Extracellular potassium dependency of block of HERG by quinidine and cisapride is primarily determined by the permeant ion and not by inactivation

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Abbreviations: HERG, human-ether-a-go-go-related gene; TEA, tetraethylammonium; DMSO, dimethyl sulfoxide; 0K, 0 mM (no added) potassium; 2K, 2 mM potassium; 20K, 20 mM potassium; 20Cs, 20 mM cesium; 20Rb, 20 mM rubidium; 40TEA, 40 mM tetraethylammonium; 0.1Ca, 0.1 mM calcium; 10Ca, 10 mM calcium

Introduction

Long QT syndrome is a potentially lethal cardiac arrhythmia characterized by a prolonged QT interval on an electrocardiogram. One form of Long QT syndrome, referred to as acquired Long QT syndrome, has been shown to primarily result from a reduction in IKr, a potassium current important in repolarizing the cardiac action potential.1 Reduction in IKr can result from block of the human ether-a-go-go potassium channel (HERG - Kv11.1), the pore forming subunit of IKr, by a large number of pharmaceutical compounds.1 In some instances, Long QT syndrome will degenerate into the potentially lethal arrhythmia torsade de pointes, characterized by a rapid heart rate and severely compromised cardiac output. Although death resulting from HERG blocking drugs is rare,2,3 the potentially lethal consequences resulting from block of HERG requires a solid understanding of the mechanisms by which different compounds interact with this channel.

Numerous studies have described block of HERG by a structurally and functionally diverse group of compounds including gastrointestinal motility agents, antipsychotics, anti-arrhythmics and antibiotics.1 These studies have revealed a number of mechanisms by which different drugs block HERG. One important component of these mechanisms has been the relationship between HERG gating and drug block of HERG. HERG gating can be approximated by a 3-state model (C ↔ O ↔ I), in which depolarization of the cell results in HERG channels slowly transitioning from a closed state (C) to an open state (O), followed by a rapid transition to an inactivated state (I).4 There are a number of reports describing the relationship between HERG block and both activation (i.e., C ↔ O) and inactivation (i.e., O ↔ I) of the channel.3-15 However, the interactions between drug-induced block of HERG and HERG gating still remain poorly resolved.

A large number of HERG blockers require channel opening in order to act.10,16 In addition, studies with a mutant HERG channel that opens upon hyperpolarization have revealed that a
number of drugs are trapped in the channel after the channel closes.\textsuperscript{15,24} These studies suggest a link between activation gating (C↔O) and drug block. Regarding inactivation, there is controversy in the literature over what role, if any, inactivation (O↔I), plays in the development of block.\textsuperscript{10} A number of studies suggest a limited role for inactivation in determining drug block of HERG (i.e., halofantrine, cisapride, quinidine)\textsuperscript{5,13,18} whereas other studies suggest that for certain compounds the inactivation state is an important determinant of HERG block (i.e., dofetilide, sotalol).\textsuperscript{6-8,11,12,15}

To evaluate the extent to which gating influences drug block of HERG, we examined the blockade of HERG by quinidine and cisapride in different extracellular electrolytes known to alter HERG gating. The extracellular divalent ions magnesium and calcium primarily alter HERG channel activation (C↔O)\textsuperscript{9,20} whereas the extracellular monovalent ions potassium, rubidium, cesium and tetraethylammonium (TEA) primarily alter HERG inactivation (I↔O).\textsuperscript{3,21-23} Functionally, HERG channels are much more selective for potassium and rubidium than for cesium and do not allow TEA to permeate (P\textsubscript{K+} > P\textsubscript{Cs+} >> P\textsubscript{TEA}) and this ion selectivity is unchanged in the presence of different extracellular divalent cations. The effects of extracellular cations on HERG gating and selectivity allows for an estimation of the relative contribution of gating to drug block. By altering the extracellular concentrations of various extracellular cations we demonstrate that channel activation plays a limited role in determining HERG block by quinidine and cisapride. Furthermore, over the range of 0 mM to 20 mM extracellular potassium, ion permeation appears to play a greater role in determining drug block compared to inactivation.

