Probing the Interaction Between Inactivation Gating and D-Sotalol Block of HERG

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Abstract—Potassium channels encoded by HERG underlie $I_{Kr}$, a sensitive target for most class III antiarrhythmic drugs, including methanesulfonanilides such as d-sotalol. Recently it was shown that these drugs are trapped in the channel as it closes during hyperpolarization. At the same time, HERG channels rapidly open and inactivate when depolarized, and methanesulfonanilide block is known to develop in a use-dependent manner, suggesting a potential role for inactivation in drug binding. However, the role of HERG inactivation in class III drug action is uncertain: pore mutations that remove inactivation reduce block, yet many of these mutations also modify the channel permeation properties and could alter drug affinity through gating-independent mechanisms. In the present study, we identify a definitive role for inactivation gating in d-sotalol block of HERG, using interventions complementary to mutagenesis. These interventions (addition of extracellular Cd$^{2+}$, removal of extracellular Na$^+$) modify the voltage dependence of inactivation but not activation. In normal extracellular solutions, block of HERG current by 300 μmol/L d-sotalol reached 80% after a 10-minute period of repetitive depolarization to +20 mV. Maneuvers that impeded steady-state inactivation also reduced d-sotalol block of HERG: 100 μmol/L Cd$^{2+}$ reduced steady-state block to 55% at +20 mV ($P < 0.05$); removing extracellular Na$^+$ reduced block to 44% ($P < 0.05$). An inactivation-disabling mutation (G628C-S631C) reduced d-sotalol block to only 11% ($P < 0.05$ versus wild type). However, increasing the rate of channel inactivation by depolarizing to +60 mV reduced d-sotalol block to 49% ($P < 0.05$ versus +20 mV), suggesting that the drug does not primarily bind to the inactivated state. Coexpression of MiRP1 with HERG had no effect on inactivation gating and did not modify d-sotalol block. We postulate that d-sotalol accesses its receptor in the open pore, and the drug-receptor interaction is then stabilized by inactivation. Whereas deactivation traps the bound methanesulfonanilide during hyperpolarization, we propose that HERG inactivation stabilizes the drug-receptor interaction during membrane depolarization. (Circ Res. 2000;87:1012-1018.)

Key Words: HERG  ■  potassium channel  ■  inactivation  ■  antiarrhythmic drugs

The acquired long-QT syndrome, which provokes sudden death from ventricular arrhythmias, has been linked to pharmacological suppression of the rapid component of delayed rectifier potassium current ($I_{Kr}$).1,2 $K^+$ channels encoded by the HERG gene recapitulate the essential characteristics of $I_{Kr}$,3,4 including sensitivity to class III antiarrhythmic agents,5,6 and many other therapeutic agents (antihistamines, prokinetic agents, antibiotics).7–10 This highlights the importance of identifying and understanding the molecular mechanisms that underlie HERG channel block.

When depolarized, HERG channels open through a relatively slow (rate-limiting) step that precedes a unique, rapid, and voltage-dependent inactivation process.11,12 Hence, depolarized HERG channels primarily occupy either the open (conducting) or inactivated (nonconducting) conformational state, and incremental depolarization increases partitioning into the inactivated state. Studies of HERG pharmacology have shown that methanesulfonanilide block develops only when the channel is depolarized,5,6,13 suggesting that one or both of the depolarization-induced conformational states (open and inactivated) form a high-affinity drug receptor. Mutation of HERG residues that disrupt inactivation11,14 also reduce HERG block by E-4031 and dofetilide,15–17 suggesting that the inactivated state may participate in methanesulfonanilide block. However, many of these mutations also alter the pore permeation properties11,18 and could reduce drug affinity through gating-independent mechanisms. Further, voltage-clamp studies found that strong depolarizations that augment HERG inactivation inhibit dofetilide block and raise serious doubt as to whether HERG inactivation actually facilitates...
block by these compounds.\textsuperscript{6,13} In addition, a recent study showed that closure of the activation gate during hyperpolarization traps drug in the channel, thus stabilizing block.\textsuperscript{19}

