Rusinova et al., http://www.jgp.org/cgi/content/full/jgp.201511470/DC1

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 $NaNO_3$, 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 $DC_{18:1}PC$/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
gA analogues used in this study

Analogue	Sequence[a]	Hydrophobic channel length[b]
		nm
$gA^-(13)$	f-ALAVVVWLWLWLW-e	1.9
gA(15)	f-AGALAVVVWLWLWLW-e	2.2

[a]The underlined residues are D amino acids; f is formyl, and e is ethanolamine.
[b]The 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

Elliott, J.R., D. Needham, J.P. Dilger, and D.A. Haydon. 1983. The effects of bilayer thickness and tension on gramicidin single-channel lifetime. *Biochim. Biophys. Acta.* 735:95–103. doi:10.1016/0005-2736(83)90264-X

Ingólfsson, H.I., and O.S. Andersen. 2010. Screening for small molecules' bilayer-modifying potential using a gramicidin-based fluorescence assay. *Assay Drug Dev. Technol.* 8:427–436. doi:10.1089/adt.2009.0250

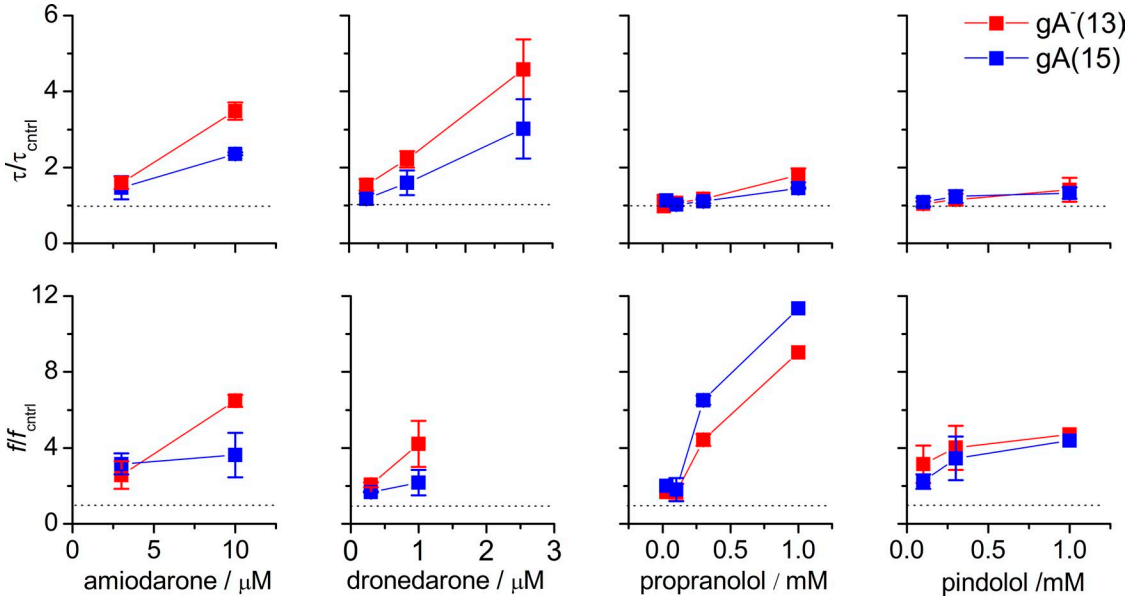

Figure S1. Antiarrhythmic drugs increase τ and *f* for $gA^-(13)$ (red squares) and gA(15) (blue squares) *f* or τ recorded in the presence of amiodarone, dronedarone, propranolol, and pindolol at the indicated concentrations and normalized to τ value in the absence of the antiarrhythmic. All data points are from $n = 2 – 4$, and error bars represent mean ± SD if $n \geq 3$ or mean ± range/2 if $n = 2$. Frequency measurements with 1 mM pindolol and 30 μM and 1 mM propranolol had $n = 1$.