**Results**

**Effect of pulse length on drug block.** Figure 1 shows that the degree of block by cisapride depends on pulse length used during repetitive pulsing, whereas the degree of block by quinidine does not. The protocols used in Figure 1 differ only by the length of the depolarization to +20 mV (1 sec versus 5 sec) and the frequency of pulsing (0.167 Hz versus 0.1 Hz). Based on the results shown in Figure 1, it is evident that cisapride-induced block is significantly greater for a pulse length of 5 sec compared to a pulse length of 1 sec. This effect on pulse length is consistent with the degree of block and the IC\textsubscript{50} value for cisapride reported in the literature using a long pulse.\textsuperscript{5,24}

**Inactivation and permeability properties of HERG.** Inactivation and permeability properties of HERG have been studied in some detail.\textsuperscript{21-23,25} However, some of the solutions used in this study to measure HERG block are slightly different than solutions used by other investigators. In order to assess the relative contribution of inactivation and permeation to drug block of HERG, channel inactivation and permeation properties were measured in the same external potassium, cesium and TEA solutions that were used in this study to evaluate quinidine and cisapride block of HERG.

**Estimation of the fraction of inactivated channels.** The fraction of inactivated channels was determined using a protocol adapted from the literature.\textsuperscript{5,25} Figure 2A shows that over a range of voltages, the fraction of inactivated channels at the end of the test pulse is lowest in 40TEA and highest in 0K. For depolarizing pulses to -10 mV and 0 mV, there is a statistically significant difference between the fraction of inactive channels in 0K compared to 20K, 20Cs and 40TEA. For a depolarizing pulse to either +10 mV or +20 mV, there was no statistically significant difference in the fraction of inactive channels in 0K compared to 20K, or 20Cs, but there was a statistically significant difference between 0K and 40TEA. The inactivation time constant shows a similar dependency on extracellular monovalent cations (τ\textsubscript{40TEA} > τ\textsubscript{20Cs} = τ\textsubscript{20K} > τ\textsubscript{0K}: Fig. 2A, bottom). However, there is a statistically significant difference in the time constant between 0K and either 20K, 20Cs or 40TEA at all voltages shown (-10 mV, 0 mV, +10 mV, +20 mV). While it is likely that the fraction of inactive channels decreases from 0K to 20K at all depolarizing voltages tested in the present study,\textsuperscript{3,15,26} the data presented here can only demonstrate that at +20 mV (the depolarizing pulse used to assess drug block), the fraction of inactivated channels is significantly less in 40TEA compared to all other solutions tested.

We did not observe a statistically significant difference in either the fraction of inactive channels or the inactivation time constant between 0K and 2K. One possible explanation is the inability of the two-electrode-voltage clamp to measure the very fast inactivation time constant in 0K. Consistent with this possibility, current recordings of HERG channels expressed in mammalian cells using patch clamp methodology showed a rightward shift in the steady state inactivation curve between 0 mM and 5 mM extracellular potassium.\textsuperscript{7}

**Estimation of permeation through HERG channels.** Permeability ratios were determined using reversal potential measurements in bionic conditions. Figure 2B shows that the permeability sequence through the HERG channel is P\textsubscript{K+} > P\textsubscript{Cs+} >> P\textsubscript{TEA} in agreement with recent reports in the literature.\textsuperscript{21,23}

