C-Linker Accounts for Differential Sensitivity of ERG1 and ERG2 K⁺ Channels to RPR260243-Induced Slow Deactivation

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ABSTRACT

Compounds can activate human *ether-à-go-go*-related gene 1 (hERG1) channels by several different mechanisms, including a slowing of deactivation, an increase in single channel open probability, or a reduction in C-type inactivation. The first hERG1 activator to be discovered, RPR260243 ((3*R*,4*R*)-4-[3-(6-methoxyquinolin-4-yl)-3-oxo-propyl]-1-[3-(2,3,5-trifluorophenyl)-prop-2-ynyl]-piperidine-3-carboxylic acid) (RPR) induces a pronounced, voltage-dependent slowing of hERG1 deactivation. The putative binding site for RPR, previously mapped to a hydrophobic pocket located between two adjacent subunits, is fully conserved in the closely related rat *ether-à-go-go*-related gene 2 (rERG2), yet these channels are relatively insensitive to RPR. Here, we use site-directed mutagenesis

and heterologous expression of channels in *Xenopus* oocytes to characterize the structural basis for the differential sensitivity of hERG1 and rERG2 channels to RPR. Analysis of hERG1-rERG2 chimeric channels indicated that the structural determinant of channel sensitivity to RPR was located within the cytoplasmic C-terminus. Analysis of a panel of mutant hERG1 and rERG2 channels further revealed that seven residues, five in the C-linker and two in the adjacent region of the cyclic nucleotide-binding homology domain, can fully account for the differential sensitivity of hERG1 and rERG2 channels to RPR. These findings provide further evidence that the C-linker is a key structural component of slow deactivation in *ether-à-go-go*-related gene channels.

Introduction

The rapid delayed rectifier K⁺ current (*I*_{Kr}) contributes to the repolarization phase of cardiomyocyte action potentials (Sanguinetti and Jurkiewicz, 1990, 1991). In the human heart, human *ether-à-go-go*-related gene 1 (hERG1) (Kv11.1) channels conduct *I*_{Kr} (Sanguinetti et al., 1995; Trudeau et al., 1995) and loss of function mutations in the hERG1 gene *KCNH2* are a common cause of long QT syndrome (Curran et al., 1995), a disorder of ventricular repolarization that is associated with life-threatening arrhythmia (Jervell and Lange-Nielsen, 1957; Schwartz et al., 1991).

In response to repolarization of the cell membrane, *ether-à-go-go*-related gene (ERG) channels close (deactivate) slowly compared with the closely related *ether-à-go-go* (eag) channels and many other voltage-gated K⁺ channels. The structural basis of slow deactivation has been intensely investigated and together many studies indicate that it involves a cytoplasmic interaction between the amino- and carboxyl-termini of adjacent subunits within the tetrameric channel (Gustina and Trudeau, 2011; Gianulis et al., 2013; Li et al., 2014; Ng

et al., 2014). The structures of amino- and carboxyl-termini of the ERG channel subunit have been solved (Morais Cabral et al., 1998; Adaixo et al., 2013; Brelidze et al., 2013). The N-terminus includes a Per-Arnt-Sim (PAS) (residues 26–135) domain and a PAS-cap (residues 1–26) domain that together are called the eag domain. The carboxyl-terminus contains a cyclic nucleotide-binding homology domain (CNBHD) that is coupled to the S6 α -helical transmembrane segment by the C-linker, a 77 amino acid structure containing four α -helices. Truncation of all (Schonherr and Heinemann, 1996) or a portion (Morais Cabral et al., 1998) of the eag domain accelerates hERG1 channel deactivation, as do specific point mutations in the PAS domain (Chen et al., 1999), PAS cap (Muskett et al., 2011), or CNBHD (Al-Owais et al., 2009).

Early in the drug discovery process, compounds are routinely screened for their propensity to block hERG1 channels, an undesirable side effect that can induce cardiac arrhythmia in susceptible individuals (Fenichel et al., 2004). Extensive screening fortuitously led to the discovery of several structurally diverse compounds that activate rather than block hERG1 channels. The first hERG1 channel activator, RPR260243 ((3*R*,4*R*)-4-[3-(6-methoxyquinolin-4-yl)-3-oxo-propyl]-1-[3-(2,3,5-trifluorophenyl)-prop-2-ynyl]-piperidine-3-carboxylic acid) (RPR) was discovered 10 years ago (Kang et al., 2005). The primary effect of RPR is a pronounced,

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ABBREVIATIONS: CNBHD, cyclic nucleotide-binding homology domain; cRNA, complementary RNA; eag, *ether-à-go-go*; ERG, *ether-à-go-go*-related gene; ICA-105574, 3-nitro-*N*-(4-phenoxyphenyl)-benzamide; PAS, Per-Arnt-Sim; PD-118057, 2-[[4-[2-(3,4-dichlorophenyl)ethyl]phenyl]amino]benzoic acid; PL, pore linker; RPR, (3*R*,4*R*)-4-[3-(6-methoxyquinolin-4-yl)-3-oxo-propyl]-1-[3-(2,3,5 trifluorophenyl)-prop-2-ynyl]-piperidine-3-carboxylic acid; S45L, S4/S5 linker; WT, wild-type.

