Mechanism of Action of a Novel Human ether-a-go-go-Related Gene Channel Activator

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Long QT syndrome (LQTS) is a disorder of ventricular repolarization that predisposes affected individuals to cardiac arrhythmia and sudden death. Although inherited LQTS can result from mutations in Na\(^+\) and Ca\(^{2+}\) channels, it is most often caused by loss of function mutations in HERG or KCNQ1 K\(^+\) channel genes (Keating and Sanguinetti, 2001). Acquired LQTS is more common and can be induced as an unintended side effect of treatment with class III antiarrhythmic drugs (Waldo et al., 1996). LQTS is associated with torsade de pointes, a peculiar tachyarrhythmia characterized by sinusoidal twisting of the QRS axis around the isoelectric line of the electrocardiogram. This arrhythmia usually reverts to normal sinus rhythm but can degenerate into ventricular fibrillation, the cause of sudden death (Schwartz et al., 2000, 2001). More recently, many noncardiovascular drugs have been shown to cause torsade de pointes and sudden death, including such common medications as terfenadine, cisapride, and erythromycin (Haverkamp et al., 2000). These drugs were developed and approved for human use without any knowledge of their untoward effects on ventricular repolarization. Only after they had been used by large numbers of patients, oftentimes when coadministered with other drugs that inhibit specific cytochrome P450 isoenzymes, were these drugs discovered to possess significant proarrhythmic risk. In the past decade, several commonly used drugs, including terfenadine, cisapride, sertraline, thioridazine, and grepafloxacin, were withdrawn from the market, or their approved use was severely restricted when it was discovered, albeit very infrequently, that they caused arrhythmia or were associated with unexplained sudden death (Pearlstein et al., 2003). In most clinically relevant cases, drug-induced torsade de pointes seem to result from block of human ether-a-go-go related gene (hERG) K\(^+\) channels. These channels conduct the rapid delayed rectifier K\(^+\) current (Sanguinetti et al., 1995; Trudeau et al., 1995), and...
activate cardiac K+ channels could be used to enhance net repolarizing current reduced by gene mutations or block of hERG channels. KATP channel activators are not used for this purpose because of side effects such as postural hypotension (Lawson, 2000). Drugs that activate KCNQ1 or hERG channels would seem preferable because these channels are highly expressed in the heart, and their activation might be associated with less severe side effects. We previously characterized the benzodiazepine derivative R-L3, the only known KCNQ1-specific activator (Salata et al., 1998; Seebohm et al., 2003). Novel and specific activators of hERG channels have been reported recently. These compounds, RPR260243 (Kang et al., 2005), PD-118057 (Zhou et al., 2005), and NS1643 (Hansen et al., 2006), seem to increase hERG current by different mechanisms. RPR260243 slows hERG deactivation, whereas PD-118057 and NS1643 enhanced current magnitude by an unknown mechanism without measurable effects on current kinetics. As expected for hERG channel activators, all three compounds shorten the action potential duration of ventricular myocytes (Kang et al., 2005; Zhou et al., 2005; Hansen et al., 2006).

Here, we have studied the mechanism of action of NS1643 on hERG channels expressed in Xenopus laevis oocytes. We find that this drug alters the rate of onset and voltage dependence of steady-state inactivation with no significant effects on the voltage dependence for activation or recovery from inactivation of hERG channels. The effect of the drug on mutant hERG channels revealed that it is also a weak blocker. Thus, NS1643 is a partial agonist.
Results

Increase in hERG Current by NS1643 Is Concentration- and Voltage-Dependent. Oocytes were voltage clamped to a holding potential of $-80$ mV, and hERG channel current was elicited with 2-s depolarizations to potentials ranging from $-90$ to $+50$ mV (Fig. 1a). Each test pulse was followed by a 2-s pulse to $-70$ mV to measure current deactivation and to determine the voltage dependence of activation. The effects of NS1643 on a single oocyte at concentrations of 10 and 30 $\mu$M are illustrated in Fig. 1, b and c. The currents were increased in a concentration-dependent manner by the drug, and this effect was most notable at the more positive test potentials. The percentage of increase in tail currents measured at $-70$ mV after a pulse to $+10$ mV was $8.8 \pm 0.05\%$ with 10 $\mu$M and $19.5 \pm 0.2\%$ with 30 $\mu$M drug ($n = 6$). The percentage of increase in hERG current was measured at the end of a 2-s pulse and plotted as a function of [NS1643] in the bath solution (Fig. 1d). The EC$_{50}$ for NS1643, determined by fitting these data to a Hill equation, was similar for all three voltages examined (Fig. 1d).

