Extracellular Sodium Interacts with the HERG Channel at an Outer Pore Site

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ABSTRACT Most voltage-gated K+ currents are relatively insensitive to extracellular Na+ (Na+o), but Na+o potently inhibits outward human ether-a-go-go-related gene (HERG)–encoded K+ channel current (Numaguchi, H., J.P. Johnson, Jr., C.I. Petersen, and J.R. Balser. 2000. Nat. Neurosci. 3:429–30). We studied wild-type (WT) and mutant HERG currents and used two strategic probes, intracellular Na+ (Na+i) and extracellular Ba2+ (Ba2+o), to define a site where Na+o interacts with HERG. Currents were recorded from transfected Chinese hamster ovary (CHO-K1) cells using the whole-cell voltage clamp technique. Inhibition of WT HERG by Na+o was not strongly dependent on the voltage during activating pulses. Three point mutants in the P-loop region (S624A, S624T, S631A) with intact K+ selectivity and impaired inactivation each had reduced sensitivity to inhibition by Na+o. Quantitatively similar effects of Na+o to inhibit HERG current were seen in the WT and S624A channels. As S624A has impaired Na+i sensitivity, this result suggested that Na+i and Na+o act at different sites. Extracellular Ba2+ (Ba2+o) blocks K+ channel pores, and thereby serves as a useful probe of K+ channel structure. HERG channel inactivation promotes relief of Ba2+ block (Weerapura, M., S. Nattel, M. Courtemanche, D. Doern, N. Ethier, and T. Heberl. 2000. J. Physiol. 526:265–278). We used this feature of HERG inactivation to distinguish between simple allosteric and pore-occluding models of Na+o action. A remote allosteric model predicts that Na+o will speed relief of Ba2+ block by promoting inactivation. Instead, Na+o slowed Ba2+ egress and Ba2+ relieved Na+o inhibition, consistent with Na+o binding to an outer pore site. The apparent affinities of the outer pore for Na+o and K+o as measured by slowing of Ba2+ egress were compatible with competition between the two ions for the channel pore in their physiological concentration ranges. We also examined the role of the HERG closed state in Na+o inhibition. Na+o inhibition was inversely related to pulsing frequency in the WT channel, but not in the pore mutant S624A.

KEY WORDS: human ether-a-go-go-related gene • potassium ion channel • ion channel gating • sodium • barium

INTRODUCTION

The human cardiac delayed rectifier K+ current IK is carried by a channel encoded by the human ether-a-go-go–related gene (HERG), (Sanguinetti et al., 1995). Suppression of IK by loss of function mutations in the HERG gene (Curran et al., 1995; Roden and Balser, 1999) or by untoward drug block (Monahan et al., 1990; Roy et al., 1996; Rampe et al., 1997) can prolong the QT interval and predispose patients to the potentially lethal arrhythmia torsades de pointes. Studies of IK and HERG have begun to illuminate the mechanisms of recognized risk factors for acquired (drug-induced) long QT syndrome, including hypokalemia (Roden, 1998). Lowering the extracellular potassium concentration ([K+]o) decreases the magnitude of IK (Sanguinetti and Jurkiewicz, 1992) and of outward HERG K+ currents (Sanguinetti et al., 1995). Further, raising serum [K+] to the high normal range can normalize QT prolongation in some congenital long QT patients with HERG mutations (Compton et al., 1996) and in patients with acquired QT prolongation (Choy et al., 1997). These effects are “paradoxical” or “anti-Nernstian” in that their direction opposes that predicted by the simple change in electrochemical driving force.

Both IK (Yang et al., 1997) and heterologously expressed HERG current (Smith et al., 1996) demonstrate inward rectification due to rapid, voltage-dependent inactivation. HERG inactivation resembles the C-type inactivation of Shaker-family K+ channels (Hoshi et al., 1991) in its sensitivity to extracellular TEA and to mutations in the P-loop (Schonherr and Heinemann, 1996; Smith et al., 1996; Herzberg et al., 1998; Fan et al., 1999). In Shaker, K+o and other small extracellular cations slow onset of C-type inactivation (Lopez-Barneo et al., 1993). Raising [K+]o may also...
produce an anti-Nernstian effect on Shaker current magnitude (Lopez-Barneo et al., 1993), but only if the frequency of activating pulses is fast enough that recovery from inactivation between pulses is incomplete (Gomez-Lagunas and Armstrong, 1994; Gomez-Lagunas, 1997). This effect is of K\textsuperscript{+}o, quantitatively well-explained by destabilization of the Shaker C-type– inactivated state (Baukrowitz and Yellen, 1995). Raising [K\textsuperscript{+}]o was found to slow HERG inactivation (Wang et al., 1996), and it was initially anticipated that the effect of [K\textsuperscript{+}]o on HERG current magnitude would be explained entirely by a destabilization of the HERG inactivated state. However, a careful quantitative analysis suggested that the change in inactivation would account for only a small fraction of the observed change in current magnitude. A K\textsuperscript{+}o-mediated increase in single-channel conductance was proposed (Wang et al., 1997), consistent with the requirement of very high [K\textsuperscript{+}]o for resolution of HERG single-channel events and dependence of single-channel conductance on [K\textsuperscript{+}]o over the examined (nonphysiological) range (Kiehn et al., 1996, 1999; Zou et al., 1997, 1998).

Recent studies support an alternative model for the mechanism of [K\textsuperscript{+}]o modulation of HERG current, that K\textsuperscript{+}o relieves inhibition of HERG current by Na\textsuperscript{+}o. This kind of model was first suggested by Scamps and Carmeliet (1989) based on work in rabbit single Purkinje cells before the separation of the delayed rectifier cardiac I\textsubscript{Ko} into I\textsubscript{Ko} and I\textsubscript{K1}, the rapidly activating and slowly activating components (Balser et al., 1990; Sanguinetti and Jurkiewicz, 1990). Working with whole-cell current in a heterologous expression system, we found that extracellular Na\textsuperscript{+} (Na\textsuperscript{+}e) potently inhibited outward HERG current (IC\textsubscript{50} = 3 mM) in the absence of K\textsuperscript{+}o, and the magnitude of current augmentation by K\textsuperscript{+}o was markedly reduced in the absence of Na\textsuperscript{+}o. Mole fraction experiments revealed a nonadditive interaction between Na\textsuperscript{+}o and K\textsuperscript{+}o. Although the data did not rule out contributions from direct K\textsuperscript{+}o effects on HERG inactivation and single-channel conductance, the magnitude of this effect in the physiologic range of [K\textsuperscript{+}]o suggested that the primary mechanism of K\textsuperscript{+}o augmentation of HERG current was to relieve inhibition by Na\textsuperscript{+}o (Numaguchi et al., 2000a).

The interaction between Na\textsuperscript{+}o, K\textsuperscript{+}o, and the HERG channel is complex and poorly understood. Recent results from a study focused on antiarrhythmic drug action indicated that [Na\textsuperscript{+}]o affects HERG inactivation gating (Numaguchi et al., 2000b). Here we have focused directly on the interaction between Na\textsuperscript{+}o and HERG, with a view to developing a more precise biophysical understanding of the Na\textsuperscript{+}o effect and thereby illuminating the Na\textsuperscript{+}o–K\textsuperscript{+}o interaction.
used to generate an average trace used for display (figures) and data analysis. In figures, error bars represent SEM, calculated from several cells. P values in the text and figure legends are from independent Student’s t tests. In some cases, one-population t tests were used to compare a normalized statistic to a mean of 0 or 1. No current rundown was observed for as long as 1 h. Na⁺/H⁻ effects could be washed out completely with our solution exchange setup.