voltage-dependent slowing of hERG1 deactivation (Kang et al., 2005; Perry et al., 2007), leading to a persistent outward current during cardiac repolarization that shortens the action potential duration. RPR has little or no effect on the voltage dependence of channel activation (Kang et al., 2005), and the modest increase in the amplitude of outward currents induced by RPR results from a small positive shift in the voltage dependence of channel inactivation (Perry et al., 2007). The effects of RPR on hERG1 channel gating have physiologic consequences in the heart. In isolated guinea pig ventricular myocytes, 10 μ M RPR alone had little effect on the action potential shape, but reversed action potential prolongation caused by prior treatment of myocytes with dofetilide, a potent hERG1 channel blocker (Kang et al., 2005). In Langendorff-perfused guinea pig hearts, 5 μ M RPR increased T-wave amplitude and shortened the QT interval (Kang et al., 2005). Thus, the slow deactivation of hERG1 channels induced by RPR accelerates the rate of cardiac repolarization.

Site-directed mutagenesis and electrophysiological analysis of mutant channels heterologously expressed in *Xenopus* oocytes were used to identify the amino acid residues in hERG1 that form the putative binding site for RPR. This approach identified several residues in the S5 segment (Val549, Leu550, Leu553, or Phe557) and S6 segment (Asn658, Val659, Ile662, Leu666, or Tyr667) of the hERG1 subunit as key determinants of RPR activity. Mutation of any one of these residues attenuated or eliminated the effects of RPR on channel deactivation. Together, the side chains of these nine residues form a binding pocket for RPR that is located between two adjacent hERG1 subunits. Due to the 4-fold symmetry of voltage-gated K⁺ channels, a homotetrameric hERG1 channel should contain four identical RPR binding sites. Characterization of concatenated hERG1 tetramers containing a defined number and positioning of L533A mutant subunits that prevent RPR activity (Perry et al., 2007) revealed that occupancy of all four binding sites was required to achieve the maximal slowing of deactivation by RPR (Wu et al., 2015).

The key residues of the putative RPR binding site defined for hERG1 are conserved in human and rat *ether-à-go-go*-related gene type 3 (rERG3) channels. However, whereas RPR reduces the current magnitude at high concentrations, it has no effect on deactivation of human (Kang et al., 2005) or rat (Perry and Sanguinetti, 2008) ERG3 channels. Two residues in the S5 segment of hERG1 (Phe551 and Thr556) that are located within the putative RPR binding pocket, although not identified as interacting with RPR based on mutational analysis, are not conserved in rERG3. Phe551 and Thr556 in hERG1 are replaced by Met553 and Ile558 in rERG3. We previously reported that a Thr in the second position (as in hERG1) was required for RPR to activate channels, whereas an Ile in this position (as in rERG3) eliminated agonist activity in either channel type and revealed a secondary inhibitory effect of RPR (Perry and Sanguinetti, 2008).

In the present study, we further investigate the mechanistic basis of channel-selective pharmacology of RPR by comparing its effects on hERG1 and rat *ether-à-go-go*-related gene type 2 (rERG2). Similar to rERG3, rERG2 channels are nearly insensitive to high concentrations of RPR; however, unlike rERG3, the amino acid sequence of the putative RPR binding site of rERG2 is identical to hERG1. Thus, the structural basis for rERG2 insensitivity to RPR must be accounted for by a region of the channel outside of the putative ligand-binding

pocket. In this study, we determined the effects of RPR on deactivation of hERG1-rERG2 chimeric channels and the cumulative effect of multiple point mutations on both hERG1 and rERG2 homotetramers to identify the specific residues that account for the differential RPR sensitivity of hERG1 and rERG2 channels.

Materials and Methods

Site-Directed Mutagenesis. cDNAs for rat *kcnh6* (rERG2, NCBI Reference Sequence NP_446389), kindly provided by David McKinnon, and human *KCNH2* (HERG1, isoform 1a, NCBI Reference Sequence NM_000238) were cloned into the pSP64 oocyte expression vector. Single or multiple point mutations in *KCNH2* or *kcnh6* were introduced into wild-type (WT) channels using the QuikChange site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA).

Construction of hERG1/rERG2 Chimeras. Four hERG1/rERG2 chimera channels were constructed by swapping between homologous regions of *KCNH2* and *kcnh6* cDNAs. The rERG2(N-S4)/hERG1 chimera was composed of the N-terminus to the end of the S4 segment of rERG2 linked to the S4/S5 linker to the C-terminus of hERG1. A HindIII site was inserted into the multiple cloning site of both hERG1 and rERG2, and an AscI restriction site was inserted into hERG1 at Thr526-Leu529 (bp 1578–1585). rERG2 had an existing AscI site at Thr377-Leu380 (bp 1131–1138). The N-terminus to the S4 region of hERG1 was excised and replaced by the homologous region of rERG2 using HindIII and AscI. The resulting chimera had Met1-Thr377 from rERG2 and Ala378-Ser1010 from hERG1.