The I-V relationships for hERG current measured at the end of 2-s test pulses before and after treatment of oocytes with NS1643 are plotted in Fig. 2a. At 3 $\mu$M, a very small increase in current amplitude was observed. Progressively larger increases in hERG current were induced by 10 and 30 $\mu$M (Fig. 2a). Regardless of the concentration, NS1643 had only a small effect on currents activated by pulses applied to potentials between $-70$ and $-30$ mV, whereas an obvious enhancement of currents was observed for test potentials greater than $-10$ mV. For example, NS1643 increased hERG current by 17% at 10 $\mu$M and 22% at 30 $\mu$M when measured at $-30$ mV. In contrast, at $+20$ mV NS1643 increased out-

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**Fig. 2.** Voltage-dependent increase of hERG current by NS1643. a, I-V relationship for normalized WT hERG current determined before (control; $n = 13$) and after treatment of oocytes with three concentrations of NS1643 ($n = 6$). Current values were normalized to the peak of the control current at $+10$ mV ($3.86 \pm 0.53$ nA). b, voltage dependence of hERG activation is not shifted by NS1643. The half-point ($V_{1/2}$) and slope factors ($k$) determined from fitting data to a Boltzmann function were as follows: WT ($V_{1/2}$ = $-31.2 \pm 0.2$ mV; $k$ = $7.6 \pm 0.2$ mV), 10 $\mu$M ($V_{1/2}$ = $-29.3 \pm 0.2$ mV; $k$ = $7.8 \pm 0.2$ mV), and 30 $\mu$M ($V_{1/2}$ = $-30.2 \pm 0.3$ mV; $k$ = $8.9 \pm 0.3$ mV).

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**Fig. 3.** Effect of NS1643 on amplitude and kinetics of hERG currents. a and b, tail currents measured using the voltage protocol illustrated above the current traces. Note that current scaling is not the same for the two sets of tracings. c and d, time constants ($\tau_{deact}$) for fast and slow components of current deactivation in the absence ($n = 12$) and presence of 10 and 30 $\mu$M NS1643 ($n = 7$ each).
ward current by 69% at 10 μM and 166% at 30 μM. At 100 μM, the agonist activity of NS1643 was attenuated at higher test potentials. At this high concentration, the increase in current was 68 ± 7% at -30 mV, 47 ± 10% at 0 mV, and 38 ± 15% at +20 mV (n = 5; not shown), suggesting that the drug is a partial agonist. Thus, the agonist activity of NS1643 on WT hERG channels was voltage- and concentration-dependent. In theory, voltage-dependent partial agonist activity might be explained by a voltage-independent agonist effect plus a drug-induced shift in the voltage dependence of channel gating. However, NS1643 at 10 and 30 μM had no effect on the voltage dependence of hERG activation determined from plots of tail current amplitudes (normalized to the peak current under each condition) versus voltage (Fig. 2b). These findings suggest that the block observed at high concentrations was voltage-dependent and that the partial agonist activity results from two opposing effects (agonist and block) on the channel.

HERG tail currents were measured at potentials between -140 and +40 mV after channel activation induced by a 1-s pulse to +40 mV (Fig. 3a, inset). Currents were inward and rapidly deactivating at potentials negative to -90 mV, reversed near -90 mV, and outward and deactivated more slowly at potentials positive to -90 mV (Fig. 3, a and b). The time course of current deactivation was determined at potentials varying from -120 to -60 mV. Deactivation was best described by a two-exponential function, and only 30 μM NS1643 caused a significant slowing in the time constants for both fast and slow deactivation (Fig. 3, c and d). Thus, NS1643 increased the amplitude of hERG without causing a shift in the voltage dependence of channel activation and slowed deactivation was observed at 30 μM.