Generating Structural Hypotheses Based on the KcsA Crystal Structure

Fig. 13, which illustrates a putative structural effect of the S624A mutation, was created using the KcsA crystal structure coordinates, accession code 1BL8 (Doyle et al., 1998) and Insight II modeling software (Molecular Simulations, Inc.) on a Silicon Graphics O₂ workstation. Putative hydrogen bonds were identified using a heavy-atom distance criterion of <3 Å.

RESULTS

Voltage Dependence of Current Inhibition by Extracellular Sodium: Absence of a Predominant Field Effect

To characterize the voltage dependence of HERG current inhibition by Na⁺/H⁻, we measured the inhibitory potency of Na⁺/H⁻ in the absence of K⁺/H⁻ at several membrane potentials (Fig. 1). 2 s depolarizing steps to a variable test potential were followed by a 2 s step to −50 mV. Channels open and inactivate in response to the depolarizing test step. The step to −50 mV elicits hooked “tail currents” due to fast recovery from inactivation, followed by slower deactivation. We chose to use 3 mM Na⁺/H⁻ because it is near the IC₅₀ for current inhibition at 20 mV (Numaguchi et al., 2000a). Therefore, any effects of membrane potential on inhibition are expected to be most prominent at this concentration, as 3 mM corresponds to the steepest part of the dose-response curve. Fig. 1, A and B, show results from an experiment in a single cell recorded in 0 Na⁺/H⁻ and 3 mM Na⁺/H⁻, respectively. Data summarizing current levels at the end of the 2 s depolarizing test step (Fig. 1, A and B, arrows) are summarized in Fig. 1 C. Current at each test potential was normalized to the value in 0 Na⁺/H⁻ in the same cell. We observed a modest trend toward increasing Na⁺/H⁻ inhibition with greater depolarization (44 ± 3% inhibition of current at 0 mV vs. 55 ± 3% at 40 mV, n = 7, P < 0.05). Notably, this trend was opposite in direction to the expectation for a simple field effect, which would predict greater inhibition at more hyperpolarized potentials. A balance between an inhibition-promoting effect of a depolarization-induced conformational change and an inhibition-opposing field effect could potentially explain the plateau in the voltage-inhibition curve (Fig. 1 C, 40–80 mV). Alternatively, the Na⁺/H⁻ binding site could be located outside the transmembrane field with a small effect of channel conformational state accounting for the observed trend.

HERG P-loop Mutants with Impaired Inactivation Are Relatively Insensitive to Inhibition by Extracellular Sodium

To gain insight into the structural basis and possible conformational-state dependence of HERG’s Na⁺/H⁻ sen-

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**Figure 1.** Effect of test potential on current inhibition by Na⁺/H⁻. Membrane potential was stepped from −80 mV to a test potential between −20 and 80 mV for 2 s, followed by stepoff to −50 mV. (A and B) Representative traces from a paired experiment in which a single cell was moved from 0 Na⁺/H⁻ to 3 mM Na⁺/H⁻ with [K⁺], held at 0. (C) Relationship of test potential to current inhibition by 3 mM Na⁺/H⁻. Current in 3 mM Na⁺/H⁻ at the end of the 2 s depolarizing test step (B, arrow) was normalized to the value in 0 Na⁺/H⁻ (arrow, A) at each test potential for several cells (n = 7). Normalized current differed significantly at only one pair of tested voltages, 0 mV vs. 40 mV (P < 0.05). Note that the observed trend of increasing inhibition at more depolarized test potentials is opposite the expectation for a simple field effect.
Na⁺/H⁺ sensitivity, we studied the Na⁺/H⁺ sensitivity of several HERG mutants. We chose to focus our attention initially on mutations in the pore-lining, P-loop region. Several HERG P-loop mutations completely derange both K⁺ selectivity and inactivation gating (Smith et al., 1996; Herzberg et al., 1998; Fan et al., 1999). Although such effects could be consistent with a mechanism in which Na⁺ preferentially interacts with the HERG inactivated state, the permeability of these mutant channels to Na⁺ limits their utility for studying inhibition of outward HERG K⁺ current by Na⁺. Therefore, we chose to focus on mutant channels that retained K⁺ selectivity in standard solution conditions. The point mutants we studied included S631A, a channel with a well-characterized gating phenotype (Schonherr and Heinemann, 1996; Zou et al., 1998), as well as S624A and S624T, which are not well-characterized. S631A HERG channels retain K⁺ selectivity and have voltage dependence of activation identical to that of WT HERG, but inactivation in S631A is shifted by 100 mV relative to WT (Zou et al., 1998). The S624A mutation was one of several reported to influence methanesulfonanilide block in an alanine-scanning mutagenesis study (Mitcheson et al., 2000a), but its gating has not been studied in detail. To our knowledge, no phenotype of S624T has been described previously.

Fig. 2 compares the responses of WT, S624A, and S624T mutant channels to a standard HERG activation protocol similar to the one shown in Fig. 1. The extracellular solution was 0 Na⁺, 0 K⁺, so the experiment provides a measure of “baseline” channel kinetics independent of external cation concentrations. The morphology of S624A and S624T HERG currents (Fig. 2, B and C, respectively) immediately suggests impaired inactivation compared with WT (Fig. 2 A). Specifically, in the mutants, current at depolarized test potentials is more similar in magnitude to peak tail current than in WT HERG, for which peak tail currents are much larger. The protocol in Fig. 2 does not provide a distinct measurement of inactivation gating, but does allow for comparison of the voltage dependence of activation in mutant and WT channels. Because recovery from inactivation is faster than channel deactivation, peak tail current (Fig. 2, A–C, arrows) provides a measure of the number of activated channels at each test potential. These values were normalized to the peak following the pulse to 100 mV, and results from several cells are summarized in D. The voltage dependence of activation for WT HERG and S624A was indistinguishable in the steep voltage-dependent region, whereas the voltage dependence of activation for S624T was significantly shifted in the positive direction for potentials between −20 mV and 10 mV (P < 0.05 vs. WT).
channels. A strong, extended initial depolarizing step inactivated most channels. Channels then equilibrated between open and inactivated states during a short (12.5-ms) step to the test potential. Finally, the number of open channels was measured immediately after stepping to 30 mV (Fig. 3, A–C, symbols). By this measurement, both S624A and S624T channels have significantly decreased baseline inactivation compared with WT channels over a range of depolarized voltages (Fig. 3 D). Slower onset of inactivation for S624A and S624T was also visible in the representative traces shown (Fig. 3, A–C, compare current decay after final step to 30 mV).

The gating phenotypes of S624A and S624T can be summarized as follows. For both mutants, inactivation was significantly impaired (vs. WT) at potentials greater than or equal to 20 mV (Fig. 3). For S624A, voltage dependence of activation was similar to WT HERG. For S624T, there were significantly fewer activated channels at potentials between 20 and 10 mV (Fig. 2).

Overall, for potentials of 20 mV and higher, S624A and S624T have significantly impaired inactivation compared with WT, but are not significantly different from WT with respect to activation.