Certain extracellular cations selectively modify the inactivation gating of HERG and therefore offer an independent means to evaluate the role of inactivation in methanesulfonanilide block. Extracellular Cd\textsuperscript{2+} at concentrations below 200 \(\mu\text{mol/L}\) inhibit HERG inactivation but do not change activation gating.\textsuperscript{20} In addition, we show that replacing extracellular Na\textsuperscript{+} with NMG\textsuperscript{+} destabilizes HERG inactivation. We tested whether these cation substitutions modify HERG block by \(\text{d-sotalol}\), a class III methanesulfonanilide known to inhibit \(I_C\).\textsuperscript{21,22} Our results show that these ionic substitutions, as well as P-loop mutations that remove inactivation, antagonize \(\text{d-sotalol}\) inhibition of HERG current. At the same time, stronger depolarizations that rapidly inactivate the channel reduce \(\text{d-sotalol}\) inhibition, similar to earlier reports with dofetilide.\textsuperscript{6,13} The findings suggest that the methanesulfonanilide receptor is accessible primarily when HERG channels are open, but once occupied, the stability of the drug-receptor interaction is increased by channel inactivation. We propose that methanesulfonanilide block of HERG is stabilized secondarily as inactivation gating ensues.

**Materials and Methods**

HERG cDNA was kindly provided by Dr Mark Keating (University of Utah), and the mutant G628C:S631C HERG cDNA was provided by Dr Gary Yellen (Harvard University). Wild-type and mutant constructs were then subcloned into the Bgl/BstEI site of vector pGFP-IRS for bicistronic expression of the channel protein and GFP reporter as previously described.\textsuperscript{23} MiRP1 cDNA was kindly provided by Dr Steve Goldstein, (Yale University) in vector pCI-neo (Promega). CHO-K1 cells were transfected with ion channel cDNAs and maintained as described previously.\textsuperscript{20} Cells exhibiting green fluorescence were chosen for electrophysiological analysis.

Whole-cell potassium currents were recorded at 22°C to 23°C (Axopatch 200B, Axon Instruments) using electrodes of 2 to 4 \(\Omega\) when filled with a pipette solution containing (in mmol/L) KCl 110, HEPES 10, K\textsubscript{2}ATP 5, K\textsubscript{4}BAPTA 5, and MgCl\textsubscript{2} 1, adjusted to pH 7.2 with KOH to yield a final intracellular \([K]\textsuperscript{+}\) of 145 mmol/L. Eighty percent of the series resistance was compensated. Unless otherwise indicated in the figure legends, the standard bath solution contained (in mmol/L) NaCl 140, KCl 5.4, MgCl\textsubscript{2} 1, CaCl\textsubscript{2} 2, HEPES 10, and glucose 10, adjusted to pH 7.4 with NaOH. CaCl\textsubscript{2} was diluted in the standard bath solution from a 1 mol/L aqueous stock. In experiments where extracellular Na\textsuperscript{+} was removed, NaCl was replaced by equimolar N-methyl-D-glucamine chloride (NMG\textsuperscript{+}). \(\text{d-Sotalol}\) was a gift from Bristol Meyers Squibb (Princeton, NJ) and was added to the bath from a 1 mol/L stock solution. A 2-minute period of equilibration was allowed between solution changes to establish equilibrium. We used a small bath volume (\(\approx 1\ mL\)) and fully exchanged the external solution at least 4 times within 2 minutes.

Data were acquired using pCLAMP (Axon Instruments). In all figures, the bottom of the scale bar indicates the zero current level. The time dependence of \(\text{d-sotalol}\) block was fitted using the exponential function \(y=A\exp(-(t-t_0)/\tau)\). Pooled data are expressed as means and standard errors, and statistical comparisons were made (Origin, Microcal Software, Northampton, Mass) with \(P<0.05\) considered significant.

