Voltage-Dependent Profile of Human Ether-a-go-go-Related Gene Channel Block Is Influenced by a Single Residue in the S6 Transmembrane Domain

JOSE A. SÁNCHEZ-CHAPULA, TANIA FERRER, RICARDO A. NAVARRO-POLANCO, and MICHAEL C. SANGUINETTI

Unidad de Investigación “Carlos Méndez” del Centro Universitario de Investigaciones Biomédicas de la Universidad de Colima, Colima, México (J.A.S.-C., T.F., R.A.N.-P.); and Department of Physiology and Eccles Program in Human Molecular Biology and Genetics, University of Utah, Salt Lake City, Utah (M.C.S.)

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ABSTRACT

Many common medications block delayed rectifier K⁺ channels and prolong the duration of cardiac action potentials. Here we investigate the molecular mechanisms of voltage-dependent block of human ether-a-go-go-related gene (HERG) delayed rectifier K⁺ channels expressed in Xenopus laevis oocytes by quinidine, an antiarrhythmic drug, and vesnarinone, a cardiotonic drug. The IC₅₀ values determined with voltage-clamp pulses to 0 mV were 4.6 µM and 57 µM for quinidine and quinine, respectively. Block of HERG by quinidine (and its iso- 


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HERG (Warmke and Ganetzky, 1994) encodes the pore-forming α subunits of channels that conduct the rapid delayed rectifier K⁺ current IKr (Sanguinetti et al., 1995; Trudeau et al., 1995). Blockers of IKr were developed to treat arrhythmia, but unintended block of HERG K⁺ channels can also be proarrrhythmic and is a serious side effect for many otherwise clinically useful drugs. Previously, we used site-directed mutagenesis and voltage clamp of mutant channels expressed in Xenopus laevis oocytes to elucidate the molecular mechanisms of HERG channel block by structurally diverse drugs, including MK-499, cisapride, and terfenadine (Mitcheson et al., 2000). These studies identified two aromatic residues (Y652 and F656) in the S6 domain. Mutations of Y652 eliminated (Y652F) or reversed (Y652A) the voltage dependence of HERG channel block by quinidine and quinine. These quinolines contain a charged N atom that might bond with Y652 by a cation–π interaction. However, similar changes in the voltage-dependent profile for block of Y652F or Y652A HERG channels were observed with vesnarinone, a cardiotonic drug that is uncharged at physiological pH. Together, these results suggest that voltage-dependent block of HERG results from gating-dependent changes in the orientation of Y652, a critical component of the drug binding site, and not from a transmembrane field effect on a charged drug molecule.

Like chloroquine, micromolar concentrations of quinidine are required for the block of HERG channels expressed in oocytes. Both drugs are substituted quinolines, however, chloroquine has two positively charged alkylamines with pH₅₀ values of 8.4 and 10.8, whereas quinidine has a single tertiary N in a quinuclidine group with a pH₅₀ of 8.6. These N atoms are predominantly protonated at physiological pH and conceivably could mediate cation–π interaction with Y652. Here, we also examined the effects of the uncharged drug vesnarinone on Y652A and Y652F HERG channels to determine whether a drug must possess an ionizable N atom to block HERG channels in a voltage-dependent manner.

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ABBREVIATIONS: HERG, human ether-a-go-go-related gene; WT, wild type; Mes, 2-[(N-morpholino)ethanesulfonic acid; MK-499, [(+)-N-[(6-cyano-1,2,3,4-tetrahydro-2(R)-naphthalenyl)-3,4-dihydro-4(R)-hydroxy spiro (2H-1-benzpyran-2,4'-piperidin)-6-yl)methane sulfonamide]-HCl.
Vesnarinone blocks wild-type HERG with little (Katayama et al., 2000) or no (Kamiya et al., 2001) dependence on transmembrane voltage.

In this study, we find that quinidine and quinine block wild-type and mutant HERG channels in a manner similar to that shown previously for chloroquine, confirming the importance of Y652 in voltage-dependent block by quinolines. Although the block of wild-type and Y652F HERG channels by vesnarinone, an uncharged drug, was relatively insensitive to voltage, block of Y652A HERG was surprisingly decreased at greater depolarized potentials, similar to the charged quinolines.