Having characterized the gating phenotype of S624A and S624T, we next studied the sensitivity of these channels and S631A channels to inhibition by Na\(^+\)o (Fig. 4). We compared currents at 20 mV in several [Na\(^+\)]o, all in the absence of K\(^+\)o. Representative traces for WT HERG, S624A, S624T, and S631A are shown in Fig. 4, A–D, respectively. Note the large outward tail currents in S624A (Fig. 4 B) and S624T (Fig. 4 C) present at 50 mV even in very high (146 mM) [Na\(^+\)]o. This feature is indicative not only of reduced sensitivity of these mutant channels to inhibition by Na\(^+\)o, but also of their high K\(^+\) selectivity. For each record in Na\(^+\)o, current at the end of the 2-s step was normalized to the value from the same cell in 0 Na\(^+\)o. Data from several cells are summarized in Fig. 4 E. The three mutant channels each demonstrated significantly reduced sensitivity to inhibition by Na\(^+\)o versus WT at all concentrations studied. Interestingly, the dose-response relationships for the three mutant channels are quite similar, and for S624A and S624T the relationships appear to reach a plateau far short of complete inhibition (Fig. 4 E). This result is not easily explained, but we offer a possible interpretation in the discussion, informed by data presented later in the paper.

The sensitivity of Na\(^+\)o inhibition to P-loop mutations (Fig. 4) would be expected if Na\(^+\)o bound to a site in the HERG pore. However, the lack of a predominant field effect on Na\(^+\)o inhibition (Fig. 1) and the correlation of potent Na\(^+\)o inhibition with an intact inactivation mechanism (Fig. 4) are also potentially consistent...
Na\textsuperscript{+} interaction with HERG probed with Na\textsuperscript{+} and Ba\textsuperscript{2+}

with Na\textsuperscript{+}o acting allosterically to stabilize the inactivated state at a site remote from the pore. Further, it is also conceivable that Na\textsuperscript{+}o could stabilize the inactivated state by binding in the pore. We chose to utilize intracellular Na\textsuperscript{+}o (Na\textsuperscript{+}i) and extracellular barium (Ba\textsuperscript{2+}o) as probes to begin to define the location of a binding site for Na\textsuperscript{+}o. We considered three possibilities for the site of Na\textsuperscript{+}o action: inner pore, outer pore, and remote from the pore.

**Intracellular Sodium Inhibits HERG Current in a Manner Consistent with Voltage-dependent Block and Independent of Extracellular Sodium**

The action of intracellular Na\textsuperscript{+}o (Na\textsuperscript{+}i) to block voltage-gated K\textsuperscript{+} currents is well described (Bezanilla and Armstrong, 1972; French and Wells, 1977; Yellen, 1984a,b) and, in light of the KcsA crystal structure (Doyle et al., 1998), interpreted to occur in a large intracellular cavity (Heginbotham et al., 1999; Zhou et al., 2001). A macroscopic Na\textsuperscript{+}i block with features in common with previous descriptions of Na\textsuperscript{+}i block (Bezanilla and Armstrong, 1972; French and Wells, 1977; Yellen, 1984a,b), including antagonism by K\textsuperscript{+}o, was reported recently for the I\textsubscript{Ks} (KCNQ1/KCNE1) channel (Pusch et al., 2001).

We have suggested several nonexclusive possibilities for the mechanism of Na\textsuperscript{+}o inhibition of outward HERG K\textsuperscript{+} current. As a prerequisite to limiting the number of possible models, we considered approaches that could potentially identify the region of the channel where Na\textsuperscript{+}o acts. Reasoning that observation of similar effects of Na\textsuperscript{+}i and Na\textsuperscript{+}o on the HERG channel would implicate the pore, we examined the impact of Na\textsuperscript{+}o on HERG channel function.

Fig. 5, A and B, show the basic effect of adding 10 mM Na\textsuperscript{+}o to the standard (high-K\textsuperscript{+}o) pipette solution on WT HERG currents. Results from several cells, quantified as the ratio of current measured at a depolarized potential to the peak tail current, are summarized in Fig. 5 C. Addition of 10 mM Na\textsuperscript{+}o to the pipette solution significantly reduced this ratio compared with the standard internal solution (at 20 mV, 0.39 ± 0.04 vs. 0.14 ± 0.01 for 0 and 10 mM Na\textsuperscript{+}i, respectively, P < 0.01), consistent with current inhibition at depolarized potentials.

The change in magnitude of current at depolarized potentials when 10 mM Na\textsuperscript{+}o was included (Fig. 5 D) closely resembled the change in current ratio with Na\textsuperscript{+}i (Fig. 5 C). This suggested that the change in ratio primarily reflected current inhibition by Na\textsuperscript{+}o at depolarized potentials. We next tested the effect of Na\textsuperscript{+}o on the magnitude of current augmentation by K\textsuperscript{+}o (Fig. 6).
Given the ability of K⁺o to relieve current inhibition by Na⁺o (Numaguchi et al., 2000a), a simple model in which Na⁺i acts by the same mechanism as Na⁺o predicts that the magnitude of current augmentation by K⁺o will be greater in the presence of Na⁺i. Notably, K⁺o has also been reported to relieve Na⁺i inhibition in other voltage-gated K⁺ channels (Bezanilla and Armstrong, 1972; Yellen, 1984a,b; Pusch et al., 2001). Fig. 6, A and B, show results from representative cells expressing WT HERG in 0 Na⁺i and 10 mM Na⁺i, respectively. Maximum current augmentation by K⁺o was more than sixfold in the presence of 10 mM Na⁺i and approximately twofold with the standard (0 Na⁺i) internal solution (Fig. 6 C). The significant reduction in current magnitude in cells with 10 mM Na⁺i in the absence of K⁺o (see Fig. 5 D, also compare traces in Fig. 6, A and B) suggests that the data should be interpreted as relief of Na⁺i block by K⁺o rather than enhancement of a K⁺o effect by Na⁺i. Further, the change shown in Fig. 5 D is a 2–3-fold reduction in current magnitude in the presence of 10 mM Na⁺i, which corresponds to the threecold increase in the magnitude of the K⁺o augmentation effect when 10 mM Na⁺i is present (Fig. 6 C). The observation that physiologically relevant [Na⁺i] inhibited HERG current in a manner sensitive to physiologically relevant [K⁺i], (note low [K⁺i] region of graph, Fig. 6 C) is interesting and will be revisited in the discussion.