**Results**

Figure 1A shows HERG currents recorded in standard solutions (left) and after addition of 100 \(\mu\text{mol/L}\) Cd\textsuperscript{2+} (right). Addition of Cd\textsuperscript{2+} increased the magnitude of current during the depolarizing steps and also hastened the tail current kinetics. As shown previously,\textsuperscript{20} these effects both result from a destabilizing effect of Cd\textsuperscript{2+} on HERG inactivation. Figure 1B shows an analogous experiment where HERG currents were recorded prior to (left) and after (right) removal of the extracellular Na\textsuperscript{+}. Similar to Cd\textsuperscript{2+} addition, removing Na\textsuperscript{+} increased the magnitude of the outward current recorded during depolarization. However, removal of Na\textsuperscript{+} greatly reduced tail current amplitude after strong (\(>+20\ mV\)) depolarizations, an effect not seen with Cd\textsuperscript{2+}.

Figure 2A shows the effects of Cd\textsuperscript{2+} addition or Na\textsuperscript{+} removal on the current-voltage (I-V) relationship at the end of the 2-second depolarizing pulse (paired observations). In both cases, the interventions substantially increased the current at membrane potentials \(\geq+20\ mV\) where the I-V curve rectifies. The effects of the two interventions were qualitatively and quantitatively similar. In addition, there was little effect of either intervention on the I-V curve at less depolarized potentials, consistent with selective effects on inactivation. Figure 2B plots the tail current amplitudes after each depolarization, normalized to the maximal tail current amplitude in the same bath solution, to illustrate the effects of Cd\textsuperscript{2+} addition or Na\textsuperscript{+} replacement on the voltage dependence of activation. As previously shown (see Figures 2 and 3 in Johnson et al\textsuperscript{20}), 100 \(\mu\text{mol/L}\) Cd\textsuperscript{2+} did not significantly change the voltage dependence of activation (Figure 2B, top). Although removal of Na\textsuperscript{+} had a somewhat different effect on the tail current magnitude than did Cd\textsuperscript{2+} addition (Figure 1), removal of Na\textsuperscript{+} still did not alter the voltage dependence of activation (Figure 2B, bottom). In Figure 2B, the solid lines are fits to a Boltzmann function to these activation data. The fitted parameters (legend, Figure 2B) revealed no statistically significant effect of either intervention on the \(V_{1/2}\) or the slope
factor. Although we cannot entirely exclude the possibility that removing Na\(^+\) has a small effect on activation gating, the effect of Na\(^+\) removal on the voltage dependence of inactivation is substantial, as shown below.

We recently showed that [Cd\(^{2+}\)] \(\leq 200 \mu \text{mol/L}\) induces a depolarizing shift in the voltage dependence of HERG inactivation,\(^{20}\) and it was postulated that this destabilized inactivation gating was responsible for the Cd\(^{2+}\)-induced increase in HERG current. We tested whether a similar mechanism could underlie the enhancement of HERG current on removal of extracellular Na\(^+\) (Figure 3). To assess this, a 3-pulse voltage-clamp protocol was used (Figure 3A).\(^{11,24}\) Figure 3B plots the voltage-dependent partitioning of HERG channels between noninactivated states (closed or open) and the inactivated state. Replacing Na\(^+\) with NMG\(^+\) caused a rightward depolarizing shift in the voltage dependence of inactivation. The effect of 100 \(\mu \text{mol/L Cd}^{2+}\) was also tested (Figure 3B) and also exhibited a rightward shift, as shown previously with 200 \(\mu \text{mol/L Cd}^{2+}\) (Johnson et al\(^{20}\)). A Boltzmann function provided an inadequate fit to these data (not shown) because of the competing influence of two gating processes (deactivation and inactivation), primarily at the hyperpolarized membrane potentials. Hence, the effects of the two interventions (Cd\(^{2+}\) addition or Na\(^+\) removal) were assessed in a model-independent manner at each membrane potential (Figure 3B). Cd\(^{2+}\) significantly reduced the extent of inactivation at nearly all the membrane potentials tested (−110 to +50 mV, \(P<0.05\)). Conversely, the effects of Na\(^+\) to destabilize inactivation predominated at membrane potentials \(\geq -70 \text{ mV}\), consistent with the distinctive kinetic effects of this intervention (see below, Figure 4).