Materials and Methods

Molecular Biology. Point mutations were introduced into HERG (Y652A, Y652F, F656A) in the pSP64 plasmid expression vector (Promega, Madison, WI), as described previously (Mitcheson et al., 2000). Constructs were confirmed with restriction mapping and DNA sequencing. Complementary RNAs for injection into oocytes were prepared with SP6 Cap-Scricle (Roche Diagnostics, Indianapolis, IN) after linearization of the expression construct with EcoRI.

Voltage-Clamp of Oocytes. Isolation and maintenance of X. laevis oocytes and cRNA injection were performed as described previously (Sanguinetti and Xu, 1999). Currents were recorded at room temperature (22–24°C) with a GeneClamp 500 amplifier (Axon Instruments, Union City, CA) 2 to 3 days after cRNA injection using standard two microelectrode voltage-clamp techniques (Stühmer, 1992). Oocytes were bathed in a low Cl− solution containing 96 mM 2-(morpholino)ethanesulfonic acid (NaMes), 2 mM KMes, 2 mM CaMes, 5 mM HEPES, and 1 mM MgCl2, adjusted to pH 7.6 with methanesulfonic acid. Current-voltage (I-V) relationships were determined with test pulses applied to voltages of −70 to +40 mV at a frequency of 0.05 Hz from a holding potential of −80 mV. Deactivating (tail) currents were measured at −70 mV.

The time-dependent block of current was determined by dividing the step current recorded during a 4-s pulse in the presence of the drug (Istep/drug) by the current recorded before application of the drug (Istep/control). The resulting ratio, Istep/drug/Istep/control, was fit with a single exponential function to obtain the time constant for the onset of the HERG current blockade. The relative steady-state block of current as a function of test potential was determined by dividing the control currents by currents recorded in the presence of drug.

Quinidine and quinine (Sigma Chemical, St. Louis, MO) and vesnarinone (Otsuka Pharmaceutical Co., Tokyo) were dissolved in a solution containing 96 mM 2-(morpholino)ethanesulfonic acid (NaMes), 2 mM KMes, 2 mM CaMes, 5 mM HEPES, and 1 mM MgCl2, adjusted to pH 7.6 with methanesulfonic acid. Current-voltage (I-V) relationships were determined with test pulses applied to voltages of −70 to +40 mV at a frequency of 0.05 Hz from a holding potential of −80 mV. Deactivating (tail) currents were measured at −70 mV.

For example, 3 μM quinidine had little effect on currents activated by pulses up to −40 mV, but it reduced currents by 50% or more when activated by pulses to test potentials ≥−20 mV (Fig. 1C). In addition, the peak of the I-V relationship was shifted to the left, suggesting a negative shift in the voltage dependence of activation. This was confirmed by tail current analysis (Fig. 1E) and the finding that current activation was faster in the presence of the drug (Fig. 1F). Quinidine shifted the voltage dependence of channel activation assayed by 4-s pulses by −5.9 ± 0.7 mV at 3 μM, −8.3 ± 0.9 mV at 10 μM, and −9.5 ± 1.1 mV at 30 μM (Fig. 1E).

We next investigated whether block requires channel activation. After a control pulse to +20 mV, the membrane potential was held constant at −80 mV to maintain HERG channels in the closed state during a 10-min equilibration with 10 μM quinidine. After the equilibration period, a single pulse to +20 mV was applied again, eliciting a current that initially activated with a time course similar to that of the control, but subsequently displayed a time-dependent decline (Fig. 1G). This finding indicates that at 10 μM, quinine blocks open channels but has no significant effect on closed channels.

The time- and voltage-dependent block of WT HERG current by quinidine was studied in greater detail under steady-state conditions. Superimposed traces of currents recorded

Results

Voltage-Dependent Block of WT HERG Current by Quinidine. Block of HERG channel current by quinidine was first characterized using WT channels. An example of HERG currents recorded before and after block by 10 μM quinidine is shown in Fig. 1, A and B. The normalized I-V relationship for currents measured at the end of a 4-s test pulse (Fig. 1C) and for tail currents measured at −70 mV (Fig. 1D) was reduced by quinidine in a similar concentration-dependent manner. Block was also voltage-dependent.