**NS1643 Slows the Rate of Inactivation and Reduces the Extent of hERG Channel Rectification.** The increase in WT hERG current by NS1643 was greatest at positive potentials, suggesting that the drug might decrease the extent of channel inactivation. The onset rate of hERG current inactivation was determined using a three-pulse protocol described previously (Smith et al., 1996; Spector et al., 1996). In brief, current was first activated and inactivated by a 1-s pulse to +40 mV. During the second pulse to -120 mV for 12 ms, channels were allowed to recover from inactivation. A third pulse was applied to a potential that was varied from -40 to +50 mV to observe the re-onset of current inactivation (Fig. 4a, inset). The currents during the third pulse were fitted to a single exponential function to estimate the time constants for inactivation (Fig. 4b). NS1643 at 10 and 30 μM slowed the rate of hERG inactivation throughout the voltage range examined. The $V_{1/2}$ for the voltage dependence of recovery from inactivation was $-74.1 ± 3.8$ mV under control conditions and $-73.1 ± 2.5$ mV in the presence of 10 μM drug (n = 3). The slope factor for this relationship was also not altered by the drug ($19.6 ± 1.3$ mV for control versus $18.9 ± 1.3$ mV after drug). A higher concentration of NS1643 (30 μM) was reported to cause a $+11$-mV shift in $V_{1/2}$ (Hansen et al., 2006). Thus, NS1643 slowed the onset of inactivation but did not alter the voltage dependence for recovery from inactivation sufficiently to explain its agonist activity.

Slow activation coupled with rapid inactivation of channels results in rectification of the I-V relationship for hERG (Smith et al., 1996; Spector et al., 1996). The fully activated I-V for hERG (Fig. 4c) was determined by measuring the peak amplitude of tail currents over a wide range of mem-

![Fig. 4.](image-url)
brane potentials after a 1-s pulse to +40 mV. NS1643 at 10 and 30 μM increased tail current amplitudes at test voltages positive to the reversal potential, reducing the rectification of hERG current. This effect was quantified by measuring the rectification factor, defined as the deviation (reduction) of current amplitudes relative to that expected from extrapolation of the linear portion of the I-V relationship (between −140 and −110 mV) to more positive potentials. NS1643 shifted the half-point of the rectification-voltage relationship by +21 mV at 10 μM and +35 mV at 30 μM (n = 5; Fig. 4d). Thus, the agonist activity of NS1643 was associated with a decrease in the rate of onset and extent of hERG channel inactivation.

NS1643 Does Not Affect Noninactivating hERG Channels. HERG channels with impaired inactivation were used to confirm the importance of altered inactivation as the mechanism of action for NS1643. Inactivation of hERG can be completely removed by the double mutation G628C/S631C (Smith et al., 1996). Because G628C/S631C hERG channels do not inactivate, current is increased progressively when the membrane is depolarized to potentials positive to 0 mV (Fig. 5a). In contrast to WT hERG, NS1643 had no effect on currents at all potentials, and the peak increase of the I-V relationship (between −50 mV, the peak of the I-V relationship) that exhibited inactivation (Fig. 6a).

Thus, the agonist activity of NS1643 was associated with a decrease in the rate of onset and extent of hERG channel inactivation.