The hERG1/rERG2(S45L-PL) chimera was constructed by swapping the S4/S5 linker (S45L) to the pore linker (PL) of hERG1 with the homologous region of rERG2 using AscI and BglII enzymatic cuts. The AscI sites are described above, located on the 5' end of the chimera swap. A pre-existing BglII site in hERG1 at Lys638-Phe640 (bp 1913–1918) was located on the 3' end of the swap. A BglII site was introduced into rERG2 at Lys490-Phe492 (bp 1469–1474) and resulted in a V491I mutation in rERG2 (matching the residue in hERG1). The resulting chimera had Met1-Thr526 of hERG1, Ala527-Lys639 from rERG2, and Ile640-Ser1160 from hERG1.

The hERG1/rERG2(S6-C_{term}) chimera was constructed by swapping the S6 segment to the C-terminus of hERG1 with the homologous region of rERG2. A BglII site was introduced into rERG2 as described above. An existing BglII site in hERG1 (bp 1913–1918) was located at the beginning of S6. The S6 to C-terminus of rERG2 was excised from the pSP64 vector using BglII and EcoRI (located in the vector region, bp 2905–2910; rERG2 stop codon at bp 2851–2853), and inserted into hERG1 using BglII and EcoRI (also located in the pSP64 vector region, bp 3532–3537; hERG1 stop codon at 3478–3480) to remove the hERG1 S6 to the C-terminus. The resulting chimera had Met1-Lys638 from hERG1 and Ile639-Ser1098 from rERG2.

The hERG1/rERG2(CNBHD β -roll-C_{term}) chimera was constructed by swapping a portion of CNBHD to the C-terminus of hERG1 with the homologous region of rERG2. New SpeI sites were mutated into rERG2 at Thr620-Val622 (bp 1860–1865) and hERG1 at Thr768-Val770 (bp 2304–2309). These sites are located 10 amino acids into the β -roll of the CNBHD. The remaining region of the β -roll to the C-terminus was removed from hERG1 using SpeI and EcoRI (bp 3532–3537 in the vector), and the same region of rERG2 was inserted using the same enzymes (bp 2905–2910 for EcoRI). The resulting chimera had Met1-Thr768 from hERG1 and Leu769-Ser1098 from rERG2.

All mutant constructs were verified by DNA sequence analysis. To prepare complementary RNA (cRNA) for use in oocyte expression studies, pSP64 plasmids were linearized with EcoRI prior to in vitro transcription using the mMessage mMachine SP6 kit (Ambion, Austin, TX).

Isolation, cRNA Injection, and Voltage Clamp of *Xenopus* Oocytes. Procedures used for the surgical removal of ovarian lobes from *Xenopus laevis* and isolation of oocytes was approved by the

University of Utah Institutional Animal Care and Use Committee and performed as described previously (Abbruzzese et al., 2010). Single oocytes were injected with 5–40 ng cRNA encoding WT or mutant ERG subunits and studied 2–7 days later. Ionic currents in oocytes were recorded using standard two-electrode voltage-clamp techniques (Goldin, 1991; Stühmer, 1992) and agarose-cushion microelectrodes (Schreibmayer et al., 1994), with tip resistances that ranged from 0.6 to 1.8 MΩ when back-filled with a 3 M KCl solution. A GeneClamp 500 amplifier, Digidata 1322A data acquisition system, and pCLAMP 8.2 software (Molecular Devices, Inc., Sunnyvale, CA) were used to produce command voltages and record current and voltage signals.

Voltage Pulse Protocols and Data Analysis. ERG channel deactivation was quantified by comparing the integral of leak-subtracted tail currents ($\int I_{\text{tail}}$) before and after treatment of an oocyte with a variable concentration of RPR. Channel currents were initially activated with a 1-second pulse to +40 mV, and tail currents were elicited by 9-second pulses to a return potential (V_{ret}) that was applied in 10-mV increments from –60 to –110 mV. The holding potential was either –80 or –90 mV. A 9-second pulse was long enough for channels to completely deactivate for each V_{ret} under control conditions. However, deactivation was often incomplete after 9 seconds in the presence of high concentrations of RPR, especially when V_{ret} was more positive than –90 mV. An analysis of digitized data, including fitting of I_{tail} kinetics and determination of $\int I_{\text{tail}}$, were performed with pCLAMP 8.2 software. For some experiments, I_{tail} was fitted with a two-exponential function

$$I_{\text{tail}}(t) = A_f e^{-t/\tau_f} + A_s e^{-t/\tau_s} + C$$

where A_f and τ_f are the amplitude and time constant for the fast component, A_s and τ_s are the amplitude and time constant for the slow time component of I_{tail} deactivation, and C is the maintained current component. The relative amplitude of the fast component of I_{tail} was defined as $A_f/(A_f + A_s)$.