For example, 3 μM quinidine had little effect on currents activated by pulses up to −40 mV, but it reduced currents by 50% or more when activated by pulses to test potentials ≥−20 mV (Fig. 1C). In addition, the peak of the I-V relationship was shifted to the left, suggesting a negative shift in the voltage dependence of activation. This was confirmed by tail current analysis (Fig. 1E) and the finding that current activation was faster in the presence of the drug (Fig. 1F). Quinidine shifted the voltage dependence of channel activation assayed by 4-s pulses by −5.9 ± 0.7 mV at 3 μM, −8.3 ± 0.9 mV at 10 μM, and −9.5 ± 1.1 mV at 30 μM (Fig. 1E).

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The time- and voltage-dependent block of WT HERG current by quinidine was studied in greater detail under steady-state conditions. Superimposed traces of currents recorded

Fig. 1. Effect of quinidine on HERG current in Xenopus laevis oocytes. A and B, representative HERG currents recorded from an oocyte before (A) and after (B) incubation with 10 μM quinidine. Currents were recorded at test potentials between −60 and +40 mV. Tail currents were recorded after repolarization to −70 mV. C, I-V relationships for currents measured at the end of the 4-s test pulse before and after application of 3, 10, and 30 μM quinidine (n = 5). Currents were normalized to the control current at −20 mV for each oocyte. D, I-V relationships for peak tail currents before and after application of 3, 10, and 30 μM quinidine. Currents were normalized to the peak current measured in control conditions for each oocyte. E, effect of quinidine on the isochronal activation curves for HERG. Tail currents were normalized to the peak current under each condition, and the data were fit with a Boltzmann function. The V1/2 and slope factors, respectively, were the following: control, −35.8 ± 0.8 mV and 9.8 ± 0.6 mV, n = 5; 3 μM quinidine, −41.7 ± 0.9 mV and 8.0 ± 0.6 mV, n = 5; 10 μM quinidine, −44.1 ± 0.8 mV and 7.0 ± 0.5 mV, n = 5; and 30 μM quinidine, −45.3 ± 0.9 mV and 6.8 ± 0.5 mV, n = 5. F, superimposed and scaled traces of currents activated by a 4-s pulse to −10 mV in control and after 10 μM quinidine. G, quinidine blocks open channels. A 1-s pulse to +20 mV was elicited from a holding potential of −80 mV before and 10 min after a pulse-free exposure of the oocyte to 10 μM quinidine.
during a 4-s pulse to −50 mV or +10 mV before and after equilibration with 10 μM quinidine are shown in Fig. 2, A and B. The ratio $I_{\text{drug}}/I_{\text{control}}$ as a function of time during the pulse was used to estimate initial block and the rate for onset of block. For the test pulse to −50 mV (Fig. 2C), the current ratio had an initial value of 1.0, indicating that channels completely recovered from block between test depolarizations. Block of current during a depolarizing step to −50 mV developed slowly compared with a step to +10 mV (Fig. 2D). The time constant for the onset of block by quinidine decreased with membrane depolarization from 200 ms at −50 mV to 10 ms at +40 mV (Fig. 2E). Steady-state reduction of HERG current by 10 μM quinidine varied as a function of test potential, with the fractional decrease varying from 0.35 at −50 mV to 0.8 at +40 mV (Fig. 2F). These data indicate that quinidine preferentially blocks open HERG channels and that steady-state block was increased by membrane depolarization.

**Characterization of the Putative Binding Site for Quinidine.** We reported previously that mutation to Ala of certain residues in the S6 domain (Y652, F656) or V625 in the pore helix reduced the block of HERG by MK-499 (Mitcheson et al., 2000). Mutation of the S6 residues, but not V625, also greatly reduced channel block by other compounds such as terfenadine, cisapride, and chloroquine (Mitcheson et al., 2000; Sanchez-Chapula et al., 2002). Therefore, we determined the concentration-effect relationship for quinidine on V625A, Y652A, and F656A HERG channels and compared the potency for block with that of the WT HERG channel. Peak tail current was measured at −70 mV after a 4-s pulse to 0 mV for WT, V625A, and Y652A HERG channels. The effect of 10 μM quinidine on WT, V625A, and Y652A HERG channel current is shown in Fig. 3, A through C. As reported previously (Mitcheson et al., 2000), the tail currents for V625A HERG channels were inward at −70 mV because of a change in ion selectivity. The IC$_{50}$ values were 4.6 ± 1.2 μM for WT, 17.5 ± 1.9 μM for V625A, and 16 ± 1.7 μM for Y652A HERG (Fig. 3D). To increase the amplitude of poorly expressing F656A mutant channels, tail currents were recorded at 140 mV.

