquid-ordered domains is so much larger than in the liquid-disordered domains (e.g., Lundbæk et al., 2003) that we observe gA channel activity only in the liquid-disordered domains—and that the antiarrhythmics do not soften the liquid-ordered domains sufficiently to allow for channel formation.

Dronedarone produced similar changes in the reference DC_{18:1}PC bilayers and in bilayers formed from DC_{18:1}PC/bSM/Chol 1:1:1 (Fig. 6), as a mimic of the extracellular leaflet of the plasma membrane (Feigenson, 2007). Dronedarone increased both τ and f for gA(15) channels over a similar concentration range as in DC_{18:1}PC membranes, but to a greater extent (Table 2 and Figs. S1 and 6, A and B): at 3 µM dronedarone, ΔG_{bilayer} was doubled from 5.2 kJ/mole in DC_{18:1}PC to 10.5 kJ/mole in DC_{18:1}PC/bSM/Chol (compare Figs. 3 B and 6 C). The increased effect on ΔG_{bilayer} most likely reflects that the liquid-ordered domains in the ternary mixture are stiffer than the DC_{18:1}PC bilayers, which would lead to larger absolute changes in the amphiphile-induced changes in bilayer elasticity (Bruno et al., 2013). In any case, these results show that there are no qualitative differences in the antiarrhythmics’ effects on single-component and multicomponent bilayers.

**DISCUSSION**

A hallmark of many current and in-development antiarrhythmics is their effects on numerous, diverse membrane...
proteins (Singh, 1983; Dobrev et al., 2012; Zimetaubam, 2012), as is the case for amiodarone and dronedarone (Kodama et al., 1997; Zimetaubam, 2012; Heijman et al., 2013a). Although well established, the mechanism(s) underlying these multi-target effects remains unresolved. The prevailing paradigm attributes drug regulation of protein function to direct interactions (binding) of a drug to its target, but this paradigm does not readily explain the multi-targeting behavior over a narrow effective concentration range. So, alternatively, amiodarone and dronedarone could act through a common, more general mechanism such as drug-induced changes in the energetic coupling between the bilayer-embedded proteins and their host bilayer (Sackmann et al., 1984; Andersen et al., 1992; Keller et al., 1993; Lundbæk and Andersen, 1994). To explore this possibility, we took advantage of the gA channels’ sensitivity to changes in lipid bilayer properties to determine whether amiodarone and dronedarone, as well as propranolol and pindolol (Chatelain et al., 1989), might alter the energetic coupling between a well-defined reporter channel and the host lipid bilayer.

Amiodarone and dronedarone alter lipid bilayer properties at their clinical, pharmacologically relevant (Kodama et al., 1997; Zimetaubam, 2012; Heijman et al., 2013a) concentrations (Table 1). In contrast, propranolol and pindolol block β-adrenergic receptors (Zimetaubam, 2012) and have their clinical effects in the micromolar concentration range (Anavekar et al., 1975; Woosley et al., 1979), which does not overlap with the concentrations at which they alter lipid bilayer properties (Table 1).

We first discuss the generality of drug-induced changes in lipid bilayer properties. We then consider how, despite the generality, different amphiphiles alter different bilayer properties and the time-dependent effects of amiodarone. We finally discuss the implications of our results for target promiscuity and a bilayer-dependent mechanism for polypharmacology.

**Generality of amphiphile-induced changes in lipid bilayer properties**

Dronedarone is a more potent bilayer modifier in DC_{18:1}PC/bSM/Chol 1:1:1 bilayers, producing a two-fold larger reduction in ΔG_{bilayer} than in DC_{18:1}PC bilayers (Figs. 3 B and 6 C). Similarly, we have shown previously that amphiphiles produce greater increases in gA channel activity in cholesterol-containing bilayers, as compared with cholesterol-free membranes (Bruno et al., 2007; Rusinova et al., 2011). The increased effect in cholesterol-containing bilayers reflects that cholesterol increases bilayer thickness (Simon et al., 1982; Gandhavadi et al., 2002) and elastic moduli (Needham and Nunn, 1990), making it energetically more costly to deform cholesterol-containing bilayers (Lundbæk et al., 2003). The increased stiffness further causes the amphiphile-induced changes in bilayer stiffness to be increased (Bruno et al., 2013), which also contributes to the greater changes in channel function.