**Block of HERG by quinidine and cisapride in different extracellular calcium and potassium solutions.** In order to assess the role of HERG gating in drug block, HERG channel blockade by either 3 μM quinidine or 1 μM cisapride was tested in solutions of low and high extracellular calcium, and low and high extracellular potassium. External calcium is known to influence HERG channel activation, whereas the external potassium concentration affects primarily the degree of channel inactivation. Figures 3 and 4 show the time course and degree of block for representative oocytes in low and high extracellular calcium and potassium solutions. Due to the manner in which drug was added (i.e., bath application), this time course reflects the time for the drug to reach the inner mouth of the channel and is not solely determined by the kinetics of drug block. Figure 5 summarizes the results from a number of different experiments and shows that the extent of HERG block by either 1 μM cisapride or 3 μM quinidine is similar in low or high calcium, whereas HERG block by either 1 μM cisapride or 3 μM quinidine is significantly reduced in elevated extracellular potassium. The amount of block by 3 μM quinidine is similar to that previously reported,\textsuperscript{27} whereas we observed a lesser amount of block by cisapride compared to earlier studies.\textsuperscript{5,24} However, as noted in Figure 1, block by 1 μM cisapride appears to be highly sensitive to the length of the test pulse used. To
quinidine and cisapride was reduced in extracellular cesium, rubidium and potassium, but not TEA. In addition HERG block by both quinidine and cisapride was similar in 0K and 40TEA solutions, suggesting that channel inactivation relative to ion permeation, does not play a large role in determining HERG block by either cisapride or quinidine.

It is noteworthy that HERG block by quinidine and cisapride shows slightly different quantitative dependencies on monovalent extracellular cations. In the case of quinidine, there is a statistically significant difference in the amount of block between 0K compared to 2K and between 2K compared to 20K (Fig. 8). In addition, compared to block in 0K, block in 20Cs shows a smaller reduction than block in 20K. In the case of cisapride, there is no statistically significant difference in the degree of cisapride block between 2K and 20K or between 20K and 20Cs. These data are in agreement with recent observations that HERG blockers do not all show the same sensitivity to extracellular potassium.31

Figure 9 shows dose response curves for both quinidine and cisapride in 0K, 2K, 20Cs and 40TEA. The dependency of block on extracellular cations is qualitatively similar for quinidine compared to cisapride, but quantitatively different. For quinidine, the reduction in HERG block is smaller for the less permeant ion cesium compared to potassium, whereas for cisapride, the reduction in HERG block is similar for potassium compared to cesium (see discussion). However, for both quinidine and cisapride, block is similar in 0K and 40TEA, suggesting that, in both cases, the

evaluate the mechanism by which extracellular potassium alters HERG block by both quinidine and cisapride, we carried out the series of experiments described below.

Block of HERG by quinidine and cisapride in different extracellular monovalent cation solutions. Reduction of drug-induced blockade of HERG in the presence of high extracellular potassium has been reported by a number of investigators.14,28-30 Biophysically, at least two mechanisms could account for this reduction in block: (1) Permeant potassium could destabilize or “knock-off” the drug from its binding site; (2) cisapride and quinidine binding could preferentially block the inactivated state or be stabilized by the inactivated state and potassium ions could reduce the fraction of inactive channels. Figures 2 and 5 suggest that ion permeation through HERG plays a greater role in block by quinidine and cisapride compared to channel inactivation, since the fraction of drug blocked channels is significantly less in 20K, yet the fraction of inactive channels at +20 mV is similar in 0K compared to 20K. To further evaluate the relative contributions of channel inactivation and ion permeation to block of HERG by quinidine and cisapride, drug-induced block of HERG was tested in solutions containing rubidium, cesium and TEA. The time course and degree of drug block in these solutions are shown in Figures 6 and 7 and summarized in Figure 8. Consistent with a molecular “knock-off” mechanism, HERG blockade by both quinidine and cisapride was reduced in extracellular cesium, rubidium and potassium, but not TEA. In addition HERG block by both quinidine and cisapride was similar in 0K and 40TEA solutions, suggesting that channel inactivation relative to ion permeation, does not play a large role in determining HERG block by either cisapride or quinidine.