![Fig. 2. Voltage-dependent block of WT HERG channel current by quinidine. A and B, superimposed traces of currents elicited during 4-s test pulses to −50 mV (A) and +10 mV (B) before and after exposure to 10 μM quinidine. Tail currents were measured upon repolarization to −70 mV. C and D, plot of the ratio $I_{\text{drug}}/I_{\text{control}}$ during the 4-s pulses shown in A and B. E, time constants for onset of block by quinidine plotted as a function of test potential ($V_t$). F, fractional block of WT HERG currents plotted as a function of the preceding test potential.](molpharm.aspetjournals.org/get-pdf)

![Fig. 3. Concentration-dependent block of WT and mutant HERG channels by quinidine. A through C, superimposed traces of WT HERG (A), V625 HERG (B), and Y652A HERG (C) currents elicited by the application of depolarizing pulses to 0 mV before and after exposure to 10 μM quinidine. D, concentration-effect relationship for the block of HERG current by quinidine at 0 mV. The IC$_{50}$ value was 4.6 ± 1.2 μM for WT, 17.5 ± 1.9 μM for V625A, and 16 ± 1.7 μM for Y652A ($n = 5$ for each group). E and F, superimposed traces of WT HERG (E) and F656 HERG (F) currents elicited by application of depolarizing pulses to 0 mV and upon repolarization to −140 mV before and after exposure of quinidine at 10 μM (E) or 100 μM (F). G, concentration-effect relationship for peak current inhibition by quinidine at 0 mV. The IC$_{50}$ value was 5.2 ± 0.9 μM for WT HERG and 650 ± 116 μM for F656 HERG ($n = 5$ for each group).](molpharm.aspetjournals.org/get-pdf)
−140 mV instead of −70 mV (Fig. 3, E and F). Using this protocol, the IC50 was 5.2 ± 0.9 µM for WT current and 650 ± 116 µM for F656A HERG current (Fig. 3G). Thus, mutation of F656 caused a 125-fold reduction in drug potency, whereas mutation of either Y652 or V625 reduced potency by a factor of approximately 3. We reported previously that the block of F656A channels by chloroquine was also greatly (approximately 1000 times) reduced (Sanchez-Chapula et al., 2002). However, Y652A channels were 500 times less sensitive to chloroquine and the V625A mutation had no effect on potency of block. These findings indicate that although quinidine and chloroquine share structural features, the binding site for the two drugs is not identical.

Voltage-Dependent Block of HERG Channels by Quinidine Is Altered by Mutation of Y652. We reported previously that the voltage dependence for block of HERG channels by chloroquine was reversed by the Y652A mutation (Sanchez-Chapula et al., 2002). The effect of quinidine on Y652A HERG current elicited at test potentials of −40 and +20 mV in the same cell is shown in Fig. 4, A and B. Quinidine blocked Y652A HERG current more effectively at −40 mV than at +20 mV, and the apparent rate of deactivation was faster in the presence of drug. For example, the time constants for deactivation at −70 mV after a pulse to +20 mV, respectively, were 129 ± 15 ms and 571 ± 63 ms before drug and 87 ± 9 ms and 295 ± 35 ms after 10 µM quinidine (n = 5). The ratio Idrug/Icontrol during 4-s test pulses to −40 or +20 mV is plotted in Fig. 4, C and D. For the test pulse to −40 mV, the current ratio had an initial value of 1.0 and decreased gradually throughout the test pulse. In contrast, for the pulse to +20 mV, the time course of the current ratio was biphasic. After an initially rapid decrease to a ratio of 0.75, the ratio increased to 0.9, indicating a rapid block followed by a partial recovery. The time constants for the rapid onset and the slower recovery from block were voltage-dependent and decreased at more depolarized potentials (Fig. 4E). Steady-state block of Y652A HERG was also voltage-dependent and varied from 0.7 at −50 mV to 0.04 at +40 mV (Fig. 4F). Thus, quinidine blocked HERG channels only after opening the activation gate, and in opposition to results observed with WT HERG, the block of Y652A HERG decreased with increasing membrane depolarization. Also in opposition to the WT HERG results, quinidine shifted the voltage dependence of Y652A HERG channels to more positive potentials (Fig. 5A).