Using current augmentation by K⁺o as an index of sensitivity to inhibition by Na⁺o, we also tested the effect of Na⁺i on the S624A channel (Fig. 6 C). A simple model in which Na⁺i and Na⁺o both act at an inner pore site that predicts that S624A channels, shown to have decreased sensitivity to inhibition by Na⁺o (Fig. 4, B and E), will also have reduced sensitivity to inhibition by Na⁺i. Instead, S624A and WT channels had very similar sensitivity to Na⁺i, as inferred from K⁺o augmentation (Fig. 6 C). Further, the small difference may be attributable to an intrinsic difference in K⁺o sensitivity independent of Na⁺i (see 25 mM K⁺o in the absence of Na⁺o, Fig. 6 C). This result suggests that Na⁺i and Na⁺o act by fundamentally different mechanisms. We also measured the effect of Na⁺i on the potency of current inhibition by Na⁺o. Na⁺i inhibition appeared unaffected by 10 mM Na⁺i (Fig. 6 D). This result is also consistent with different mechanisms for Na⁺o and Na⁺i. It is theoretically possible that Na⁺o and Na⁺i could act at the same (internal) site with inactivation serving as a rate-limiting determinant of Na⁺i access to the site. However, given the similar Na⁺i effect in S624A and WT HERG (Fig. 6 C), this interpretation requires that the S624A mutation impair inactivation without changing the structure of the relevant pore site. Further, this interpretation also requires that a 10–15% difference in steady-state inactivation (see Fig. 3 D) be translatable to a difference of up to 50% in fractional site occupancy (see Fig. 4 E).
Overall, the features of the Na\textsuperscript{+} inhibition of HERG, including sensitivity to K\textsuperscript{+}, are broadly consistent with the kind of voltage-dependent block by Na\textsuperscript{+}, described for other K\textsuperscript{+} channels (Bezanilla and Armstrong, 1972; French and Wells, 1977; Yellen, 1984a,b; Pusch et al., 2001) and interpreted to occur in the cavity (Heginbotham et al., 1999; Zhou et al., 2001). Moreover, Na\textsuperscript{+} and Na\textsuperscript{+} appear to act by distinct mechanisms. Thus, Na\textsuperscript{+} is unlikely to act by reaching the inner pore or cavity of HERG. These experiments using Na\textsuperscript{+} as a probe do not shed light on the question of whether Na\textsuperscript{+} reaches the outer pore or acts at a remote site. Therefore, we employed an alternative probe, Ba\textsuperscript{2+}, to further investigate the location of the Na\textsuperscript{+} receptor.

Barium Attenuates Current Inhibition by Extracellular Sodium, and Extracellular Sodium Slows Barium Egress from the Channel

Barium (Ba\textsuperscript{2+}) was used as a probe in a pair of elegant studies (Neyton and Miller, 1988a,b) that predicted the K\textsuperscript{+} channel pore structure (Doyle et al., 1998; Jiang and MacKinnon, 2000; Morais-Cabral et al., 2001; Zhou et al., 2001) based on functional data. Extracellular barium (Ba\textsuperscript{2+}) block of HERG exhibits strong voltage dependence consistent with a binding site deep in the pore (Ho et al., 1999; Weerapura et al., 2000), similar to the site now confirmed crystallographically for KcsA (Jiang and MacKinnon, 2000). Classical C-type inactivation traps Ba\textsuperscript{2+} in the channel (Basso et al., 1998; Harris et al., 1998), whereas HERG channel inactivation relieves Ba\textsuperscript{2+} block with kinetics that are measurable (Weerapura et al., 2000). Here, we utilize this unusual interaction between Ba\textsuperscript{2+} and HERG inactivation to distinguish between a model in which Na\textsuperscript{+} blocks the HERG pore and a simple remote allosteric model in which Na\textsuperscript{+} stabilizes the inactivated state. Our experimental design was a macroscopic version of an approach first employed by Neyton and Miller (1988b) in single channel measurements of BK channels. This approach is to measure the effects of external cations on the rate of Ba\textsuperscript{2+} egress at depolarized potentials. For a classical C-type--inactivating channel, the interpretation of a result in which Na\textsuperscript{+} slows Ba\textsuperscript{2+} egress would be problematic because of the potential trapping of Ba\textsuperscript{2+} by C-type inactivation. For HERG, however, a result in which Na\textsuperscript{+} slows Ba\textsuperscript{2+} egress would be informative, and would strongly support a pore-occluding model for current inhibition. The prediction of the remote allosteric model is that Na\textsuperscript{+} will speed relief of Ba\textsuperscript{2+} block by promoting HERG channel inactivation. Notably, a speeding of Ba\textsuperscript{2+} egress could also be consistent with a pore-occluding model. For example, Na\textsuperscript{+} binding in...
the pore could destabilize Ba\(^{2+}\) via a repulsive mechanism. If the off-rate for Na\(^+\) were faster than the Ba\(^{2+}\) off-rate, then such a mechanism might explain Na\(^+\)\(_o\) inhibition of HERG. Cells were stepped from a holding potential of −80 to 20 mV for 2 s, followed by a 2 s step to −50 mV. [K\(^+\)], was held at 0 in these experiments. (A) Representative WT currents from a cell washed through four consecutive solutions as shown. Dotted arrow indicates fast tail current decay in presence of Ba\(^{2+}\), reflecting Ba\(^{2+}\) loading at hyperpolarized potentials. (B) currents from A at 20 mV scaled to show the rate of current rise. Arrows indicate the \(t_{1/2}\) (time to half-maximal current value). The cartoon underneath the scaled traces illustrates the simplest model that can explain the data. In this model, Na\(^+\) inhibits Ba\(^{2+}\) egress by occupying a site external to the Ba\(^{2+}\) site (explaining the effect on normalized \(t_{1/2}\) and Ba\(^{2+}\) clears Na\(^+\)\(_o\) as it exits the pore.

Ba\(^{2+}\) would sweep Na\(^+\)\(_o\) from the pore as it leaves inactivated channels. The remote allosteric model, by contrast, does not make a straightforward prediction as to the effect of combining Ba\(^{2+}\) and Na\(^+\)\(_o\) on current magnitude.
Figure 8. Effects of combining Ba$^{2+}$ and Na$^{+}$ in HERG S624A. Cells were stepped from a holding potential of −80 to 20 mV for 2 s, followed by a 2s step to −50 mV. [K$^{+}$]o was held at 0 in these experiments. (A) Representative S624A currents from a cell washed through four consecutive solutions as shown. (B) Currents from A at 20 mV scaled to show the rate of current rise. Arrows indicate the $t_{1/2}$ (time to half-maximal current value). (C) For several cells as in A ($n = 5$), current at the end of the 2s depolarizing step (A, arrows) was normalized to the value in 0 Na$^{+}$o, 0 Ba$^{2+}$o. Normalized current for S624A in 2 mM Ba$^{2+}$o was significantly smaller than the value for WT in 2 mM Ba$^{2+}$o (compare with Fig. 7 C, P < 0.01). Normalized S624A current in 100 mM Na$^{+}$o + 2 mM Ba$^{2+}$o was significantly smaller than the S624A values in 100 mM Na$^{+}$o alone (P = 0.05) and 2 mM Ba$^{2+}$o alone (P < 0.01). (D) For several cells as in B ($n = 5$), $\Delta t_{1/2}$ values were calculated by subtracting the $t_{1/2}$ in 0 Na$^{+}$o from the $t_{1/2}$ in 100 mM Na$^{+}$o. The calculation was made for 0 and 2 mM Ba$^{2+}$o, and results were compared. $\Delta t_{1/2}$ was greater than 0 for both 0 Ba$^{2+}$o and 2 mM Ba$^{2+}$o (P < 0.01 in both cases), but the values in 2 mM Ba$^{2+}$o and 0 Ba$^{2+}$o were indistinguishable from one another (P = 0.55).

The fast tail current decay in the presence of Ba$^{2+}$ (dotted arrow, Fig. 7 A) reflects this loading.