Figure 1. Effect of pulse length on drug block. (A) Time course and degree of drug block by either quinidine (left) or cisapride (right) assessed in 2K with repetitive pulsing using different depolarizing pulse lengths. Top row shows block using a depolarization to +20 mV for 1 second every 6 seconds. Bottom row shows block using a depolarization to +20 mV for 5 seconds every 10 seconds. All other aspects of the repetitive puling protocol are identical and described in materials and methods. The insets show two raw current traces: just prior to addition of drug and after steady state block. Scale bars are for the time course plot only. For the 1 second protocol data were sampled at 500 μsec and filtered at 1 kHz. For the 5 second protocol data were sampled at 1,000 μsec and filtered at 500 Hz. (B) Comparison of fractional block using the 2 different protocols described in (A). Fractional block was calculated as 1 - (Iis/Ipre), where Ipre is the current level before addition of drug and Iss is the steady state current level after block. Error bars represent standard error of the mean. n values: quinidine-1 sec (5), quinidine-5 sec (4), cisapride-1 sec (3), cisapride-5 sec (4).
Voltage dependence of quinidine block of HERG. To examine the voltage dependence of HERG channel blockade, block of HERG by 10 μM quinidine was measured at 0 mV and at +60 mV in both 2 mM and 20 mM extracellular potassium. Channel block was assessed with the same pulsing protocol used in Figures 3, 4, 6 and 7 except that the voltage during the step to +20 mV was changed to either 0 mV or +60 mV. In 2K, the fraction of HERG channels blocked at +60 mV was 0.53 ± 0.02, compared to 0.54 ± 0.02 at 0 mV. This is consistent with a previous study which showed no difference in quinidine block of HERG in 2 mM extracellular potassium at voltages between 0 mV and +40 mV.25 The fraction of HERG channels blocked in 20K at +60 mV was 0.54 ± 0.03 compared to 0.40 ± 0.03 at 0 mV. This decrease in block observed in 20K at 0 mV compared to +60 mV is statistically significant (p < 0.01).

Discussion

The data presented here support three conclusions concerning HERG block by quinidine and cisapride over an extracellular potassium concentration range of 0 mM to 20 mM: (1) neither deactivation gating nor inactivation gating predominantly determine HERG block; (2) ion permeation through the HERG channel contributes a greater amount to the extent of drug block than channel inactivation; (3) HERG block by quinidine and cisapride show a similar qualitative, but different quantitative dependence on monovalent extracellular cations.
cisapride. This is consistent with the fact that neither quinidine nor cisapride are thought to be trapped in the channel after the activation gate has closed.17,27,32

A number of investigators have demonstrated that some HERG blockers can be trapped in the channel after channel closure.9,17 Slowing of channel deactivation could allow greater dissociation of drugs that are trapped in the channel and result in reduced drug block. Experiments described in our study show that decreasing the rate of deactivation approximately 2- to 3-fold by increasing extracellular calcium from 0.1 mM to 10 mM had minimal effects on block of HERG by either quinidine or cisapride. This is consistent with the fact that neither quinidine nor cisapride are thought to be trapped in the channel after the activation gate has closed.17,27,32

A number of studies have attempted to correlate drug block with channel inactivation.10 Some of these report limited or no reduction in drug block with reduced inactivation,5,13,18 while others show significant reduction in drug block with reduced inactivation.6,8,11,12,15 Most of these studies have utilized inactivation

Figure 3. Quinidine block of HERG in calcium and potassium. After extracellular solution change from ND96 to either low or high calcium or low or high potassium, oocytes were repetitively pulsed as described in Materials and Methods. Current level was assessed at the beginning of the pulse to -60 mV and is plotted as a function of time. After a steady state current level was reached in the absence of drug, 3 μM quinidine was perfused into the bath. The vertical arrow and larger diamond indicates the time point when perfusion of drug into the bath was initiated. The insets show two raw current traces: just prior to addition of drug and after steady state block. Each time course and inset represents one representative oocyte for that solution. Scale bars are for the time course plot only. Data were sampled at 500 μsec and filtered at 1 kHz. The units for the Y-axis are μA.