We reported previously that mutation of Y652 to Phe reduced the potency of chloroquine by more than 10-fold and greatly reduced the voltage dependence of block (Sanchez-Chapula et al., 2002). Like chloroquine, quinidine block of Y652F HERG channels was also weakly voltage-dependent (Fig. 6), but the potency (IC50 = 3.8 ± 0.7 µM, n = 5) was nearly the same as that observed for WT HERG. Examples of currents elicited at −40 and +20 mV before and after exposure of an oocyte to 10 µM quinidine are shown in Fig. 6, A and B. The rate of Y652F HERG channel deactivation was only slightly altered by quinidine, but the rate was best fit with a single exponential function rather than the biexponential function required for the WT HERG current. The time constants for deactivation at −70 mV after a pulse to +40 mV were 173 ± 19 and 1102 ± 123 ms in control and 734 ± 91 ms after exposure of oocytes to 10 µM quinidine (n = 5). Similar to rates obtained with WT and Y652A HERG channels, the rate for the onset of block of Y652F channels was faster at more depolarized potentials (Fig. 6, C–E). However, unlike Y652A HERG (Fig. 4D), block of Y652F HERG channels by quinidine at +20 mV was not followed by a recovery from block (Fig. 6D), and the half-point for activation of Y652F HERG was only slightly affected (Fig. 5B). Steady-state fractional block of Y652F channels was weakly voltage-dependent at potentials between −30 and +10 mV (Fig. 6F).

Voltage-Dependent Block of WT and Mutant HERG Channels by Quinine. We also determined the effects of quinine, a stereoisomer of quinidine, on WT, Y652A, and Y652F HERG channels. Quinine was less potent than quinidine, reducing WT HERG with an IC50 of 57 ± 3.3 µM (Hill coefficient = 1.1 ± 0.07, n = 5). Like quinidine, quinine shifted the voltage dependence of HERG channel activation to more negative potentials. The V1/2 was shifted by −2.4 ± 0.4 mV with 30 µM and by −6.1 ± 0.7 mV with 100 µM quinine (n = 5; data not shown). At a concentration of 100 µM, quinine blocked WT HERG more at depolarized potentials (Fig. 7, A and D), blocked Y652A HERG less at depolarized potentials (Fig. 7, B and E), and blocked Y652F HERG independent of voltage (Fig. 7, C and F). Together with our previous findings for chloroquine,
the present findings with quinidine and quinine confirm a crucial role for Y652 in the voltage-dependent block of HERG by quinolines.

Voltage-Dependent Block of WT and Mutant HERG Channels by Vesnarinone. Vesnarinone is an uncharged cardiotonic drug that weakly blocks HERG channels expressed in oocytes with an IC50 of approximately 18 μM at 0 mV (Kamiya et al., 2001). At a concentration of 30 μM, vesnarinone caused a slightly greater block of WT HERG at depolarized potentials (Fig. 8, A and D), a dramatically less block of Y652A HERG at depolarized potentials (Fig. 8, B and E), and a slightly less block of Y652F HERG at depolarized potentials (Fig. 8, C and F). These data indicate that the voltage-dependent block of an uncharged drug is also altered by the mutation of Y652.