In the first experiment with Ba$^{2+}$/o, individual cells were recorded in four different external solution conditions (all 0 K$^{+}$/o): (a) 0 Na$^{+}$/o, 0 Ba$^{2+}$/o, (b) 100 mM Na$^{+}$/o, 0 Ba$^{2+}$/o, (c) 0 Na$^{+}$/o, 2 mM Ba$^{2+}$/o, (d) 100 mM Na$^{+}$/o, 2 mM Ba$^{2+}$/o. Two major effects of combining Na$^{+}$/o and Ba$^{2+}$/o were observed: (a) Ba$^{2+}$/o attenuates Na$^{+}$/o inhibition (Fig. 7, A and C), and (b) Na$^{+}$/o slows Ba$^{2+}$/o egress as reflected in a delay in the current rise time (Fig. 7, B and D). Fig. 7, A and B, show raw current records from a representative cell. Records are scaled in Fig. 7 B to accentuate the similarities and differences in rate of current rise.

Although 2 mM Ba$^{2+}$/o alone caused a small reduction in the current at the end of a 2s pulse compared with baseline (0 Na$^{+}$/o, 0 Ba$^{2+}$/o), current in 2 mM Ba$^{2+}$/o + 100 mM Na$^{+}$/o was significantly greater than current in 100 mM Na$^{+}$/o alone (Fig. 7 C). This effect of Ba$^{2+}$/o to relieve Na$^{+}$/o inhibition was dramatic, yielding approximately threefold more current than was seen for Na$^{+}$/o alone (mean I$_{norm} = 0.48 \pm 0.04$ vs. 0.15 ± 0.02 for 100 mM Na$^{+}$/o alone).

Because many current records did not reach steady-state by the end of a 2s pulse, we quantified the rate of current rise (which we interpret to reflect the rate of Ba$^{2+}$/o egress) using a $t_{1/2}$ measurement (the time necessary for current to reach half of its end level, see Fig. 7 B, arrows). 100 mM Na$^{+}$/o alone did not affect the $t_{1/2}$ measurement (compared with 0 Na$^{+}$/o, 0 Ba$^{2+}$/o), but 100 mM Na$^{+}$/o + 2 mM Ba$^{2+}$/o did significantly increase the $t_{1/2}$ compared with 2 mM Ba$^{2+}$/o alone (Fig. 7 D). The results of these experiments, then, indicated that Na$^{+}$/o and Ba$^{2+}$/o effects are not functionally independent. The data suggested a model in which Na$^{+}$/o inhibits Ba$^{2+}$/o egress by occupying a site external to the Ba$^{2+}$/o site (explaining the effect on $t_{1/2}$) and Ba$^{2+}$/o clears Na$^{+}$/o as it exits the pore (explaining the relief of Na$^{+}$/o inhibition by Ba$^{2+}$/o). This model is illustrated in the cartoon shown under the scaled currents in Fig. 7 B.

Barium Relief of Current Inhibition by Extracellular Sodium Is Absent in the Inactivation-impaired Mutant S624A

We performed Ba$^{2+}$/o-Na$^{+}$/o combination experiments similar to those described for WT in the point mutant S624A, which is impaired in both inactivation (Fig. 3 D) and Na$^{+}$/o sensitivity (Fig. 4 E). Fig. 8, A and B, show raw and scaled current traces, respectively, and Fig. 8, C and D, summarize current magnitude and $t_{1/2}$ data, respectively. The model of Ba$^{2+}$/o action proposed by Weerapura et al. (2000), who studied effects of Ba$^{2+}$/o on the inactivation-impaired mutant S631A, predicts...
current at the end of the 2s depolarizing step was measured. Note that in B, current increased after Ba2+ of 2 mM Ba2+ in WT HERG (compare Figs. 8 C and 7 C, 2 mM Ba2+). In contrast to WT (Fig. 7 D), adding 100 mM Na+ served in the absence and presence of Na+/H11001 and Na+ or 2 mM Ba2+. For experiments in which Na+ was washed in, peak tail current was measured. For experiments in which Na+ was washed in, the time necessary for current level to equilibrate to new solution conditions was much greater for condition B.

Figure 9. Na+ slows onset of Ba2+ effect, but Ba2+ does not slow onset of Na+ effect. Cells were held at –80 mV and stepped to 20 mV for 2 s followed by a 2-s step to –50 mV. Current was first recorded in one solution (τ = –90 s), and the cell was then held at –80 while the solution was exchanged, adding either 100 mM Na+ or 2 mM Ba2+. At t = 0, cells were repeatedly pulsed for 10 min. Representative cells for each of four solution permutations are shown in A–D. For each solution permutation, similar results were obtained in four different cells. (A) Adding Ba2+ without Na+. (B) Adding Ba2+ with Na+. (C) Adding Na+ without Ba2+. (D) Adding Na+ with Ba2+. For experiments in which Ba2+ was washed in, peak tail current was measured. For experiments in which Na+ was washed in, the Na+ inhibition by Ba2+, observed in Fig. 7. The time necessary for current level to equilibrate to new solution conditions was much greater for condition B.

that Ba2+ unblock will be impaired in any inactivation-impaired mutant. We found this prediction to be fulfilled for S624A; sustained Ba2+ block of this inactivation-impaired channel was greater than that observed in WT HERG (compare Figs. 8 C and 7 C, 2 mM Ba2+). Further, in S624A, Ba2+ and Na+ together yielded a degree of current inhibition greater than that seen with either cation alone (Fig. 8, A and C), unlike the relief of Na+ inhibition seen in WT (Fig. 8 C). That is, Ba2+ attenuation of Na+ inhibition was absent in the inactivation-impaired mutant S624A, consistent with the idea that strong relief of Ba2+ block (due to intact inactivation) is of central importance to the attenuation phenomenon.

Given the decreased sensitivity of S624A to inhibition by Na+, a model in which Na+ binds at an external site where it opposes Ba2+ egress predicts a reduced effect of Na+ on Ba2+ egress in S624A relative to WT HERG. That is, Na+ should evoke less slowing of the HERG current rise time in 2 mM Ba2+ in the mutant compared with WT HERG. Interpretation of the S624A data is clouded by the fact that 100 mM Na+ in the absence of Ba2+ increases the t1/2 (Fig. 8, B and D), a phenomenon not observed for WT HERG. However, in contrast to WT (Fig. 7 D), adding 100 mM Na+ on top of 2 mM Ba2+ increases the t1/2 in a manner indistinguishable from adding 100 mM Na+ alone (Fig. 8 D, Δt1/2 = 228 ± 12 ms vs. 252 ± 37 ms, respectively, P = 0.55). Thus, the t1/2 data for S624A are also consistent with a model in which Na+ inhibits current at an external site where it opposes Ba2+ egress.