As is the case for other amphiphiles (Lundbæk et al., 2005, 2010b; Greisen et al., 2011), all four antiarrhythmics produce greater relative changes in f than in τ, consistent with the notion that gA dimer formation involves a large decrease in local membrane thickness, whereas dimer dissociation involves only a modest axial separation of the monomers (Huang, 1986; Lundbæk et al., 2010b). This is evident also when comparing the effects of dronedarone on gA(15) in DC_{18:1}PC and DC_{18:1}PC/bSM/Chol bilayers. Indeed, 1 µM dronedarone increased f by a factor of 2.2 in DC_{18:1}PC versus 12.7 in DC_{18:1}PC/bSM/Chol bilayers; yet the relative changes in τ were only 1.6 versus 2.3, respectively (Table 2 and Figs. S1 and 6, A and B), reflecting the greater decrease in ΔG_{bilayer} (and greater stiffness) in the ternary mixture as compared with DC_{18:1}PC bilayers (Figs. 3 B and 5 C). Not surprisingly, therefore the ΔG_{bilayer} versus (k_{diss} − δ)/k_{diss} relation for gA(15) channels in DC_{18:1}PC/bSM/Chol bilayers is steeper than the relation in DC_{18:1}PC bilayers (Fig. S3 C).

We conclude that amphiphiles alter lipid bilayer properties generally, but that the magnitude of the changes in ΔG_{bilayer} varies with changes in the membrane lipid composition. We further note that amphiphile-induced changes in integral membrane protein function are related to the changes in gA channel function in single-component bilayers (Hwang et al., 2003; Lundbæk et al., 2005; Bruno et al., 2007; Ingólfssson et al., 2007, 2014; Rusinova et al., 2011), and changes in (NaV) function in cell membranes scale with the changes in gA function in single-component bilayers (Lundbæk et al., 2005; Rusinova et al., 2011; Herold et al., 2014). That is, the results with gA channels in well-defined bilayer systems relate well to the results in the much more complex situation in cellular membranes, even though the conformational changes in membrane proteins are far more complex than the gA monomer-dimer transitions. In either case, however, the bilayer deformation energies for the different states are functions of the same changes in bilayer properties, which allows for the use of gA channels as probes for changes in lipid bilayer properties and in membrane protein function.

**What bilayer properties are altered by amphiphiles?**

Amphiphile-induced changes in lipid bilayer properties (Schreier et al., 2000) have often been attributed to changes in bilayer fluidity (e.g., Gordon et al., 1980; Chatelain et al., 1989), but changes in membrane fluidity per se cannot account for changes in the energetics of membrane protein conformational change or membrane protein function (Lee, 1991). Moreover, apparent changes in fluidity may reflect changes in the free volume of the membrane, which would be proportional to changes in membrane tension (Markin and Sachs, 2015) and, using the polymer brush model (Rawicz et al.,
mimic the effect of amiodarone in planar bilayers (Fig. S4, on nuclear receptors (Kodama et al., 1997). Changes in agonism of thyroid hormone (triiodothyronine) action from gene expression regulation by amiodarone’s an-
tol effect of amiodarone in planar bilayers (Fig. S4), it did not do so in LUVs (Fig. S5), where the lipid/amiodarone ratio is 10-fold higher, which could underlie some of its pharmacologic/toxic effects during chronic use.