ORIGIN 8.5 software (OriginLab, Northampton, MA) was used to construct graphs and analyze concentration-effect relationships for RPR. The fold change in $\int I_{\text{tail}}$ was plotted as a function of [RPR], and the relationship was fitted with the logistic equation

$$fc = fc_{\text{max}} + (fc_{\text{min}} - fc_{\text{max}})/(1 + [\text{RPR}]/\text{EC}_{50})^H$$

where fc is the fold change in $\int I_{\text{tail}}$, EC_{50} is the concentration of RPR that produced a half-maximal effect, and H is the Hill coefficient. Plots of fold change in $\int I_{\text{tail}}$ versus time constants of deactivation at –70 mV were analyzed by linear regression, and the adjusted R^2 was used to estimate the degree of relationship between the two variables. All data in the text and figures are presented as mean $\pm$ S.E.M. (n = number of oocytes).

Solutions and Drugs. The extracellular solution used for voltage-clamp experiments contained (in millimolar): 96 NaCl, 2 KCl, 1 CaCl₂, 1 MgCl₂, and 5 HEPES, and the pH was adjusted to 7.6 with NaOH. RPR260243 (ChemShuttle, Wuxi, China) was dissolved in dimethylsulfoxide to make a 10 mM stock solution, and final drug concentrations (3–50 μM) were obtained by dilution of the stock solution with the extracellular solution.

Results

RPR Has Differential Effects on hERG1 and rERG2 Channels. The effects of RPR on hERG1 channel currents have been characterized in detail (Kang et al., 2005; Perry et al., 2007; Wu et al., 2015). For this study, we did not analyze the effects of RPR on the kinetics of activation or the voltage dependence of activation or inactivation. Instead, we focused on how RPR dramatically slows the rate of deactivation of hERG1 channels but has very little effect on the deactivation of closely related rERG2 channels. Deactivation of ERG

channel currents in the absence of RPR is best described as a two exponential process; however, slow deactivation induced by RPR is characterized by complex kinetics that are not amenable to analysis by exponential fitting. Therefore, to simplify our analysis of the effects of RPR on deactivation, we measured the integral of tail currents ($\int I_{\text{tail}}$) over a limited voltage range (–60 to –110 mV) and duration (9 seconds) before and after treatment of an oocyte with a solution containing RPR at a concentration ranging from 3 to 50 μM . At 10 μM , RPR slowed the rate of deactivation of hERG1 channels measured as outward I_{tail} in response to repolarization of the membrane potential to –60, –70, –80, and –90 mV or inward I_{tail} in response to a V_{ret} of –100 or –110 mV (Fig. 1, A and B). I_{tail} was integrated (Fig. 1, C and D), and the value of $\int I_{\text{tail}}$ at 9 seconds was normalized to that measured at –60 mV under control conditions and plotted as a function of V_{ret} (Fig. 1E). At –60 mV, 10 μM RPR increased $\int I_{\text{tail}}$ of hERG1 channels by a factor of 3.2 ± 0.2 ($n = 7$). Using the same experimental protocol and method of analysis indicated that deactivation of rERG2 channel currents are barely affected by 10 μM RPR (Fig. 1F–I). At –60 mV, $\int I_{\text{tail}}$ of rERG2 channels was only increased by $16 \pm 6\%$ ($n = 5$; Fig. 1J). At the highest soluble concentration of 50 μM , RPR dramatically slowed the deactivation of hERG1 and reduced peak I_{tail} , measured a few milliseconds after repolarization to a V_{ret} that is more negative than –60 mV (Fig. 2A). A plot of the average normalized $\int I_{\text{tail}}$ of hERG1 for several oocytes as a function of V_{ret} is presented in Fig. 2B. At –60 mV, $\int I_{\text{tail}}$ for hERG1 was increased by a factor of 4.1 ± 0.5 ($n = 5$). For rERG2 channels, peak I_{tail} was also reduced, but deactivation was only modestly slowed by 50 μM RPR (Fig. 2C). At –60 mV, $\int I_{\text{tail}}$ for rERG2 was increased by only $27 \pm 9\%$ ($n = 5$; Fig. 2D). Thus, even a high concentration of RPR has only a small effect on deactivation of rERG2 compared with hERG1 channels. Reduced RPR sensitivity was also evident for I_{tail} measured at more negative potentials, where the rate of rERG2 channel deactivation is much faster (Fig. 2, C and D).