Both removal of Na\(^+\) and addition of Cd\(^{2+}\) destabilized steady-state inactivation over a range of membrane potentials (Figure 3). Examination of Figure 1 indicates that although the two interventions have similar effects during depolarization (increasing the current magnitude), on hyperpolarization to −50 mV, Na\(^+\) removal markedly reduced the tail current amplitude. We therefore examined the kinetic features of Na\(^+\) removal in greater detail during hyperpolarization (Figures 4A through 4C) and depolarization (Figure 4D). The large amplitude of HERG current tails on sudden hyperpolarization, relative to the small current size during the preceding depolarization, results from rapid recovery from inactivation.

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**Figure 2.** I-V relationships in 100 \(\mu \text{mol/L Cd}^{2+}\) and 0 Na\(^+\) solutions. I-V relationships were generated with the voltage-clamp protocol and bath solutions described in Figure 1. The symbols used here (see Figure 1) indicate whether current during the depolarizing pulse (squares, A) or tail current amplitudes (circles, B) was measured. Solid symbols indicate the control bath solution before adding 100 \(\mu \text{mol/L Cd}^{2+}\) (top, \(n=5\), paired observations) or removing Na\(^+\) (bottom, \(n=6\), paired observations). A, Current in each cell was normalized to the maximum current recorded in the same cell. B, Tail current amplitudes were measured in each condition. Each tail current was normalized to the maximal tail current recorded in the same bath solution. The solid lines show fits of a Boltzmann function to these data. For the Cd\(^{2+}\) experiments, \(V_{1/2}=0.4\pm 0.5\) mV in control conditions and \(0.0\pm 0.5\) mV in 100 \(\mu \text{mol/L Cd}^{2+}\). Slope factors were \(8.5\pm 0.3\) in control and \(8.7\pm 0.5\) in Cd\(^{2+}\). For Na\(^+\) removal, \(V_{1/2}=5.6\pm 2.8\) mV in control conditions and \(0.0\pm 0.4\) mV in 0 Na\(^+\). Slope factors were \(9.1\pm 1.2\) in control and \(8.7\pm 2.0\) in 0 Na\(^+\). Neither Cd\(^{2+}\) addition nor Na\(^+\) removal significantly changed either the \(V_{1/2}\) or the slope factor of the voltage dependence of activation (\(P=\text{NS}\)).

**Figure 3.** Voltage dependence of channel availability. A, Voltage-clamp protocol is shown at the top, and a typical family of currents in the standard bath solution is shown at the bottom. B, Peak current observed immediately after stepping to +30 mV is plotted (paired observations) before and after addition of Cd\(^{2+}\) or removal of Na\(^+\). Each value was normalized to the current recorded at +120 mV in the same cell, under the same cationic conditions. The deactivation that occurred during the brief 12.5-ms period at test potentials negative to 0 mV was corrected by fitting a single exponential to the measured rate of deactivation at each potential, in each solution (data not shown), using established methods.\(^{11}\) Addition of 100 \(\mu \text{mol/L Cd}^{2+}\) and removal of Na\(^+\) produced a depolarizing shift. \(P<0.05\) vs control.
before a slower deactivation process.\textsuperscript{11,24} We postulated that an effect of Na\textsuperscript{+} removal to slow recovery from inactivation at negative membrane potentials would reduce the amplitude of the tail currents. To assess this, we fitted a single exponential to the early recovery phase of HERG current during a hyperpolarization step that followed a depolarization to +50 mV\textsuperscript{22}; the rapid recovery phase was resolved using a fast sampling rate (20 kHz) in Na\textsuperscript{+}-free and control solutions (Figures 4A and 4B). Removal of Na\textsuperscript{+} slowed recovery from inactivation at all negative membrane potentials tested (−30 to −100 mV; \(P<0.05\), Figure 4C). At the same time, Figure 4D plots representative currents recorded at +30 mV after activation and recovery from inactivation (3-pulse protocol from Figure 3, top). These currents reflect the developing rate of HERG inactivation during depolarization\textsuperscript{11,24}; removal of Na\textsuperscript{+} slowed the +30-mV time constant of inactivation (derived from single-exponential fits to the decaying currents) from 11.3±1.2 ms to 14.6±1.5 ms (\(n=3\), \(P<0.05\)). Consistent with these observations, Na\textsuperscript{+} removal destabilized steady-state inactivation (Figure 3B) at depolarized voltages but had no significant effect at potentials more negative than −70 mV where the rate constant for recovery from inactivation likely predominates. In summary, the findings suggest that extracellular Na\textsuperscript{+} hastens the development of HERG inactivation during depolarization and the rate of recovery from inactivation during hyperpolarization.