Discussion

Quinidine is a widely used class IA antiarrhythmic drug that inhibits Na+ and K+ currents and prolongs cardiac repolarization both in vivo and in vitro (Weld et al., 1982; Packer et al., 1989; Salata and Wasserstrom, 1988). Prolongation of cardiac action potential duration has been attributed to the blockade of several potassium currents, including the transient outward current and the delayed rectifier currents Ito, IKs, and IKur (Furukawa et al., 1989; Sanguinetti and Jurkiewicz, 1990; Snyders and Hondeghem, 1990). K+ channel block explains its propensity to induce long QT syndrome and torsades de pointes arrhythmia (Grace and Camm, 1998). The blocking effect of quinidine on these K+ channels is always voltage-dependent, increasing with progressive membrane depolarization. Quinidine preferentially blocks open Na+ and K+ channels and gains access to its binding site from the intracellular side of the membrane (Snyders et al., 1992; Clark et al., 1995; Zhang et al., 1998; Lees-Miller et al., 2000). Site-directed mutagenesis of several cloned K+ channels suggests that residues of the S6 domain which line the central cavity constitute an important component of the binding site for quinidine (Yeola et al., 1996; Zhang et al., 1998; Lees-Miller et al., 2000); however, the structural basis of voltage-dependent channel block is unknown. Our findings provide a possible molecular explanation for the voltage-dependent block of HERG by quinidine.

Block of HERG Channels by Quinolines. Quinidine, quinine, and chloroquine are substituted quinolines that cause voltage-dependent block of HERG channels. The block of WT channels by these drugs was enhanced by increasing membrane depolarization. However, an analysis of drug effects on WT and mutant HERG channels

Fig. 5. Effect of quinidine on the voltage dependence of Y652A and Y652F HERG channel activation. A, effect of quinidine on the isochronal activation curves for Y652A HERG. Tail currents were normalized to the peak current under each condition, and the data were fit with a Boltzmann function. The V1/2 and slope factors, respectively, were the following: control, −25.6 ± 0.7 mV and 9.5 ± 0.5 mV, n = 5; 3 μM quinine, −18.6 ± 0.9 mV and 9.1 ± 0.6 mV, n = 5; 10 μM quinidine, −14.9 ± 1.0 mV and 8.8 ± 0.6 mV, n = 5; and 30 μM quinidine, −13.6 ± 0.8 mV and 8.4 ± 0.5 mV, n = 4. B, the effect of quinidine on the isochronal activation curves for Y652F HERG. The V1/2 and slope factors, respectively, were the following: control, −35.2 ± 1.0 mV and 8.5 ± 0.5 mV, n = 5; 3 μM quinidine, −36.7 ± 1.1 mV and 7.3 ± 0.5 mV, n = 5; 10 μM quinidine, −36.5 ± 1.0 mV and 7.1 ± 0.5 mV, n = 4; and 30 μM quinidine, −35.8 ± 0.9 mV and 6.8 ± 0.5 mV, n = 4.

Fig. 6. Voltage-dependent block of Y652F HERG currents by quinidine. A and B, superimposed traces of currents elicited during application of depolarizing pulses to −40 mV (A) or +20 mV (B) and upon repolarization to −70 mV before and after exposure to 10 μM quinidine. C and D, onset of HERG channel block by 10 μM quinidine assessed during depolarizing voltage steps to −40 and +20 mV, respectively. E, time constants for onset of block of Y652F HERG current by quinidine plotted as a function of the test potential (Vt). F, fractional block of Y652F HERG currents plotted as a function of Vt.
indicates several significant differences in the mechanism of the block. First, quinidine shifted the voltage dependence of activation of WT HERG to more negative potentials. This effect was significant enough to result in an increase in HERG current by 3 μM quinidine when activated with pulses to −60 mV, despite a decrease in current at test pulses of −40 mV or more positive potentials. In contrast, chloroquine did not cause a measurable shift in the voltage dependence of HERG channel activation (Sanchez-Chapula et al., 2002). Second, chloroquine slowed the rate of deactivation of WT HERG by a factor of 4, whereas quinidine slowed deactivation only 2-fold. The