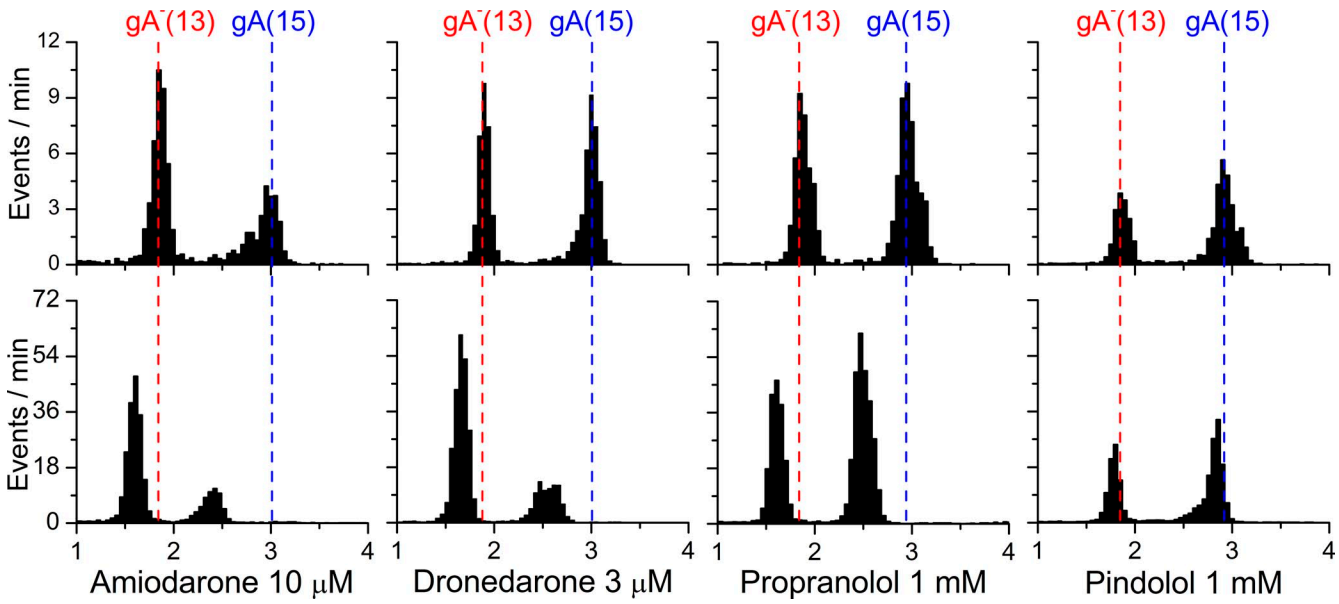

Figure S2. Effects of antiarrhythmic drugs on gA single-channel current transition amplitudes. Single-channel current transition amplitude histograms without (top) and with (bottom) amiodarone, dronedarone, propranolol, and pindolol. Red and blue dashed lines indicate the average $gA^-(13)$ and gA(15) 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.

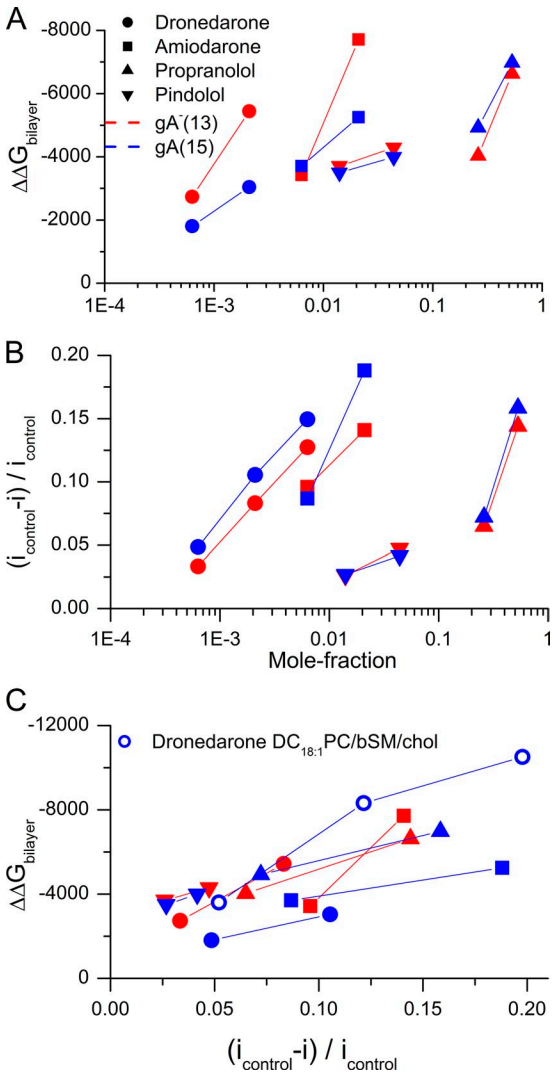

Figure S3. (A) Plot of $\Delta\Delta G^{M\to D}_{bilayer}$ (Eq. 4) as a function of the mole fraction of antiarrhythmics in the bilayer. (B) Relative changes in current transition amplitudes (the average current transition amplitude in the absence of the drug [i_{cntrl}] minus the average current transition amplitude in the presence of the drug [i] divided by i_{cntrl}). 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 $\Delta\Delta G^{M\to D}_{bilayer}$ as a function of $(i_{cntrl} - i)/i_{cntrl}$. 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 $DC_{18:1}$/bSM/Chol membranes.