Figure 4. Cisapride block of HERG in calcium and potassium. Data were collected as described in Figure 3. After a steady state current level was reached in the absence of drug, 1 μM cisapride was perfused into the bath. The vertical arrow and larger diamond indicates the time point when perfusion of drug into the bath was initiated. The insets show two raw current traces: just prior to addition of drug and after steady state block. Each time course and inset represents one representative oocyte for that solution. Scale bars are for the time course plot only. Data were sampled at 500 μsec and filtered at 1 kHz. The units for the Y-axis are μA.
Cisapride block of an inactivation deficient HERG mutant (S620T) and two mutations at residue 656 known to be a major determinant in HERG drug block, shows reduced sensitivity to external potassium. This result suggests that a conformational change during channel inactivation stabilizes cisapride block of HERG. At least for S620T, the largest reduction in potassium deficient HERG mutants to test drug-induced channel blockade. Many of these inactivation deficient mutants also show reduced ion selectivity and it is not clear if reductions in drug block are due to changes in inactivation or to changes in the drug binding site. In the experiments described in this paper, monovalent cations were used to alter the degree of HERG inactivation. Monovalent cations are thought to slow inactivation by binding to a site in the outer mouth of the channel, although the exact mechanism by which this binding alters inactivation is still not clear. Our data have demonstrated that slowing inactivation with extracellular TEA did not reduce HERG block by either quinidine or cisapride. This result is consistent with a study using a series of inactivation mutations, which concluded that inactivation per se does not determine cisapride-induced blockade of HERG. However, another study has reported that decreasing the fraction of inactive channels with either changes in extracellular cadmium or extracellular sodium reduced HERG block by sotalol. While the difference between these studies is not clear, a more recent study found that sotalol exhibited preferential binding to the inactivated state whereas quinidine did not. In addition, Perrin et al. found a 2- to 3-fold decrease in the IC50 value for cisapride with an 84% reduction in the fraction of inactive channels and calculated that cisapride exhibits an 8-fold preferential binding to the inactivated state compared to the open state. These findings are complementary to the data reported in our study in which a smaller reduction in the fraction of inactive channels was used—an approximately 15% reduction in the fraction of inactive channels in 40TEA compared to 0K. Taken together, these results suggest that channel inactivation plays a relatively minor role in determining HERG block by cisapride within an extracellular potassium concentration range between 0 mM and 20 mM. A larger reduction in the fraction of inactivated HERG channels is needed in order to reduce block of HERG by cisapride.

Figure 5. Summary of Quinidine and Cisapride block of HERG in the presence of external potassium and calcium. Fractional block by 3 µM quinidine (right) and 1 µM cisapride (left) in calcium and potassium solutions. Error bars indicate standard error of the mean; n values are quinidine: 0K(3), 20K(8), 0.1Ca(4), 10Ca(3); cisapride: 0K(4), 20K(3), 0.1Ca(3), 10Ca(4). An asterisk indicates a statistically significant difference between low and high solutions (p < 0.05).

Figure 6. Quinidine block of HERG in potassium, rubidium, cesium and TEA. After extracellular solution change from ND96 to 0 mM potassium, 2 mM potassium, 20 mM potassium, 20 mM rubidium, 20 mM cesium or 40 mM TEA, oocytes were repetitively pulsed as described in Materials and Methods. Current level was assessed at the beginning of the pulse to -60 mV and is plotted as a function of time. After a steady state current level in the absence of drug was reached, 3 µM quinidine was perfused into the bath. The vertical arrow and larger diamond indicates the time point when perfusion of drug into the bath was initiated. The insets show two raw current traces: just prior to addition of drug and after steady state block. Each time course and inset represents one representative oocyte for that solution. Scale bars are for the time course plot only. Data were sampled at 500 µsec and filtered at 1 kHz. The units for the Y-axis are µA.