Sodium Slows Onset of Barium Effect, but Barium Fails to Slow Onset of the Sodium Effect

The model described above postulates simultaneous occupancy of discrete sites for Na+ and Ba2+ with Na+ external to Ba2+. The relief of Na+ inhibition of WT HERG by Ba2+ (Fig. 7, A and C) could conceivably be explained by competition of the two cations for a common site. The lack of a predominant field effect in Na+ inhibition (Fig. 1) argues against a common site, but it is possible that a field effect could be masked by inactivation favoring Na+ inhibition (see earlier discussion of Fig. 1). The model with discrete sites predicts that Na+ should protect channels from the onset of Ba2+ effects, whereas Ba2+ should be relatively ineffective at protecting channels from Na+ (Fig. 9, A and B, show the results from representative experiments in which the time course of onset of Ba2+ effects was observed in the absence and presence of Na+, respectively. Because Ba2+ block is most prominent at hyperpolarized potentials, peak tail currents were used to
quantify the time course for each cell. Cells were pre-equilibrated to 0 Na\textsuperscript{+} or 100 mM Na\textsuperscript{+} solutions before a 90-s period in which 2 mM Ba\textsuperscript{2+} was washed in, and then pulsed repeatedly to 20 mV for 10 min. Upon exposure to Ba\textsuperscript{2+}, peak tail currents decreased in the absence of Na\textsuperscript{+} (Fig. 9 A) but increased in the presence of Na\textsuperscript{+} (Fig. 9 B), consistent with the previously described relief of Na\textsuperscript{+} inhibition by Ba\textsuperscript{2+} (Fig. 7, A and C). Currents were much slower to equilibrate to Ba\textsuperscript{2+} in the presence of Na\textsuperscript{+} (Fig. 9 B). In complementary experiments (Fig. 9, C and D), the time course of onset of Na\textsuperscript{+} effects was observed in the presence and absence of Ba\textsuperscript{2+}. Because Na\textsuperscript{+} effects in the presence of Ba\textsuperscript{2+} are most prominent at depolarized potentials, currents at the end of the 2-s depolarizing pulse were used to quantify the time course for each cell. Onset of Na\textsuperscript{+} effects occurred quickly both in the absence (Fig. 9 C) and the presence (Fig. 9 D) of Ba\textsuperscript{2+}. The results are consistent with a model of the HERG pore that includes two discrete sites, a more internal Ba\textsuperscript{2+} site and a more external site for Na\textsuperscript{+}.

**Apparent Affinities of the HERG Outer Pore for Sodium and Potassium Differ by 30–50-fold**

Neyton and Miller (1988b) first applied the method of monitoring Ba\textsuperscript{2+} off-rate to determine outer pore affinities of BK channels for K\textsuperscript{+}. Our studies of the Na\textsuperscript{+} interaction with HERG began with attempts to understand the anti-Nernstian effect of K\textsuperscript{+} and suggested that K\textsuperscript{+} acts to potently relieve inhibition of outward HERG K\textsuperscript{+} current by Na\textsuperscript{+} (Numaguchi et al., 2000a). We performed dose-response experiments with Na\textsuperscript{+} (Fig. 10) and K\textsuperscript{+} (Fig. 11) in the presence of 2 mM Ba\textsuperscript{2+} to define the relative sensitivities of Ba\textsuperscript{2+} egress to the two cations. These sensitivities are most parsimoniously interpreted as relative affinities of Na\textsuperscript{+} and K\textsuperscript{+} for the HERG outer pore. Panels A and B of these figures show raw and scaled traces, respectively, while panels C and D summarize data with respect to current magnitude and t\textsubscript{1/2}. 30 mM Na\textsuperscript{+} or more was required to significantly increase t\textsubscript{1/2} (Fig. 10 D). Current magnitude declined in parallel with increasing t\textsubscript{1/2} (Fig. 10 C). Lower [K\textsuperscript{+}], was required to increase t\textsubscript{1/2} (Fig. 11 D). A large increase in t\textsubscript{1/2} was observed between 1 and 5 mM, which corresponds roughly to the physiologic range of serum K\textsuperscript{+}. In the absence of Ba\textsuperscript{2+}, 5 mM K\textsuperscript{+} caused only a modest increase in t\textsubscript{1/2} (54 ± 12 ms vs. 231 ± 12 ms for 5 mM K\textsuperscript{+} with 2 mM Ba\textsuperscript{2+}, P < 0.01), indicating that the K\textsuperscript{+} effect on t\textsubscript{1/2} like the Na\textsuperscript{+} effect (Fig. 7), reflects slowing of Ba\textsuperscript{2+} egress. Current magnitude was largely unaffected by [K\textsuperscript{+}], in the presence of 2 mM Ba\textsuperscript{2+} (Fig. 11 C), despite the reduction in driving force as [K\textsuperscript{+}] was raised.
The capacity for precise quantitative analysis of Ba$^{2+}$ off-rates and site affinities for Na$^{+}$o and K$^{+}$o is limited by our making population measurements, by the relatively slow speed of HERG channel activation, and by the fact that a Ba$^{2+}$ ion occupies the channel pore at a site near the Na$^{+}$o/K$^{+}$o site. Still, by assuming that equal fractional occupancy of an outer pore site leads to equal change in $t_{1/2}$ in the presence of Ba$^{2+}$, we can make an estimate of the relative site affinities. The effect of 100 mM Na$^{+}$o ($\Delta t_{1/2} = 154 \pm 21$ ms) falls between those of 2 mM K$^{+}$o (130 ± 8 ms) and 3.5 mM K$^{+}$o (173 ± 7 ms). Overall, then, the data suggest a difference of 30–50-fold in the affinities of the HERG outer pore for the two cations.

Extended Time at the Holding Potential Promotes Current Inhibition by Extracellular Sodium in WT HERG, but not in the Mutant S624A

The localization of Na$^{+}$o to the HERG outer pore (Figs. 7 and 9) and the low apparent K$^{+}$ affinity of the HERG outer pore (Fig. 11), together with our desire to understand the plateau in the Na$^{+}$o dose-response curve for S624A and S624T (Fig. 4 E), prompted us to reconsider the role of the closed state in Na$^{+}$o inhibition. To quickly deactivation channels after an activating pulse, we modified our pulse-train protocol to include only an activating pulse to 20 mV and the holding potential of −80 mV (Fig. 12, inset). Steady-state block of WT current by 1 mM Na$^{+}$ at the end of 2-s steps to 20 mV was compared for several intervals at −80 mV (Fig. 12, A–C). Block was significantly greater for longer intervals (P < 0.05 for 2 s vs. each longer interval, Fig. 12 C) and the effect of closed time appears to be nearing saturation at 16 s. For experiments with S624A, 20 mM Na$^{+}$o was used in order to start at a level of fractional block similar to that observed with 1 mM for the WT channel. In contrast to WT HERG, no effect of pulsing frequency on Na$^{+}$o inhibition was observed for the mutant S624A (P > 0.18 for 2 s vs. each longer interval, Fig. 12, D–F). Models with the potential to explain these data are considered in the discussion section.

**DISCUSSION**

**Sodium Permeation in Other Potassium Channels**

Na$^{+}$o can block inward rectifier (K$_{ir}$) channels (Owen et al., 1999), and has been shown to block Ca$^{2+}$ influx through the ciliary P2X receptor (Ma et al., 1999). However, Na$^{+}$o does not generally block voltage-gated K$^{+}$ channels (Hille, 2001). Intracellular Na$^{+}$ (Na$^{+}$i) block of voltage-gated K$^{+}$ channels, by contrast, is well-described (Bezanilla and Armstrong, 1972; French and Wells, 1977; Yellen, 1984a,b; Pusch et al., 2001).
Indeed, sidedness of Na\(^+\) block was one of the criteria used to determine the absolute orientation of the bacterial channel KcsA in lipid bilayers (Heginbotham et al., 1999) and the membrane topology of the K\(^+\)-selective prokaryotic glutamate receptor GluR0 (Chen et al., 1999). Still, several studies have shown that voltage-gated K\(^+\) channels can interact with Na\(^+\) (Lopez-Barneo et al., 1993) and even allow Na\(^+\) permeation in permissive (low K\(^+\)) conditions (Callahan and Korn, 1994; Korn and Ikeda, 1995; Starkus et al., 1997, 1998, 2000; Kiss et al., 1998, 1999; Ogielska and Aldrich, 1998, 1999; Wang et al., 2000a,b). Further, this Na\(^+\) permeation is often most prominent in the C-type–inactivated state. Systematic variation of internal and external cation concentrations in Shaker has led to the following reinterpretation of the mechanism of C-type inactivation: C-type–inactivated channels are nonconducting in physiological conditions because a conformational change renders an outer pore site relatively Na\(^+\) selective, whereas an inner site remains potently blocked by K\(^+\) (Starkus et al., 1997, 1998). Shaker C-type inactivation lacks intrinsic voltage dependence in standard solution conditions (Hoshi et al., 1991), but intrinsic voltage dependence can appear under conditions of balanced competition between Na\(^+\) and K\(^+\). This apparent voltage dependence of inactivation may arise from the voltage-dependent changes in relative K\(^+\) and Na\(^+\) permeabilities now thought to define the C-type inactivation process (Starkus et al., 2000).