We next examined block of HERG current by D-sotalol in the presence of these inactivation-destabilizing interventions. Figure 5A plots the development of D-sotalol block in the standard bath solution during a train of depolarizing pulses (Figure 5, top). In each case, the outward current was measured at the end of the 0.5-second step to +20 mV. Similar to the block kinetics of dofetilide in \textit{Xenopus} oocytes\textsuperscript{13} and cultured mammalian cells,\textsuperscript{6} D-sotalol block (300 \(\mu\)mol/L) develops very slowly. Identical experiments were performed with the addition of 100 \(\mu\)mol/L CD\textsuperscript{2+} (Figure 5B) or Na\textsuperscript{+} replaced with NMG\textsuperscript{+} (Figure 5C). In both cases, steady-state D-sotalol block was significantly reduced. These findings are summarized for a number of cells in Figure 6. Figure 6A plots the current remaining after 10 minutes of D-sotalol exposure during a pulse train, relative to the predrug control period. In the standard bath solution, the remaining current was only 20±2% at +20 mV but was 45±7% (\(P<0.05\)) in 100 \(\mu\)mol/L CD\textsuperscript{2+} and 56±6% (\(P<0.05\)) in 0 Na\textsuperscript{+}. In addition, the rate of D-sotalol block development was quantified in each condition by fitting a single exponential function to the data when plotted as in Figure 5. Figure 6B shows that neither Cd\textsuperscript{2+} addition nor Na\textsuperscript{+} removal significantly influenced the rate of block development.

Figure 7A examines the rate of recovery from inactivation in the absence and presence of D-sotalol in a manner similar to Figure 4; a single exponential was fitted to the early rising phase of current elicited during a hyperpolarizing step after a 0.5-ms depolarization. There was no significant difference in
the rate of recovery from inactivation. This would imply that either the drug has no effect on recovery from inactivation, or alternatively, that drug unbinding is so slow that blocked channels remain nonconducting on hyperpolarization and therefore do not participate in the measurement. Figure 5 shows that block accumulates with successive 0.5-second depolarizations, despite a 1-second hyperpolarizing interpulse interval, supporting the notion that the drug unbinding rate is slow (τ1 second). We examined d-sotalol block in additional cells using an 8-second period of hyperpolarization between pulses (n=3, not shown) and still found no relief of block between pulses. Moreover, there was no relief of d-sotalol block 5 minutes after drug washout, consistent with the irreversible block seen with other methanesulfonanilide compounds and HERG.19

As an alternative means to examine the influence of HERG inactivation on d-sotalol block, we used a mutant HERG channel (G628C-S631C) that does not appreciably inactivate (inset, Figure 5D). The effect of the mutation on the time course of block development is shown in Figure 5D, and the data summarizing steady-state d-sotalol block are plotted relative to wild-type HERG in Figure 6A. Steady-state d-sotalol block was nearly eliminated in the double mutant (89±7%, P<0.05 versus wild type), consistent with previous findings for E-403116 and dofetilide.15 Conversely, we find that more strongly inactivating the wild-type HERG channel with depolarizing pulses to +60 mV (Figure 5A) also inhibits the development of d-sotalol block. The remaining current after 10 minutes of d-sotalol perfusion was 51±4% using the +60-mV pulse train (versus only 20±2% at +20 mV, P<0.05).