Cisapride block of an inactivation deficient HERG mutant (S620T) and two mutations at residue 656 known to be a major determinant in HERG drug block, shows reduced sensitivity to external potassium. This result suggests that a conformational change during channel inactivation stabilizes cisapride block of HERG. At least for S620T, the largest reduction in potassium
sensitivity occurs when extracellular potassium is raised from 5 mM to 135 mM. It is thus possible that between 5 mM and 135 mM, inactivation plays a larger role in determining drug block than between 0 mM and 5 mM. Furthermore, Lin et al. assessed cisapride block by pulsing to +50 mV. The results presented by Lin et al. (i.e., Fig. 7 of their paper) and ourselves (Fig. 2) would suggest that at +50 mV, the difference in the fraction of inactivated channels is similar between 0 mM and 5 mM extracellular potassium. Consistent with the study by Perrin et al. discussed above, it is likely that permeation is the larger determinant of drug block at lower extracellular potassium concentrations and that channel inactivation plays a greater role at higher extracellular potassium concentrations. Interestingly, a recent study using a double mutation of HERG provides evidence of a correlation between inactivation and block of HERG when the fraction of inactive channels is increased from about 80% inactive channels to 90% inactive channels.

Wang et al. showed that E-4031 induced blockade of an inactivation deficient HERG channel was sensitive to

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**Figure 7.** Cisapride block of HERG in potassium, rubidium, cesium and TEA. Data were collected as described in Figure 6. After a steady state current level in the absence of drug was reached, 1 μM cisapride was perfused into the bath. The vertical arrow and larger diamond indicates the time point when perfusion of drug into the bath was initiated. The insets show two raw current traces: just prior to addition of drug and after steady state block. Each time course and inset represents one representative oocyte for that solution. Scale bars are for the time course plot only. Data were sampled at 500 μsec and filtered at 1 kHz. The units for the Y-axis are μA.

**Figure 8.** Summary of Quinidine and Cisapride block of HERG in the presence of external potassium, rubidium, cesium or TEA. Fractional block by 3 μM quinidine (left) and 1 μM cisapride (right) in 0 mM potassium, 2 mM potassium, 20 mM potassium, 20 mM rubidium, 20 mM cesium and 40 mM TEA. Fractional block was calculated as 1 - (Ipre/Iss), where Ipre is the current level before addition of drug and Iss is the steady state current level after block. n values are Quinidine: 0K(3), 2K(4), 20K(8), 20Rb(4), 20Cs(4), 40TEA(4); Cisapride: 0K(3), 2K(4), 20K(7), 20Rb(4), 20Cs(7), 40TEA(4). Error bars indicate standard error of the mean. An asterisk indicates a statistically significant difference compared to 0K solution (p < 0.05).
extracellular potassium between 2 mM and 98 mM potassium and suggested that, relative to a direct electrostatic interaction between potassium and E-4031, conformational changes during inactivation contributed little to the free energy of drug binding. The results of our study show that this scenario is also true for the block of wild-type HERG channels by quinidine and cisapride over a smaller range of extracellular potassium. The free energy reduction of drug binding due to increased extracellular potassium for quinidine and cisapride was found to be significantly larger than that reported for E-4031 (i.e., 1.19 kcal/mole for cisapride and 1.04 kcal/mole for quinidine compared to 0.44 kcal/mole for E-4031). These energies are consistent with an electrostatic repulsion, but suggest that different drugs are more or less easily destabilized by extracellular potassium.