Activation of hERG by NS1643 Is Enhanced by Disruption of the Binding Site for hERG-Blocking Drugs. Many drugs that block hERG channels interact with specific residues (i.e., Y652 and F656) that are located on the S6 transmembrane domain in positions that face toward the central cavity of the channel (Lees-Miller et al., 2000; Mitcheson et al., 2000; Sanchez-Chapula et al., 2002, 2003; Fernandez et al., 2004). Mutation of these residues, especially F656, reduces the potency of blockers. Therefore, we determined whether Y652A or F656V mutations might also reduce the effectiveness of NS1643 to enhance hERG current. The effect of NS1643 at 10 and 30 μM on Y652A hERG (Fig. 7a) was nearly identical to the effect of the drug on WT hERG (compare with Fig. 2a). Similar to WT hERG, the increase in Y652A hERG current was most evident at potentials greater than −20 mV, and 30 μM increased outward current by an average of 64 ± 2% at 0 mV. Thus, mutation of Y652A did not alter the agonist activity of NS1643. By contrast, NS1643 enhanced F656V hERG channels at all potentials, and the peak increase obtained with 30 μM at 0 mV was much larger (208 ± 75%; Fig. 7b). Similar results were obtained with F656T and F656M hERG channels (Fig. 7, c and d). Mutation of F656 to another aromatic residue (Tyr) resulted in agonist activity that was intermediate compared with WT and the other F656 mutant channels (Fig. 7e). Thus, mutation of residues known to be important for drug binding did not prevent the action of NS1643, and in fact, mutation of F656 to nonaromatic residues potentiated the agonist activity of the drug by more than 3-fold.

Discussion

Treatment of acute drug-induced and congenital LQTS is inadequate. HERG channel activators might be useful for the treatment of both forms of LQTS. It has been proposed that specific activation of hERG channels might prevent arrhythmia by suppressing action potential duration alternans (Hua and Gilmore, 2004; Hua et al., 2006). Three different compounds have recently been reported to possess such activity: RPR260243 (Kang et al., 2005), PD-118057 (Zhou et al., 2005), and NS1643 (Hansen et al., 2006). Understanding the molecular mechanism of action of these structurally diverse compounds could facilitate the discovery and development of additional compounds. The development of safe and effective hERG
Fig. 6. Effect of NS1643 on mutant hERG channels with partially impaired inactivation gating. a, NS1643 enhances S631A hERG currents when expression is low. b, NS1643 does not enhance S631A hERG when currents are large. c, I-V relationships for S631A hERG when expressed at low (left, peak current <4 µA at +10 mV) or high (right, average peak current 18 µA at +50 mV) levels in oocytes. Left, S631A hERG peak control current, 2.07 ± 0.7 µA (n = 6); 10 µM (n = 6); and 30 µM (n = 3). Right, hERG peak control current, 21.3 ± 1.07 µA (n = 3 for all). d, I-V relationships for S620T hERG when expressed at low (left) or high (right) levels in oocytes. Left, peak control current, 2.2 ± 0.5 µA (n = 7); 10 µM (n = 6); and 30 µM (n = 5). Right, peak control current, 14.4 ± 1.0 µA (n = 6); 10 µM (n = 5); and 30 µM (n = 4).
channel activators may provide a pharmacological treatment for LQTS and perhaps surpass the effectiveness of magnesium sulfate for this purpose (Tzivoni et al., 1984; Martinez, 1987).

Activation of hERG channels and shortening of action potential duration can be achieved by different mechanisms. RPR260243 was reported to cause a slight slowing of activation and approximately a 20% increase in hERG current magnitude. The main mechanism of action potential shortening by this drug is the pronounced delay in channel closure (deactivation); it is without any significant effects on the voltage dependence of activation or inactivation (Kang et al., 2005). PD-118057 (Zhou et al., 2005) and NS1643 (Hansen et al., 2006) also have no effect on the voltage dependence of activation. However, in contrast to RPR260243, NS1643 activates hERG primarily by altering the voltage dependence of channel inactivation with little (present study) or no effect (Hansen et al., 2006) on the rate of deactivation. Similar to NS1643, the hERG agonist activity of PD-118057 was voltage-dependent, and the drug did not significantly alter the voltage dependence of activation or recovery from inactivation of channels (Zhou et al., 2005). The agonist effects of PD-118057 were attributed to an unspecified alteration of single channel activity; however, the decrease in rectification suggests that this compound may have a mechanism similar to that described here for NS1643.