The effect of cumulative concentrations (3–50 μM) of RPR on the fold change in $\int I_{\text{tail}}$ of hERG1 and rERG2 channels as a function of V_{ret} is summarized in Fig. 3. For hERG1, $\int I_{\text{tail}}$ was increased as a function of V_{ret} from –60 to –90 mV (Fig. 3A). The apparent reduced effect of RPR on $\int I_{\text{tail}}$ at the less negative potentials is because I_{tail} was measured for only 9 seconds and not at steady-state levels, which can take >30 seconds (Wu et al., 2015). The fold change in $\int I_{\text{tail}}$ for hERG1 plotted as a function of [RPR] at five different voltages is presented in Fig. 3B. Although the efficacy of RPR (peak response) was greater when determined at the more negative voltages, where deactivation was faster and neared completion even in the presence of RPR, the potency (EC_{50} , summarized in Table 1) did not vary much over the voltage range of –60 to –90 mV. To determine if the potency or efficacy of RPR was voltage dependent when measured under near steady-state conditions, I_{tail} was measured using 60-second pulses at a V_{ret} of –70 and –110 mV (Fig. 3C). The fold change in $\int I_{\text{tail}}$ for these long pulses was larger when measured at –70 mV compared with –110 mV (Fig. 3D), but there was no voltage-dependent difference between the values when normalized to the fold change in $\int I_{\text{tail}}$ at 50 μM RPR (Fig. 3E).

In contrast to the maximum 30-fold increase in $\int I_{\text{tail}}$ of hERG1 induced by 50 μM RPR at –110 mV, $\int I_{\text{tail}}$ of rERG2

Fig. 1. Effects of 10 μM RPR on deactivation of hERG1 and rERG2 channel currents. (A) Voltage pulse protocol and representative hERG1 current traces under control conditions. (B) Currents from the same cell after treatment with 10 μM RPR. (C and D) $\int I_{\text{tail}}$ determined from current traces shown in (A and B). In (A–D), colors correspond to the voltages shown in the inset of (A). Small horizontal arrow indicates the 0 current level (A and B) or charge level (C and D). (E) Effect of RPR on $\int I_{\text{tail}}$ determined over a 50-mV range of V_{ret} . $\int I_{\text{tail}}$ was normalized to the value determined at V_{ret} of -60 mV under control conditions ($n = 7$). (F) Voltage pulse protocol and representative rERG2 current traces under control conditions. (G) Currents from same cell after treatment of oocyte with 10 μM RPR. (H and I) $\int I_{\text{tail}}$ determined from current traces shown in (F and G). In (F–I), colors correspond to the voltages shown in the inset of (F). Small horizontal arrow indicates 0 current level (F and G) or charge level (H and I). (J) RPR has only a minimal effect on $\int I_{\text{tail}}$ determined over a 50-mV range of V_{ret} . $\int I_{\text{tail}}$ was normalized to the value determined at V_{ret} of -60 mV under control conditions ($n = 5$). In (E and J), the S.E. bars are smaller than the symbol size for some of the data points.

was only enhanced by a maximum of 2.3-fold (Fig. 3F). The fold change in $\int I_{\text{tail}}$ for rERG2 plotted as a function of [RPR] at the different voltages is presented in Fig. 3G, with EC_{50} and Hill coefficient values summarized in Table 1. The average EC_{50} for RPR across all voltages, when measured with 9-second pulses, was similar for rERG2 channels (13.5 ± 1.9 μM) compared with hERG1 (9.3 ± 1.2 μM ; t test, $P > 0.05$). We next examined hERG1-rERG2 chimeric channels to determine the structural domain(s) that might account for the differential sensitivity of hERG1 and rERG2 channel gating to RPR.

C-Linker Is a Critical Determinant of Differential ERG Channel Sensitivity to RPR. The protein sequences of hERG1 and rERG2 channel subunits are 55.2% identical. Four different hERG1/rERG2 chimeric channel subunits (Fig. 4) were constructed by substituting the coding sequence of a specific region of *KCNH2* with the homologous coding sequence from *kcnh6*. The first chimera, rERG2(N-S4)/hERG1, contained rERG2 from the N-terminus to the end of the S4 segment. $\int I_{\text{tail}}$ was increased by 10 μM RPR at all potentials examined, and the increase was 1.6-fold at a V_{ret} of -60 mV (Fig. 4A), which is about half of the effect observed for WT hERG1 channels (Fig. 1E). The second chimera, hERG1/rERG2(S45L-PL), contained the rERG2 sequence from the end of the S4 segment to the start of the S6 segment. Under control conditions, these chimeric channels deactivated about 10 times faster than WT hERG1 channels. The fast and slow time constants for deactivation at -60 mV were 38 ± 3 and 219 ± 24 milliseconds for the chimeric channel ($n = 5$), as compared with 471 ± 19 milliseconds and 2.92 ± 0.11 seconds for WT hERG1 ($n = 7$). The accelerated rate of deactivation resulted in a larger than normal (7-fold) RPR-induced increase in $\int I_{\text{tail}}$ at a V_{ret} of -60 mV (Fig. 4B). Thus, the

hERG1/rERG2(S45L-PL) chimera retained RPR sensitivity. The third chimera, hERG1/rERG2(S6-C_{term}), contained the rERG2 sequence from the start of the S6 segment to the

Fig. 2. Effects of 50 μM RPR on deactivation of hERG1 and rERG2 channel currents. (A) Superimposed hERG1 current traces recorded before and after treatment of an oocyte with 50 μM RPR. Small horizontal arrow indicates 0 current level. (B) Effect of RPR on $\int I_{\text{tail}}$ of hERG1 as a function of V_{ret} . $\int I_{\text{tail}}$ was normalized to the value determined at V_{ret} of -60 mV under control conditions ($n = 7$). (C) Superimposed rERG2 current traces recorded before and after treatment of an oocyte with 50 μM RPR. (D) Effect of RPR on $\int I_{\text{tail}}$ of rERG2 as a function of V_{ret} . $\int I_{\text{tail}}$ was normalized to the value determined at V_{ret} of -60 mV under control conditions ($n = 7$). In (B and D), the S.E. bars are smaller than the symbol size for some of the data points.