Although the effects of extracellular cations on the blockade of HERG by quinidine and cisapride are qualitatively similar, there are at least two quantitative differences. (1) There is a statistically significant difference in quinidine block of HERG between 2 mM K and 20 mM K, whereas there was no such difference noted for cisapride block of HERG under the same conditions. This suggests that the sensitivity of HERG block to extracellular potassium is not the same for all drugs. This could be important clinically depending on whether or not the range of potassium sensitivity falls within clinical values. (2) For the case of quinidine, cesium, a less permeant ion than potassium, reduces drug block to a lesser extent compared to potassium. In contrast, in the case of cisapride, cesium and potassium reduce HERG block by approximately the same amount. The mechanism behind this difference is not clear but may be related to the difference in relative affinities for the inactivated versus open states of the channel for quinidine compared to cisapride.

Measurements of the voltage dependence of HERG blockade by quinidine shows that there is no difference in block between +60 mV and 0 mV in 2 mM extracellular potassium whereas there is a difference in block between +60 mV and 0 mV in 20 mM extracellular potassium. These data can be explained by noting that in 20K, there is a greater difference in the driving force between +60 mV and 0 mV (E\text{rev}\text{-}\text{20K} = -45 mV, 67% reduction in the driving force from +60 mV to 0 mV) compared to the difference in driving force between +60 mV and 0 mV in 2K (E\text{rev}\text{-}\text{2K} = -99 mV, 48% reduction in the driving force from +60 mV to 0 mV). As a result one would expect that in 20K there would be a greater difference in the single channel current between +60 mV and 0 mV, compared to the difference in single channel current between +60 mV and 0 mV in 2K. In addition, any increase in the single channel conductance from 2K to 20K would enhance the difference in the single channel current between +60 mV and 0 mV in 20K. Taken all together, these observations are consistent with contributions to block of HERG by quinidine from both the single channel conductance and the driving force. Other factors such as the intrinsic voltage dependence of drug block or any single channel rectification at positive voltages may also play a role in determining HERG block.

A number of experiments in our study were carried out with no added potassium, which could introduce the following complications. (1) There is mostly likely some potassium accumulation as evidenced by channel run-up in some 0K experiments before drug addition. However, reversal potential measurements indicate that the reversal potential is significantly more negative with no added potassium compared to all other solutions with higher extracellular potassium used in this study (Fig. 2). In addition, in all experiments, drug-induced HERG blockade in 0K was significantly greater than block with higher extracellular potassium concentrations. (2) It is possible that gating conformational changes in 0K are different compared to 2K and 20K. One study suggested that a slow form of HERG inactivation (in addition to
the well studied fast C-type inactivation) is increased in 0 mM extracellular potassium and thus drug block in 0K might be determined in part by conformational changes during this novel type of inactivation. While the experiments reported here do not address this particular issue, blockade of HERG by quinidine shows a proportional relationship between permeability of the cation and drug block, suggesting that it is the permeant ion that is the major determinant of drug block.

Many patients requiring medication present with abnormal serum electrolyte levels due to a variety of conditions including gastrointestinal dysfunction, renal and endocrine disorders, diuretic use, alcoholism and aging. Given the potentially lethal nature of decreased HERG channel numbers, it is important to understand if such patients will have altered risk for drug induced disarrhythmias. The results of our study suggest that not all drugs will be equally sensitive to changes in the extracellular electrolyte environment. Consistent with this conclusion, recent experiments suggest that HERG block by some drugs show opposite dependency on extracellular potassium. Further studies will be needed to determine the mechanisms behind these differences in potassium sensitivity, thereby allowing for accurate predictions as to which drugs will show increased risks for arrhythmias with altered serum electrolytes.

Materials and Methods

Oocyte isolation and cRNA injection. Oocytes were harvested using standard dissociation techniques. Briefly, oocytes were removed in a Ca2+ free solution and enzymatically defolliculated for about 1 hour with 1.5 mg/ml type 2 collagenase (Worthington Biochemical Corporation, Lakewood, NJ) and then stored in ND96 (ND96—98 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 5 mM Hepes). Between 25–50 ng of Herg cRNA was injected into defolliculated oocytes.