As a counterpoint to interventions that inhibit drug block, a recent study found that coexpression of the MiRP1 protein augmented E-4031 rapid (first pulse) block of HERG.26 Figure 7B shows that MiRP1 had no effect on the voltage-dependent partitioning between inactivated and noninactivated states. We also find that MiRP1 coexpression did not increase first-pulse block by 300 μmol/L d-sotalol. In additional cells transected and studied concurrently, first-pulse HERG current after a 4-minute d-sotalol perfusion period at −80 mV was 98.6±2% (n=4) of control for HERG+MiRP1 compared with 92.8±2% (n=3) for HERG alone. We have confirmed MiRP1 coexpression by assaying for an accelerated rate of HERG deactivation26 (data not shown). We propose that the blocking kinetics of d-sotalol may be slower than E-4031, making it insensitive to the effects of MiRP1.
**Discussion**

In the human heart, suppression of current through channels encoded by HERG has both therapeutic and proarrhythmic consequences. Methanesulfonanilides, including dofetilide, E-4031, and d-sotalol, are among the most potent and selective inhibitors of HERG current. Hence, a mechanistic understanding of HERG block by this class of compounds may prove useful in understanding, and potentially avoiding, the untoward suppression of \(I_{Kr}\) by newer therapeutic agents. We explore the controversy over the role of the unique inactivation gating process of HERG in class III drug block.6,13 In addition to pore mutations (G628C-S631C) that disable inactivation (Figures 5D and 6A), cation substitutions that selectively inhibit HERG inactivation (Figures 1 through 3) also decrease block of HERG current by d-sotalol (Figures 5A through 5C and 6A). These findings suggest that the effects of inactivation-disabling pore mutants to disrupt block by members of the methanesulfonanilide class6,13 do not result solely from nonspecific effects of these mutations on the pore permeation properties but rather are attributable, at least in part, to the inactivation gating effects of the mutations. Although both \(\text{Cd}^{2+}\) addition and Na\(^{+}\) removal slow the development of inactivation gating at depolarized potentials (and share the effect of reducing \(d\)-sotalol block), the molecular mechanisms whereby these interventions destabilize inactivation may differ. In addition to its effects on inactivation gating, extracellular Na\(^{+}\) was recently shown to be a potent blocker of HERG in the absence of K\(^{+}\), and this blocking effect is potently inhibited through a complex interaction with extracellular K\(^{+}\).27 Conversely, addition of Cd\(^{2+}\) destabilizes HERG inactivation gating over a lower (micromolar) concentration range and does not appear to block the channel.20 The unique functional effects of the monovalent (Na\(^{+}\)) and divalent (Cd\(^{2+}\)) cations suggest that their gating effects may be linked to distinctive binding mechanisms or interaction sites.

Taken together, the findings with pore mutations and cation substitution (Figures 5 and 6) suggest that the HERG inactivated state stabilizes methanesulfonanilide block. Although a direct competitive interaction between extracellular Cd\(^{2+}\) and d-sotalol binding could confound this interpretation, the fact that Na\(^{+}\) removal has effects on both inactivation gating (during depolarization) and d-sotalol block that are similar to Cd\(^{2+}\) addition suggests this is unlikely. Hence, the effects of the inactivation-disabling interventions could be explained by the following scheme:

Closed→Open→Inactivated→Inact-D,

where Inact-D is an inactivated, drug-bound state.