The P-loop region, which mediates ion selectivity (Heginbotham et al., 1994) and classical C-type inactivation (Liu et al., 1996) in K\(^+\) channels, has also been implicated in HERG’s rapid, voltage-dependent inactivation process (Schonherr and Heinemann, 1996; Smith et al., 1996; Herzberg et al., 1998; Zou et al., 1998; Fan et al., 1999). Therefore, the studies in Shaker suggested that a fruitful approach to understanding both Na\(^+\) effects on HERG and the HERG inactivation gating mechanism would be to lower or remove K\(^+\) in the experimental system, record Na\(^+\) currents through HERG channels, and interrogate the relationship between Na\(^+\) permeation and inactivation gating. There is at least one published report of drug-induced Na\(^+\) permeability of HERG channels in oocytes (Ulens et al., 1999). In our studies, conducted in CHO cells and in the absence of drugs, Na\(^+\) currents through HERG could not be detected despite attempts with several internal/external solution combinations in WT and S624A (unpublished data).

Figure 12. Effect of pulsing frequency on Na\(^+\) inhibition of WT HERG and S624A. Cells were stepped from a holding potential of −80 to 20 mV for 2 s, followed by an immediate step back to the holding potential of −80 mV. [K\(^+\)]\(_o\) was held at 0 in these experiments. The interval at −80 mV was varied between 2 and 16 s. A and B show representative WT HERG cells recorded while pulsing with 4- and 8-s intervals at −80 mV, respectively. Cells were first recorded in 0 Na\(^+\)\(_o\) and subsequently in 1 mM Na\(^+\)\(_o\). Current in 1 mM Na\(^+\)\(_o\) at the end of the 2-s depolarizing step (A and B, arrows) was normalized to the value in 0 Na\(^+\)\(_o\). Data from several experiments (n = 4–6 for each interval) are summarized in C. For WT HERG, normalized current was significantly smaller for longer intervals at −80 mV (P < 0.05 for 2 s vs. each longer interval). D and E show representative S624A cells recorded while pulsing with 4- and 8-s intervals at −80 mV, respectively. Cells were first recorded in 0 Na\(^+\)\(_o\) and subsequently in 20 mM Na\(^+\)\(_o\), to give a level of fractional block similar to that tested in WT (A–C). Current in 20 mM Na\(^+\)\(_o\) at the end of the 2-s depolarizing step (D and E, arrows) was normalized to the value in 0 Na\(^+\)\(_o\). Data from several experiments (n = 4–6 for each interval) are summarized in F. For S624A, there was no significant difference in normalized current as the interval at −80 mV was increased (P > 0.18 for 2 s vs. each longer interval).
Extracellular Sodium Interacts with the HERG Channel at an Outer Pore Site

In this study, Na\textsuperscript{+}\textsubscript{o} and Ba\textsuperscript{2+}\textsubscript{o} were used as probes to narrow the field of possible locations for Na\textsuperscript{+}\textsubscript{i} interaction with the HERG channel. Observation of similar effects of Na\textsuperscript{+}\textsubscript{o} and Na\textsuperscript{+}\textsubscript{i} would implicate the channel pore. We found that physiologic [Na\textsuperscript{+}]\textsubscript{i}, inhibited WT HERG current in a voltage-dependent manner similar to the inner cavity block described for other voltage-gated K\textsuperscript{+} channels (Bezanilla and Armstrong, 1972; French and Wells, 1977; Yellen, 1984a,b; Pusch et al., 2001). Further, Na\textsuperscript{+}\textsubscript{i} effects on WT and S624A HERG channels were similar, and the presence of Na\textsuperscript{+}\textsubscript{i} did not affect Na\textsuperscript{+}\textsubscript{o} inhibition in the WT channel. These results suggested that Na\textsuperscript{+}\textsubscript{o} was unlikely to act by binding in the inner pore or by permeating the channel in an occlpent manner to reach the inner cavity. The results with Na\textsuperscript{+}\textsubscript{i} did not directly address the question of whether Na\textsuperscript{+}\textsubscript{o} acts by a remote allosteric mechanism or by occluding the outer pore.

Three HERG point mutants (S624A, S624T, S631A) with impaired inactivation had reduced sensitivity to inhibition by Na\textsuperscript{+}\textsubscript{o} (Fig. 4). This result suggested that the HERG-inactivated state might be important to the mechanism by which Na\textsuperscript{+}\textsubscript{o} inhibits current. There is precedent for an external cation (Ca\textsuperscript{2+}\textsubscript{o}) to inhibit HERG current via an allosteric mechanism by binding to a site remote from the pore (Johnson et al., 1999, 2001). We used Ba\textsuperscript{2+}\textsubscript{o} as a probe to distinguish between two simple models for Na\textsuperscript{+}\textsubscript{o} inhibition of HERG current, remote allosteric stabilization of the inactivated state versus outer pore occlusion. Ba\textsuperscript{2+}\textsubscript{o} block is relieved by HERG inactivation (Weerapura et al., 2000), a feature critical to the interpretation of our experiments combining Ba\textsuperscript{2+}\textsubscript{o} and Na\textsuperscript{+}\textsubscript{o}. A simple remote allosteric model in which Na\textsuperscript{+}\textsubscript{o} stabilizes the HERG-inactivated state predicts that Na\textsuperscript{+}\textsubscript{o} will speed Ba\textsuperscript{2+}\textsubscript{o} egress from the channel. Instead, Na\textsuperscript{+}\textsubscript{o} slowed Ba\textsuperscript{2+}\textsubscript{o} egress from HERG as inferred from the rate of current rise (Fig. 7, B and D). Further, Ba\textsuperscript{2+}\textsubscript{o} was found to relieve Na\textsuperscript{+}\textsubscript{o} inhibition of HERG (Fig. 7, A and C), and Na\textsuperscript{+}\textsubscript{o} was able to protect against onset of Ba\textsuperscript{2+}\textsubscript{o} effects (Fig. 9). These data are most consistent with discrete sites for Na\textsuperscript{+}\textsubscript{o} and Ba\textsuperscript{2+}\textsubscript{o} in the outer and inner HERG pore, respectively. The data suggest that Ba\textsuperscript{2+} sweeps Na\textsuperscript{+}\textsubscript{o} from the pore as it exits inactivated HERG channels (Fig. 7 B, cartoon).