Target-promiscuity and bilayer-mediated mechanisms for altering membrane protein function

The concepts of drug promiscuity (Insel, 1988; Mencher and Wang, 2005) and polypharmacology (Roth et al., 2004; Peters, 2015) denote the effects of a drug on multiple targets, usually with an assumption of direct binding to the target proteins. Our results, together with those in earlier studies (Hwang et al., 2003; Lundbæk et al., 2005; Bruno et al., 2007; Ingólfsson et al., 2007, 2014; Rusinova et al., 2011), suggest an alternative mechanism for drug promiscuity/polypharmacology for membrane proteins, namely that the drug in question alters the physical properties of the common feature among all membrane proteins: their host lipid bilayer. Amiodarone and dronedarone alter bilayer properties at clinically relevant concentrations and alter the function of multiple membrane proteins, suggesting that their promiscuity, and maybe even their clinical effects, may be due, at least in part, to their effects on the lipid bilayer, thus representing a novel form of polypharmacology. Although the list of protein targets for amiodarone and dronedarone is effective the same, dronedarone’s efficacy is disputable and, considering its low bioavailability (Heijman et al., 2013a) compared with amiodarone (Latini et al., 1984), pharmacokinetics may play an important role in the differences in efficacy between these two drugs.

Many other current drugs similarly exhibit multichannel effects (Heijman et al., 2013b), and some drug tar-
gets, such as NaV1.5, are mechanosensitive (Morris and Juranka, 2007; Beyder et al., 2010). For example, rano-
lazine, a novel multichannel antiarrhythmic (Heijman et al., 2013b), reduces the mechanosensitivity of NaV1.5 channels in mouse cardiomyocytes, which may be caused by changes in lipid bilayer properties (Beyder et al., 2012), as well as the shear-stretch sensitivity of endoge-
ous voltage-dependent currents in cell lines derived from human atrial myocytes (Strege et al., 2012). (Table 2 in Morris and Juranka [2007] summarizes drug effects on a variety of NaV channels.) We have previously found a correlation between drug off-target effects and bilayer-modifying potency in a family of insulin-sensitizing drugs—the thiazolidinediones—where they altered bilayer properties at the same concentration as their effects on the NaV (Rusinova et al., 2011). Moreover, the rank order of the bilayer-modifying potency coincided with severity of their side effects, with troglitazone being the most effective but also the most toxic and the most bilayer modifying.

Conclusion

The bilayer-mediated regulation of membrane protein function is fundamentally nonspecific, meaning that drugs that alter bilayer properties will have unintended
off-target and pleiotropic effects that may negatively impact their toxicity profiles but also may be beneficial in the case of drugs that alter system properties, such as antiarrhythmics.

Despite the promiscuity that is implicit with a common bilayer-mediated mechanism, the relative changes in the function of any specific protein will depend on the amphiphile-induced changes in $\Delta G_{\text{bilayer}}^{\text{calc}}$ for that protein. Our results thus suggest that a bilayer-mediated mechanism may explain the ability of antiarrhythmics (and other amphiphilic drugs) to regulate multiple targets within a narrow concentration range. These results furthermore underscore the importance of considering bilayer-mediated effects in drug development as contributing to both beneficial and detrimental off-target and pleiotropic effects.

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Methods
For the GBFA, after mixing phospholipids in chloroform and gA in methanol, the solvents were removed under a stream of nitrogen gas followed by overnight desiccation under vacuum. The lipids were hydrated at room temperature in 100 mM NaNO₃, 10 mM HEPES, and 25 mM ANTS, pH 7.0 buffer solution. LUVs were formed by five to six freeze-thaw cycles, extrusion was performed through 100-nm pore-size polycarbonate filters (GE Healthcare), and the extravesicular ANTS was removed using PD-10 columns (GE Healthcare). gA channel activity was monitored by stopped-flow spectrofluorometry using a SX-20 stopped-flow spectrofluorometer (Applied Photophysics), where the ANTS-loaded LUVs are mixed with the gA channel–permeant quencher of ANTS fluorescence thallium (Tl⁺).

In the single-channel experiments, the electrolyte solution was 1 M NaCl and 10 mM HEPES, pH 7. Once the bilayer was formed and deemed to be stable, ∼24 and ∼4 pmol gA⁻(13) and gA(15), respectively, were added to both sides of the bilayer, and the system was equilibrated for 30 min before control current traces were recorded. Approximately fourfold more gA(15) was added to the DC₁₈:₁PC/bSM/Chol bilayers to achieve comparable control appearance frequency (f). DMSO added with antiarrhythmics did not exceed 1% (vol/vol), a concentration that has no effect on gA channel function (Ingólfsson and Andersen, 2010). Recordings were done using a patch-clamp amplifier (3900A; Dagan Corporation), filtered at 2 kHz, and acquired at a 20-kHz digitization rate. Data acquisition, post-acquisition filtering, and analysis were done using a program written in Visual Basic (Microsoft).