![Graphs and images showing voltage-dependent block of WT and mutant HERG currents by quinine and vesnarinone.](image-url)
slow deactivation and crossover of tail currents observed for chloroquine suggest that channels could only close after the drug dissociated from its binding site inside the central cavity, a “foot-in-the-door” effect (Armstrong, 1971; Yeh and Armstrong, 1978). The relative lack of quinidine on the rate of deactivation indicates that drug dissociation was rapid or that channels could close normally even if bound by quinidine, and that recovery from the block of closed channels was slow compared with the rate of deactivation. Alternatively, the slowed rate of deactivation induced by quinidine could be caused by a negative shift in the voltage dependence of activation. A third difference between quinidine (or quinine) and chloroquine was revealed by an analysis of mutant HERG channels. Y652 and F656 are located on the S6 domain and face the central cavity of the channel. Both residues were proposed to be important components of the HERG drug-binding site (Lees-Miller et al., 2000; Mitcheson et al., 2000). Mutation of F656 to Val reduced the IC\textsubscript{50} for block of HERG by quinidine 27-fold (Lees-Miller et al., 2000), and mutation to Ala reduced the IC\textsubscript{50} 125-fold (this study). The F656A mutation caused a much larger shift in the IC\textsubscript{50} for chloroquine, >500-fold (Sanchez-Chapula et al., 2002). Mutation of Y652 to Ala increased the IC\textsubscript{50} for block of HERG by approximately 3-fold for quinidine but >500-fold for chloroquine. Thus, Y652 and F656 seem to be more essential for the block of HERG by chloroquine than by quinidine, despite the fact that both drugs reduce WT HERG with similar potencies and voltage dependence. V625 is located at the base of the pore helix, and its side group is modeled to face the central cavity of the HERG channel. Mutation of this residue to Ala reduced the blocking potency of MK-499 by 50-fold, but it had no effect on block by terfenadine (Mitcheson et al., 2000) or chloroquine (Sanchez-Chapula et al., 2002). In contrast, we found that V625A HERG channels were 3.8-fold less sensitive to block by quinidine than were WT HERG channels. Thus, unlike chloroquine, HERG block by quinidine is also influenced by mutation of V625A located at the base of the pore helix. This extra site of interaction for quinidine may account for the similar potencies of these two drugs.

**Mutation of Y652 Alters Voltage-Dependent Block of HERG.** Block of WT HERG current by quinidine and quinine was enhanced by progressive depolarization. In contrast, block of Y652A HERG current by these drugs was diminished by increased depolarization, whereas block of Y652F HERG current was relatively insensitive to voltage. Thus, substitution of a phenyl with a benzyl moiety (Y652F) eliminated the voltage dependence of HERG block, whereas substitution with a methyl group (Y652A) reversed the voltage dependence of the block.

We previously proposed a model to explain the block of WT and mutant HERG channels by chloroquine that featured a cation-\(\pi\) interaction between the charged N atom of the drug and the benzyl and phenyl side groups of F656 and Y652 (Sanchez-Chapula et al., 2002). However, our finding that block of Y652A channels was also voltage-dependent for vesnarinone, an uncharged drug, indicates that cation-\(\pi\) interactions are not required for voltage-dependent block of HERG channels by all drugs. Instead, for drugs like vesnarinone, perhaps \(\pi\)-stacking between aromatic groups of the drug and F656 and Y652 mediates both low-affinity/voltage-dependent block and high-affinity/voltage-independent block of HERG. Membrane depolarization might rotate the S6 domain or otherwise orient the benzyl group of F656 in an unfavorable position and the phenol group of Y652 in a favorable position for drug binding. Mutation of Y652 to Phe would still allow \(\pi\)-stacking, whereas mutation to Ala would eliminate a key component of drug binding, resulting in unblocking at depolarized membrane potentials.

In summary, the voltage-dependent profile of HERG channel block by charged quinolines and an uncharged drug (vesnarinone) was modified by amino-acid substitution of Y652. Mutation of Y652 to Ala inverted the voltage-dependent profile for block by quinolines and introduced strong voltage-dependent block by vesnarinone. Mutation of Y652 to Phe eliminated the voltage dependence of the block. These findings suggest a key role for Y652 in determining the structural basis for voltage-dependent block of HERG by chemically diverse compounds.

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**References**


Address correspondence to: Dr. Michael C. Sanguinetti, Department of Physiology, Eccles Program in Human Molecular Biology and Genetics, University of Utah, 15 N 2030 E, Room 4220, Salt Lake City, UT 84112. E-mail: Michael.sanguinetti@hmbg.utah.edu