However, the results (Figures 5A and 6A) showing stronger depolarizations (+60 versus +20 mV) actually reduce d-sotalol block (as in prior studies of dofetilide\(^{6,13}\)) poses an apparent conflict with this scheme given that stronger depolarization increases HERG inactivation. A binding mechanism more complex than this scheme is also suggested by recent studies of a HERG mutant that allows opening from hyperpolarized potentials (D540K), showing that deactivation (closure) of channels traps the methanesulfonanilide in the pore. This explains the slow HERG unblocking observed at hyperpolarized potentials.19 We therefore propose that d-sotalol accesses the drug receptor during channel opening, and that binding is secondarily stabilized by either inactivation (depolarization) or deactivation (hyperpolarization) of the drug-bound channel. Although stronger depolarization (to +60 mV) paradoxically inhibits methanesulfonanilide block (inactivation is increased), it is notable that such marked depolarization shifts the open-inactivated state equilibrium almost entirely toward occupancy of the inactivated state (Figure 3B). Because the on-rate for d-sotalol block is slow (Figure 5), the marked reduction in open-state dwell time induced by this strong depolarization may significantly reduce the open-state access of d-sotalol to its receptor. Alternatively, the strongly depolarized membrane field may have an inhibitory (gating-independent) effect on d-sotalol binding to its receptor.

Because depolarization is necessary to induce block by d-sotalol as well as other methanesulfonanilides, the recent finding that the D540K HERG channel can actually unblock when open (albeit at hyperpolarized potentials)19 supports our postulate that on depolarization, a state other than the open state (ie, the inactivated state) forms a relatively stable, drug-bound block complex. Studies of Shaker K\(^{+}\) channels with N-terminal deletions reveal a C-type inactivation gating process that is distinct from the more rapid N-type process22 and involves constriction of the pore.29 Mutagenesis studies have implicated homologous amino acid residues in the pore-forming region of HERG as structural components of inactivation,11,14 suggesting that a more rapid but related form of inactivation underlies the inward rectification of \(I_{Kr}\). Viewed in this context, the proposed mechanism of methanesulfonanilide block is reminiscent of the classic work on delayed rectifier K\(^{+}\) channels in squid axons, where tetraethylammonium accessed the channel when open and then gating (channel closure) stabilized drug binding.30 The notion that methanesulfonanilide drugs bind to a site within the pore is supported by recent evidence that porelining residues at the C-terminal end of S6 may form part of the dofetilide receptor.17 At the same time, interventions that disrupt (adding Cd\(^{2+}\), removing Na\(^{+}\)) or eliminate (G628C-S631C) HERG inactivation limit d-sotalol block during depolarization. It is noteworthy that the rate of development of d-sotalol block is not influenced by the interventions that destabilize inactivation (Figure 6B). When HERG channels are depolarized, the rate at which channels enter the inactivated state is determined mainly by the slow (rate-limiting) kinetics of the activation gating process (C→O) and not by the more rapid inactivation process (O→I). Hence, in view of the very slow rate of development of d-sotalol block (minutes), it is anticipated that the block development rate would be relatively insensitive to interventions that alter the rapid inactivation gating process.

Outward current through HERG channels is sensitive to small changes in extracellular K\(^{+}\),3 and a competitive interaction between extracellular K\(^{+}\) and dofetilide block of \(I_{Kr}\) in AT1 cells has been described.31 Hence, it is possible that extracellular Na\(^{+}\) removal, and even Cd\(^{2+}\) addition, could facilitate binding of extracellular K\(^{+}\) in the outer pore and thereby destabilize d-sotalol block indirectly. However,
steady-state d-sotalol block of HERG expressed in cultured mammalian cells was insensitive to raising extracellular K+ (IC_{50} \approx 150 \text{ mmol/L} \text{ at either 1.0 or 5.4 mmol/L K}^+),^{12} \text{ a finding consistent with reports for K}^+ \text{ insensitivity of dofetilide and E-4031 block of HERG using oocyte expression.}^{5,13} \text{ This insensitivity might indicate that outer-pore K}^+ \text{ depletion does not influence d-sotalol block. Nonetheless, the finding that removing extracellular Na+ \text{ both reduces HERG inactivation and limits d-sotalol block motivates future studies to identify sites in the pore linking cation permeation, inactivation gating, and drug action.}

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**References**

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