Table S1
<table>
<thead>
<tr>
<th>Analogue</th>
<th>Sequencea</th>
<th>Hydrophobic channel lengthb (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gA⁻(13)</td>
<td>f-ALAVVVWLWLWLW-e</td>
<td>1.9</td>
</tr>
<tr>
<td>gA(15)</td>
<td>f-AGALAVVVWLWLWLW-e</td>
<td>2.2</td>
</tr>
</tbody>
</table>

aThe underlined residues are D amino acids; f is formyl, and e is ethanolamine.
bThe hydrophobic length of the 15–amino acid gA channel is from Elliott et al. (1983); the length of gA⁻(13) was adjusted by 0.3 nm per pair of L–D residues.

References

Figure S1. Antiarrhythmic drugs increase τ and f for gA⁻(13) (red squares) and gA(15) (blue squares) f or τ recorded in the presence of amiodarone, dronedarone, propranolol, and pindolol at the indicated concentrations and normalized to τ value in the absence of the antiarrhythmic. All data points are from n = 2 – 4, and error bars represent mean ± SD if n ≥ 3 or mean ± range/2 if n = 2. Frequency measurements with 1 mM pindolol and 30 µM and 1 mM propranolol had n = 1.
Figure S2. Effects of antiarrhythmic drugs on gA single-channel current transition amplitudes. Single-channel current transition amplitude histograms without (top) and with (bottom) amiodarone, dronedarone, propranolol, and pindolol. Red and blue dashed lines indicate the average gA(13) and gA(15) single-channel current amplitudes in the absence of antiarrhythmics, respectively. The histograms are compiled from two to three independent experiments. Amiodarone reduced the current transition amplitudes of gA(13) and gA(15) channels from 1.86 ± 0.10 (mean ± SD) in control conditions to 1.60 ± 0.08 and from 2.92 ± 0.15 in control conditions to 2.37 ± 0.11, respectively. Amiodarone also increased the total number of transitions for both channels from 1,859 to 5,278 and shifted the distribution between gA(13) and gA(15) from 57 and 35% in control conditions to 71 and 24% of total transitions, respectively. Dronedarone reduced the current transition amplitudes of gA(13) and gA(15) channels from 1.90 ± 0.07 (mean ± SD) to 1.66 ± 0.08 and from 2.98 ± 0.10 to 2.54 ± 0.12, respectively. Dronedarone also increased the total number of transitions for both channels from 2,950 to 6,850 and shifted the distribution between gA(13) and gA(15) from 44 and 51% in control to 71 and 27% of total transitions, respectively. Propranolol reduced the current transition amplitudes of gA(13) and gA(15) channels from 1.88 ± 0.09 (mean ± SD) in control conditions to 1.61 ± 0.08 and from 2.97 ± 0.11 in control conditions to 2.49 ± 0.09, respectively. Propranolol also increased the total number of transitions for both channels from 2,451 to 7,536 but, unlike amiodarone and dronedarone, did not shift the distribution between gA(13) and gA(15) channels; the total number of gA(13) and gA(15) appearances distributed as 43 and 54% in control conditions and 39 and 58% in the presence of 1 mM propranolol, respectively. Pindolol modestly reduced the current transition amplitudes of gA(13) and gA(15) channels from 1.87 ± 0.08 (mean ± SD) in control conditions to 1.78 ± 0.08 and from 2.92 ± 0.12 in control conditions to 2.80 ± 0.11, respectively. Pindolol also increased the total number of transitions for both channels from 3,090 to 5,449 but did not shift the distribution between gA(13) and gA(15) channels; the total number of gA(13) and gA(15) appearances distributed as 35 and 60% in control conditions and 36 and 57% in the presence of 1 mM pindolol, respectively. We can account for >90% of the channels in the two major peaks, and the antiarrhythmic drugs did not increase the noise in current transition amplitudes. Average current transition amplitudes were determined using weighted statistics by dividing the area of each gA peak by the number of transitions in the peak.
Figure S3. (A) Plot of $\Delta \Delta G_{\text{bilayer}}$ (Eq. 4) as a function of the mole fraction of antiarrhythmics in the bilayer. (B) Relative changes in current transition amplitudes (the average current transition amplitude in the absence of the drug [$i_{\text{control}}$] minus the average current transition amplitude in the presence of the drug [$i$] divided by $i_{\text{control}}$). Red symbols and lines designate the values for gA(13) channels, and the blue lines and symbols designate the values for gA(15) channels. (C) Plot of $\Delta \Delta G_{\text{bilayer}}$ as a function of $(i_{\text{control}} - i)/i_{\text{control}}$. Symbols as in A, with red symbols and lines designating the values for gA(13) channels, and blue lines and symbols designating the values for gA(15) channels. The open blue circle denotes results obtained with dronedarone in DC18:1/bSM/Chol membranes.
Amiodarone’s modulation of the gA single-channel lifetimes increases with time in the absence, but not in the presence, of the iodine scavenger Na$_2$S$_2$O$_3$ (50 µM). The single-channel lifetimes of gA$^-(13)$ and gA(15) channels were determined after 10- and 35- or 40-min incubation with 3 µM amiodarone. Data points represent $\tau$ normalized to the $\tau$ after a 10-min incubation. $n = 2$, and error bars represent mean ± range/2. Iodine alone did not alter $\tau$. In the presence of 3 µM I$_2$, the relative changes in $\tau$ were 1.2 ± 0.1 and 1.0 ± 0.2 for gA$^-(13)$ and gA(15), respectively, which were not significantly different from control.