Fig. 3. Concentration and voltage-dependent effects of RPR on deactivation of hERG1 and rERG2 channel currents. (A) Fold change in $\int I_{tail}$ (9-second duration) for hERG1 channel currents plotted as a function of V_{ret} and [RPR] ($n = 7-8$). (B) [RPR]-response relationship for fold change in hERG1 $\int I_{tail}$ (9-second duration) measured at a variable V_{ret} . Data were fitted with a logistic equation (smooth curves) to determine EC_{50} and the Hill coefficient at each potential (values presented in Table 1). (C) Effect of 3 and 50 μM RPR on I_{tail} measured for 60 seconds at -70 and -110 mV. Upper panel depicts voltage pulse protocol. The lower panel shows outward tail currents in response to pulses to -70 mV and inward tail currents in response to pulses to -110 mV. (D) Effect of RPR on fold change in hERG1 $\int I_{tail}$ (60-second duration) measured at -70 and -110 mV. Data could not be adequately fitted with a logistic equation because the effect of RPR did not reach a maximum at the maximum soluble concentration of 50 μM . (E) Normalized hERG1 $\int I_{tail}$ (60-second duration) measured at -70 and -110 mV. (F) Fold change in $\int I_{tail}$ for rERG2 channel currents plotted as a function of V_{ret} for three concentrations of RPR ($n = 5-7$). The fold change in $\int I_{tail}$ determined at a V_{ret} of -100 mV (near the I_{tail} reversal potential of -97 mV) was extremely variable and therefore not included in these plots. (G) [RPR]-response relationship for fold change in rERG2 $\int I_{tail}$ (9-second duration) measured at a variable V_{ret} . EC_{50} and Hill coefficient values are presented in Table 1. In all panels, the S.E. bars are smaller than the symbol size for some of the data points.

C-terminus, a region that is 51% identical in hERG1. This chimera was insensitive to 10 μM RPR, with only a 10% increase in $\int I_{tail}$ at a V_{ret} of -60 mV (Fig. 4C), which is similar to the relative lack of response we observed for WT rERG2 channels (Fig. 1J). The fourth chimera, hERG1/rERG2 (CNBHD $_{\beta}$ -roll-C $_{term}$) contained rERG2 from the start of the

TABLE 1

EC_{50} and Hill coefficients (H) determined from plots of the fold change in $\int I_{tail}$ versus [RPR], as shown in Fig. 3B for hERG1 ($n = 7$) and in Fig. 3G for rERG2 ($n = 5$)

V_{ret}	hERG1		rERG2	
	EC_{50}	H	EC_{50}	H
mV	μM		μM	
-60	6.8 ± 0.3	2.2 ± 1.1	8.6 ± 2.0	2.8 ± 1.1
-70	8.2 ± 0.5	1.9 ± 0.1	11.2 ± 1.7	2.7 ± 0.7
-80	9.1 ± 0.2	1.7 ± 0.03	13.4 ± 2.0	2.6 ± 0.5
-90	8.4 ± 1.2	1.7 ± 0.2	19.9 ± 5.9	2.5 ± 0.5
-110	13.9 ± 5.3	1.5 ± 0.3	14.4 ± 4.2	2.5 ± 0.8

β -roll in the CNBHD to the C-terminus. RPR at 10 μM increased $\int I_{tail}$ of this chimera by 2.9-fold at a V_{ret} of -60 mV (Fig. 4D), similar to that observed for WT hERG1 channels (Fig. 1E). Together, these findings suggest that the structural component that accounts for the differential sensitivity of hERG1 and rERG2 channels to RPR is located between the S6 segment and the C-terminus.

The region of ERG subunits bounded by the start of the S6 segment and the C-terminus is long and significantly divergent between hERG1 and rERG2. The region contains 521 residues in hERG1 and 460 residues in rERG2, with only a 48.5% identity. However, the S6 segments for hERG1 and rERG2 differ by only a single residue (Ile639 in hERG1 versus Val491 in rERG2) located at the extracellular end, and the S6 to the end of the CNBHD is 87% identical for the two subunits. Moreover, most of the sequence divergence in the S6-CNBHD region occurs in the β -roll of the CNBHD (17 residues differ), whereas the C-linker and all regions of the CNBHD other than the β -roll are more similar. Only 7 of the 77 residues are not identical. Therefore, we concentrated our efforts on examining the residues highlighted in Fig. 5A that differ between hERG1 and rERG2: five located in the C-linker and two in the αA helix of the CNBHD. The structure of the C-linker plus the CNBHD of the *Anopheles gambiae* ERG channel subunit was recently solved by X-ray crystallography (Brelidze et al., 2012). The initial seven residues that differ between hERG1 and rERG2 within this structure are highlighted in Fig. 5B, where the *A. gambiae* residues were substituted to match the residues in hERG1.