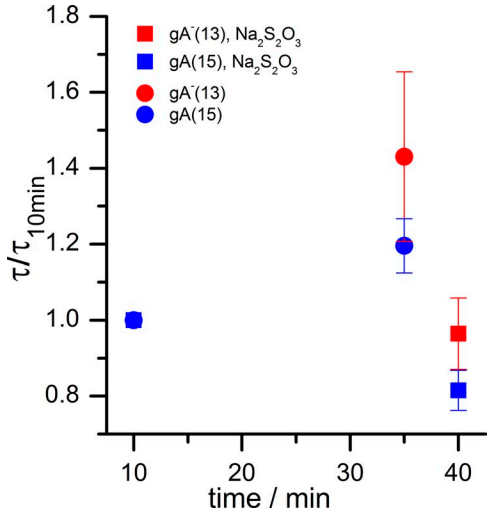

Figure S4. Amiodarone's modulation of the gA single-channel lifetimes increases with time in the absence, but not in the presence, of the iodine scavenger $Na_2S_2O_3$ (50 µM). The single-channel lifetimes of $gA^-(13)$ and gA(15) channels were determined after 10- and 35- or 40-min incubation with 3 µM amiodarone. Data points represent τ normalized to the τ after a 10-min incubation. $n = 2$, and error bars represent mean ± range/2. Iodine alone did not alter τ. In the presence of 3 µM I_2, the relative changes in τ were 1.2 ± 0.1 and 1.0 ± 0.2 for $gA^-(13)$ and gA(15), respectively, which were not significantly different from control.

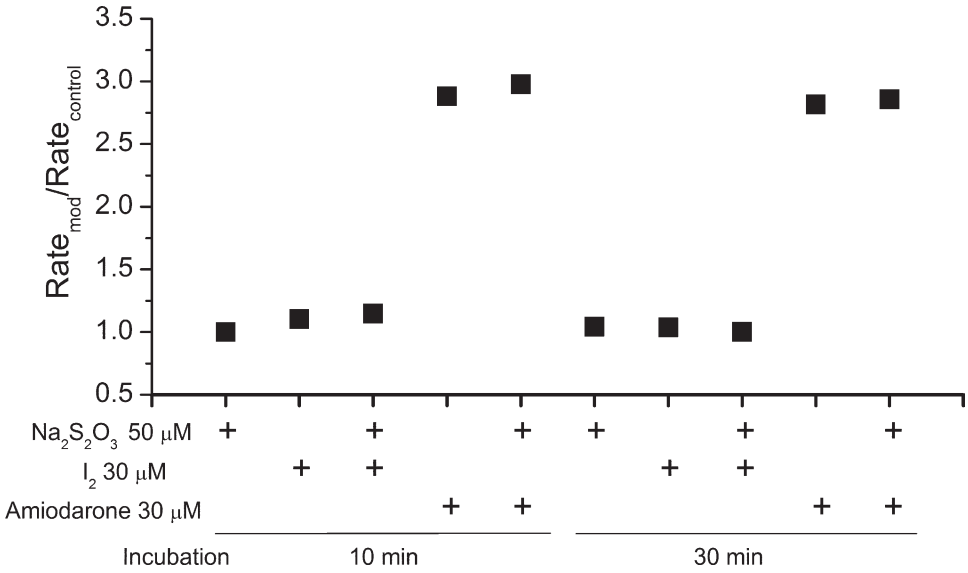

Figure S5. Amiodarone does not produce time-dependent increases in quench rate in the absence or presence of the I_2 scavenger $Na_2S_2O_3$. gA-containing LUVs were incubated with 30 µM amiodarone in the presence or absence of 50 µM $Na_2S_2O_3$ for 10 or 30 min. The relative increases in rates were indistinguishable, within instrumental error, indicating no time-dependent amiodarone effects. Incubation with 30 µM I_2 in the presence or absence of 50 µM $Na_2S_2O_3$ 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. $n = 1$.