NS1643 reduced the rectification of hERG, an effect that was best revealed by measurement of the fully activated I-V relationship before and after treatment of oocytes with the drug (Fig. 4a). NS1643 also slowed the onset rate of hERG current inactivation. Rectification is caused by fast channel inactivation. Thus, the relatively minor effect (+10-mV shift) by the drug on the voltage dependence of recovery from inactivation was unexpected (Hansen et al., 2006). However, we have previously reported that mutations of hERG can also differentially affect the onset and recovery from inactivation of hERG. A mutation in the pore of hERG (S631A) shifts the peak of the isochronal (1-s) I-V relationship by approximately +30 mV, a measure of steady-state inactivation. However, S631A shifts the voltage dependence for recovery from inactivation, determined with a triple-pulse protocol, by +102 mV (Zou et al., 1998). Thus, it is possible to differentially affect the onset and recovery from inactivation by drugs or by mutation of the channel.

Hansen et al. (2006) reported that 10 μM NS1643 increased the magnitude of hERG tail currents by 45% when measured at −60 mV. The percentage of increase in tail currents in our study were only 8.8 ± 0.05% with 10 μM and 19.5 ± 0.2% with 30 μM drug. Hansen et al. (2006) measured hERG channel deactivation at −60 mV using a bath solution containing a [KCl] of 1 mM. In contrast, we measured tail currents at −70 mV and used a bath solution with 4 mM KCl. hERG inactivation is greater at −70 mV, and its voltage dependence is leftward shifted with low [K⁺]. Therefore, it is likely that the drug-induced positive shift in the voltage

![Fig. 7. Effect of NS1643 on Y652A and F656 mutant hERG channels. a, I-V relationship for Y652A hERG for control (peak control current, 4.9 ± 0.5 μA; n = 5), 10 μM (n = 4), and 30 μM (n = 4). b, I-V relationship for F656V hERG (peak control current, 1.46 ± 0.2 μA; n = 9), 10 μM (n = 6), and 30 μM (n = 5). c, I-V relationship for F656T hERG (peak control current, 1.14 ± 0.28 μA; n = 5). d, I-V relationship for F656M hERG (peak control current, 0.85 ± 0.1 μA; n = 5). e, I-V relationship for F656Y hERG (peak control current, 0.8 ± 0.04 μA; n = 5).](molpharm.aspetjournals.org)
dependence of hERG activation resulted in a larger increase in current in the Hansen et al. (2006) experiments compared with our experiments. Moreover, as can be seen in Fig. 4c, the tail currents are larger when measured at −60 mV compared with −70 mV.

The agonist effect of NS1643 was greatly enhanced by mutation of F656 to Ala, Val, or Met and to a lesser extent by mutation to Tyr. In contrast, Y652A channels were affected by NS1643 in an almost identical manner as WT hERG channels. Most of the hERG channel blockers we have examined interact with both F656 and Y652 residues (Mitcheson et al., 2000; Sanchez-Chapula et al., 2002, 2004; Fernandez et al., 2004). However, quinidine was strongly influenced by mutation of F656 (i.e., 125-fold increase in IC₅₀ for F656A) but not by mutation of Y652A hERG (3-fold increase in IC₅₀ for Y652A) (Sanchez-Chapula et al., 2002). Thus, NS1643 most likely blocks hERG channels by low-affinity interactions with F656 but not Y652. This blocking activity is why we refer to NS1643 as a partial agonist. Furthermore, based on the intermediate effects of NS1643 on F656Y channels, the blocking of hERG by this drug may be favored by a π–π interaction involving F656 rather than a more generalized hydrophobic interaction described previously for potent blockers such as MK-499, terfenadine, and cisapride (Fernandez et al., 2004).

An important observation relative to specificity of RPR260243 and PD-118057 was that these drugs did not affect other cardiac currents, including IᵥCaL, IᵥNa, IᵥKᵢ, or IᵥKs. In addition, RPR260243 partially blocked, but did not affect the gating of ERG3, the channel most similar to hERG (ERG1). This differential effect of the drug on ERG1 and ERG3 should facilitate determination of the structural basis of the agonist effect. Future experiments will examine the binding sites for these drugs that are responsible for their distinct molecular mechanisms of hERG channel activation. More importantly, experiments in animals or isolated hearts are needed to determine whether the specific molecular mechanism of NS1643 provides unique advantages as an antiarrhythmic compared with other hERG agonists.

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References


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