hERG1 Sensitivity to RPR Can Be Incrementally Reduced by Cumulative Amino Acid Substitutions in the C-Linker to Match the Sequence of rERG2. The seven residues, located in the C-linker and the proximal region of the CNBHD domain of hERG1, were mutated in pairs or singly to match the residues in the homologous positions in rERG2. We first examined the functional consequences of substituting residues located in the C-linker. Two adjacent residues in hERG1 (Lys741 and Pro742) are located in the short region that connects the $\alpha D'$ helix of the C-linker with the αA helix of the CNBHD. Mutation of both residues to match rERG2 (K741P/P742A) had only a minor effect on RPR sensitivity. $\int I_{tail}$ was increased by 2.8-fold with 10 μM RPR at a V_{ret} of -60 mV (Fig. 6A), similar to the 3.2-fold increase measured for WT hERG1 channels. Asn733 and Ser735 are located in the $\alpha D'$ helix of the hERG1 C-linker. We mutated these two residues to match rERG2 and added them to the double mutant to obtain the quadruple mutant N733H/S735A/K741P/P742A. This mutant channel was considerably less sensitive to 10 μM RPR, exhibiting a 1.7-fold increase in

Fig. 4. Effect of RPR on hERG1-rERG2 chimeric channels. (A–D) Upper panels show a schematic of a chimeric channel subunit with six transmembrane segments (S1–S6, shown as vertical cylinders) formed by combining the indicated regions of hERG1 (blue) with rERG2 (orange). The intracellular PAS domain and CNBHD are labeled. The lower panels show normalized I_{tail} plotted as a function of V_{ret} before and after treatment of oocytes with 10 μ M RPR ($n = 5–7$). In all panels, the S.E. bars are smaller than the symbol size for some of the data points.

I_{tail} at a V_{ret} of -60 mV (Fig. 6B). The addition of a fifth mutation (R681K) produced a C-linker that fully matched the corresponding region in rERG2. This mutant channel (R681K/N733H/S735A/K741P/P742A hERG1) was even less sensitive to RPR. I_{tail} at a V_{ret} of -60 mV was only increased by 1.2-fold (Fig. 6C). The change associated with the addition of the R681K mutation was unanticipated given the conserved nature of the substitution. Therefore, we examined the effect of this isolated mutation. The single mutation R681K was equally or more effective at reducing the RPR response as that obtained with the quadruple mutant, with a 1.7-fold increase in I_{tail} at -60 mV (Fig. 6D). We next substituted Met756, located in the α A helix of the CNBHD, with a Val, and added this mutation to the five-mutant subunit to obtain the six amino acid substitution subunit R681K/N733H/S735A/K741P/P742A/M756V. This mutant channel was as insensitive to RPR as the WT rERG2 channel, with only a $9 \pm 5\%$ ($n = 5$) increase in I_{tail} at -60 mV (Fig. 6E). Finally, we mutated another residue in the α A helix of the CNBHD (T747S) to obtain a seven-mutant subunit: R681K/N733H/S735A/K741P/P742A/T747S/M756V. The seven substitutions rendered the hERG1 channel insensitive to 10 μ M RPR. I_{tail} at -60 mV was increased by only $2 \pm 2\%$ ($n = 5$; Fig. 7A). At

Fig. 5. Sequence and structure of the C-terminal region of ERG channels. (A) Sequence alignment of the proximal region of the C-terminal domain of hERG1 and rERG2. The sequence shown includes the C-linker (residues Thr670–Ala746 in hERG1) and the α A helix of the CNBHD (Thr747–Phe758 in hERG1). (B) Structure of the C-terminal region of the *Anopheles gambiae* ERG channel subunit (Brelidze et al., 2012). The hERG1 residues highlighted in red in (A) are labeled. α A'– α D' represents the C-linker. The CNBHD includes the remaining structures (α A– α C and β -roll). Side chains of Glu698 and Glu699 in the α B' helix are colored yellow.

the higher concentrations of 30 and 50 μ M, RPR caused both a reduction in current magnitude and a slight slowing of deactivation and together these effects resulted in only a minimal change in I_{tail} for hERG1 containing the seven mutations (Fig. 7, B and C), which is similar to the effects on rERG2 channels (Fig. 3C). Thus, the differences in the sequence of the C-linker and the adjoining initial α -helix of the CNBHD account for the differential sensitivities of hERG1 and rERG2 channel gating to modification by RPR.