Electrophysiology. Two electrode voltage clamp experiments were performed at room temperature (18–22°C) on HERG expressing oocytes approximately 1–3 days after injection using a Geneclamp 500 (Axon instruments/Molecular Devices, Union City, Ca). Glass electrodes with resistances between 0.5 MΩ and 5 MΩ were filled with 3 M KCl. Data were acquired using PCLAMP software (Molecular Devices, Union City, Ca).

Solutions. All oocytes were initially impaled in ND96 and subsequently one of the solutions listed in Table 1 was perfused into the bath. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO). A potassium buffer was not used and 0K refers to no added potassium. All chemicals used for the 0K solution contain trace amounts of potassium contaminant. The estimated potassium concentration due to contamination is 10 μM, which is lower than the potassium concentration estimated from reversal potential measurements. This suggests that there is most likely some potassium accumulation in 0K due to potassium efflux through open HERG channels. However, reversal potential measurements indicate that the extracellular potassium concentration with no added potassium is significantly less than 2 mM, the second lowest potassium concentration used in the experiments in this paper (Fig. 2). To minimize potassium accumulation potassium-containing solutions were continuously perfused at a rate of 1 ml/min throughout the entire experiment.

Drugs. Quinidine (Sigma-Aldrich, St. Louis, MO) and Cisapride (Research Diagnostics Inc., Concord, MA) were stored frozen at either -20°C or -80°C as a 30 mM (quinidine) or 10 mM (cisapride) stock solution in DMSO and then thawed and diluted into the appropriate electrolyte solution each day just prior to the experiment.

Data collection and analysis. Block of HERG by both quinidine and cisapride was assessed using repetitive pulsing at a frequency of 0.167 Hz. In some experiments a frequency of 0.2 kHz was used. Oocytes were held at -100 mV, and then pulsed to +20 mV for 1 sec, then to -60 mV for 300 msec, then to -130 mV for 750 msec (to close all HERG channels prior to the next pulse). In some experiments the pulse to -130 mV was shortened to 500 msec and in some experiments with cesium the second pulse was to -50 mV instead of -60 mV. Repetitive pulsing of oocytes containing HERG often shows either channel run-up or rundown. To circumvent this difficulty, oocytes were perfused at a rate of 1 ml/min and also continuously pulsed during the entire experiment. Before drug addition, oocytes were pulsed in drug free solution for 5–10 minutes until a steady state pre-drug current level was established. The amount of drug block was assessed using the current at the beginning of the pulse to -60 mV. The fractional drug block was calculated as 1 - (Iss/Ipre), where Is is the steady state current level and Ipre is the current level before addition of drug. In most experiments a 40 msec prepulse to -60 mV prior to the depolarization to +20 mV allowed for leak subtraction. No difference in the amount of block was observed in leak subtracted data versus data that was not leak subtracted and therefore only raw unsubtracted data were used. For all raw data traces shown, capacity transients were blanked for clarity. All data were analyzed using PCLAMP software (Molecular Devices, Union City, Ca).

Table 1. Solutions used to assess HERG block.

| Solution Name | Solution Ingredients                |
|---------------|------------------------------------|
| 0K            | 98 mM NaCl, 0.5 mM CaCl2           |
| 2K            | 96 mM NaCl, 2 mM KCl, 0.5 mM CaCl2 |
| 20K           | 78 mM NaCl, 20 mM KCl, 0.5 mM CaCl2|
| 20Rb          | 78 mM NaCl, 20 mM RbCl, 0.5 mM CaCl2|
| 20Cs          | 78 mM NaCl, 20 mM CaCl2, 0.5 mM CaCl2|
| 40TEA         | 58 mM NaCl, 40 mM TEACl, 0.5 mM CaCl2|
| 0.1Ca         | 96 mM NaCl, 0.2 mM KCl, 0.1 mM CaCl2|
| 10Ca          | 81 mM NaCl, 2 mM KCl, 10 mM CaCl2  |

All solutions contained 5 mM Hepes and were adjusted to pH 7.4 with NaOH.

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