Competition between Extracellular Sodium and Potassium for the HERG Channel Pore

Because K\textsuperscript{+}\textsubscript{o} and Na\textsuperscript{+}\textsubscript{o} interact with the HERG channel in a nonadditive manner (Numaguchi et al., 2000a), one attractive hypothesis is that the two cations compete for the HERG outer pore. Dose-response experiments comparing the abilities of Na\textsuperscript{+}\textsubscript{o} (Fig. 10) and K\textsuperscript{+}\textsubscript{o} (Fig. 11) to slow Ba\textsuperscript{2+} egress from HERG supported the plausibility of such a model. K\textsuperscript{+}\textsubscript{o} in the 2–10 mM range was sufficient to slow Ba\textsuperscript{2+} egress (Fig. 11 D), whereas Na\textsuperscript{+}\textsubscript{o} in the 30–140 mM range was needed (Fig. 12 D). Although these experiments do not directly measure site affinity, the simplest interpretation of the data suggests an ~50–50-fold greater affinity for K\textsuperscript{+} than Na\textsuperscript{+}, values that would permit competition of physiological [Na\textsuperscript{+}\textsubscript{o}] and [K\textsuperscript{+}\textsubscript{o}] for an outer pore site.

The 30–50-fold difference in affinity contrasts markedly with the 1,000-fold difference in site affinity described by Neyton and Miller (1988b) for the BK channel (K\textsubscript{i} = 19 mM for K\textsuperscript{+}\textsubscript{o}, 27 mM for Na\textsuperscript{+}\textsubscript{o}). Ignoring momentarily the many methodological divergences in our studies and the possibility of subtle differences in the position where Ba\textsuperscript{2+} resides in HERG versus BK channels, it would appear that the major difference between the HERG outer pore and the BK channel outer pore is not that HERG has a much greater affinity for Na\textsuperscript{+}, but instead that HERG has lower affinity for K\textsuperscript{+}. In other (non-HERG) K\textsuperscript{+} channels, very high K\textsuperscript{+} affinity could explain the inability of Na\textsuperscript{+}\textsubscript{o} to inhibit current; in such cases, even closed channels could potentially retain permeating K\textsuperscript{+}, thereby preventing Na\textsuperscript{+}\textsubscript{o} from binding. In the presence of K\textsuperscript{+}\textsubscript{o}, the outer pore would be even more likely to be occupied by K\textsuperscript{+}, as it could enter from the extracellular side.

Rate Dependence of Inhibition by Extracellular Sodium in WT HERG, but not S624A

Our desire to understand the plateau in the Na\textsuperscript{+}\textsubscript{o} dose-response curve for HERG S624A and S624T (Fig. 4 E) motivated us to more carefully study the role of the closed state in Na\textsuperscript{+}\textsubscript{o} inhibition. For WT HERG, greater inhibition was observed with extended time at the holding potential of ~80 mV (Fig. 12, A–C), whereas no such effect of holding potential was observed for S624A (Fig. 12, D–F). Earlier, we described a modest trend toward decreased Na\textsuperscript{+}\textsubscript{o} inhibition at more hyperpolarized test potentials for WT HERG (Fig. 1 C). Therefore, the effect of time at ~80 mV in WT HERG (Fig. 12 C) suggests a permissive role of the closed state in Na\textsuperscript{+}\textsubscript{o} inhibition. The closed state of the S624A channel apparently does not permit additional Na\textsuperscript{+}\textsubscript{o} inhibition (Fig. 12 F). Regardless of the underlying basis, the insensitivity of Na\textsuperscript{+}\textsubscript{o} inhibition to closed time in S624A helps reconcile this mutant’s unusual Na\textsuperscript{+}\textsubscript{o} dose-response curve (Fig. 4 E). The insensitivity to closed time must, on some level, reflect a reduced affinity of Na\textsuperscript{+}\textsubscript{o} for the S624A closed state as compared with WT. A lower closed state affinity could arise directly from a change in the structure of the channel protein resulting from the amino acid substitution. Alternatively, the insensitivity of S624A to time at ~80 mV could be the indirect
Na\(^+\) interaction with HERG probed with Na\(^+\), and Ba\(^{2+}\)

A Model that Combines Permeation and Gating

This section is speculative and focuses primarily on the mutant channels S624A and S624T. Inactivation of S631A is shifted by >100 mV (Zou et al., 1998), whereas the effects in the S624 mutations are much more modest (compare Fig. 4, A–D) and therefore more likely to reflect a pore structure similar to the WT HERG channel. Fig. 13 shows the predicted locations of HERG residues 631 and 624, using the crystal structure coordinates from the bacterial channel KcsA (Doyle et al., 1998). Serine 624 corresponds to the innermost selectivity filter position in KcsA (KcsA threonine 75). Application of a heavy atom distance criterion to locate putative hydrogen bonds within the KcsA structure identified a ring of predicted bonds between backbone carbonyl and side-chain hydroxyl moieties of threonine 75 encircling the inner selectivity filter (Fig. 13 B). By analogy with KcsA, the elimination of side chain hydroxyl moieties by the HERG S624A mutation would be expected to disrupt this putative ring of hydrogen bonds encircling the lower selectivity filter.

In the nomenclature of a recent crystallographic study of KcsA, the backbone carbonyl and side-chain hydroxyl moieties at KcsA T75 form “position 4,” the innermost selectivity filter position (Morais-Cabral et al., 2001). In the model of permeation proposed by Morais-Cabral et al. (2001) independently supported by a
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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Physiological Implications of Sodium Effects on HERG Currents

Bradycardia is a recognized risk factor for QT prolongation and torsades de pointes (Roden, 1998). Much of the risk associated with bradycardia likely arises from marked depression of IKS resulting from complete deactivation during increased time spent at hyperpolarized potentials. In the study defining the rate-dependence of IKs, performed in guinea pig myocytes, Jurkiewicz and Sanguinetti (1993) reported that the magnitude of IKs was rate independent. Interestingly, their measurements of IKs were made in the absence of Na+. Our data showing rate dependence of Na+ inhibition of WT HERG current (Fig. 12, A–C) raise the possibility that the magnitude of IKs may be rate dependent in the presence of Na+, and that this mechanism could underlie part of the torsades risk in bradycardic patients.

Physiologic [Na+]i blocks HERG current at depolarized potentials (Fig. 5) and block is relieved by physiologic [K+]o (Fig. 6). This suggests that the anti-Nernstian effect of [K+]o on IKs in vivo is likely to reflect not only relief of inhibition by Na+, but also relief of Na+ block. Further, [Na+]i in beating cells may be higher than usually thought (Wellis et al., 1990), raising the possibility that Na+ block of IKs and relief of that block by K+ could assume greater importance in vivo. It is also conceivable that K+ relief of Na+ block of cardiac K+ channels other than HERG could contribute to the salutary effect of raising serum [K+] to normalize QT interval (Compton et al., 1996; Choy et al., 1997). Although the physiological relevance of Na+ block in other K+ channels has remained somewhat unclear (Yellen, 1984b), a functional role for block of HERG by Na+ can be rationalized in a satisfying way. Voltage-dependent block by Na+ would be expected to preferentially limit HERG current in the early phases of the cardiac action potential when the membrane is strongly depolarized, and to be relieved in phase III, as IKs carries out its function of promoting rapid repolarization.

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