Amiodarone does not produce time-dependent increases in quench rate in the absence or presence of the I$_2$ scavenger Na$_2$S$_2$O$_3$. gA-containing LUVs were incubated with 30 µM amiodarone in the presence or absence of 50 µM Na$_2$S$_2$O$_3$ for 10 or 30 min. The relative increases in rates were indistinguishable, within instrumental error, indicating no time-dependent amiodarone effects. Incubation with 30 µM I$_2$ in the presence or absence of 50 µM Na$_2$S$_2$O$_3$ for 10 or 30 min yielded rates that were indistinguishable from control, indicating that I$_2$ by itself does not affect the bilayer at this concentration. $n = 1$. 

Figure S4. Amiodarone’s modulation of the gA single-channel lifetimes increases with time in the absence, but not in the presence, of the iodine scavenger Na$_2$S$_2$O$_3$ (50 µM). The single-channel lifetimes of gA$^-(13)$ and gA(15) channels were determined after 10- and 35- or 40-min incubation with 3 µM amiodarone. Data points represent $\tau$ normalized to the $\tau$ after a 10-min incubation. $n = 2$, and error bars represent mean ± range/2. Iodine alone did not alter $\tau$. In the presence of 3 µM I$_2$, the relative changes in $\tau$ were 1.2 ± 0.1 and 1.0 ± 0.2 for gA$^-(13)$ and gA(15), respectively, which were not significantly different from control.

Figure S5. Amiodarone does not produce time-dependent increases in quench rate in the absence or presence of the I$_2$ scavenger Na$_2$S$_2$O$_3$. gA-containing LUVs were incubated with 30 µM amiodarone in the presence or absence of 50 µM Na$_2$S$_2$O$_3$ for 10 or 30 min. The relative increases in rates were indistinguishable, within instrumental error, indicating no time-dependent amiodarone effects. Incubation with 30 µM I$_2$ in the presence or absence of 50 µM Na$_2$S$_2$O$_3$ for 10 or 30 min yielded rates that were indistinguishable from control, indicating that I$_2$ by itself does not affect the bilayer at this concentration. $n = 1$. 

S4 General mechanism for drug promiscuity