rERG2 Sensitivity to RPR Can Be Incrementally Enhanced by Cumulative Amino Acid Substitutions in the C-Linker to Match the Sequence of hERG1. If the C-linker and adjoining initial α -helix of the CNBHD account for the relative insensitivity of rERG2 channels to RPR, then we should be able to make cumulative substitutions of these key residues in rERG2 to match hERG1 and enhance RPR sensitivity. To test this hypothesis, we used the same approach described above for hERG1 channels, but instead, we introduced paired or single mutations into the rERG2 subunit to match the corresponding residues in hERG1. The mutant rERG2 channels expressed poorly in oocytes and limited our ability to accurately record currents at negative potentials without interference from endogenous currents. Therefore, we only analyzed I_{tail} recorded at a V_{ret} of -60 and -70 mV. Representative current traces for each of the mutant rERG2 channels, containing two, four, five, six, or seven amino acid substitutions, before and after treatment of an oocyte with 10 μ M RPR, are presented in Fig. 8. The mutations alone did not

Fig. 6. Effect of RPR on hERG1 channels containing mutations in the proximal region of the C-terminal domain to match the sequence of rERG2. (A–E) Normalized I_{tail} plotted as a function of V_{ret} before and after 10 μ M RPR for hERG1 channels with the indicated number of amino acid substitutions (aa subs) to match residues in homologous positions in rERG2 (see Fig. 5A). In (A), dashed blue line indicates effects of RPR on WT hERG1 (from Fig. 1E). Two aa subs = K741P/P742A ($n = 5$); four aa subs = N733H/S735A/K741P/P742A ($n = 5$); five aa subs = R681K/N733H/S735A/K741P/P742A ($n = 5$); six aa subs = R681K/N733H/S735A/K741P/P742A/M756V ($n = 5$); R681K hERG1 ($n = 5$). In all panels, the S.E. bars are smaller than the symbol size for some of the data points.

appreciably alter the kinetics of channel deactivation, but the ability of RPR to slow deactivation was incrementally enhanced by cumulative addition of two to seven mutations. As illustrated in Fig. 9A, 50 μ M RPR had dramatic effects on I_{tail} of rERG2 channels containing the seven amino acid substitutions. In addition to slower deactivation, peak I_{tail} at -60 mV was increased by $68 \pm 2\%$ ($n = 5$), which is twice as much as the 32% observed for WT hERG1 channel currents. The EC_{50} for the RPR-induced increase in I_{tail} at -60 mV for the rERG2 plus seven amino acid substitutions channel was 11.6 ± 0.7 μ M (Fig. 9B), which is higher than the EC_{50} for WT hERG1 (6.8 μ M) (Table 1) determined at the same V_{ret} . To verify that this activity resulted from RPR binding to the homologous site previously defined for hERG1, we introduced an eighth mutation (L404A) into rERG2. This mutation is equivalent to the L553A substitution in hERG1 that abolishes the agonist effects of RPR, presumably because it disrupts the RPR binding site (Perry et al., 2007). As shown in Fig. 9C, deactivation of rERG2 containing all eight mutations was not slowed by 50 μ M RPR. The only obvious effect was a decrease in peak I_{tail} (e.g., $-38 \pm 5\%$ at -60 mV; $n = 6$). Thus, analogous to our finding with hERG1 (Wu et al., 2015), mutation of a specific residue in the

Fig. 7. hERG1 channels harboring seven amino acid substitutions in the C-terminus region are highly insensitive to RPR. (A) RPR at 10 μ M has no effect on the I_{tail} - V_{ret} relationship of hERG1 channels containing seven amino acid substitutions (aa subs) (R681K/N733H/S735A/K741P/P742A/T747S/M756V) to match rERG2 sequence. Data were normalized to the value of I_{tail} at -60 mV under control conditions. (B) Normalized I_{tail} plotted as a function of V_{ret} before and after 30 and 50 μ M RPR. (C) Fold change in I_{tail} plotted as a function of V_{ret} at three concentrations of RPR ($n = 5$ for all panels, except 50 μ M RPR, in which $n = 7$). The fold change in I_{tail} determined at a V_{ret} of -100 mV (near the I_{tail} reversal potential of -97 mV) was extremely variable and therefore not included in this plot. In all panels, the S.E. bars are smaller than the symbol size for some of the data points.

S5 segment (L404A in rERG2) eliminates the effects of RPR on deactivation and reveals an inhibitory effect of the compound.

The fold change in I_{tail} induced by RPR at a V_{ret} of -70 mV for WT channels and five mutant hERG1 and five mutant rERG2 channels is summarized in Fig. 10. This figure illustrates that substitution of five residues in the C-linker of hERG1 to match the corresponding residues in rERG2 is sufficient to account for almost all of the reduced sensitivity to RPR of WT rERG2 relative to WT hERG1 channels. Conversely, substitution of five residues in the C-linker of rERG2 to match the corresponding residues in hERG1 is sufficient to account for almost all of the enhanced sensitivity to RPR of WT rERG2 relative to WT hERG1 channels. Further substitution of a sixth and seventh residue in the α A helix of the CNBHD completed the transformation of channel sensitivity to RPR in either channel. The altered sensitivity of mutant hERG1 and rERG2 channels was not correlated with the rate of current deactivation measured in the absence of the drug. This is illustrated in Fig. 11A, where the fast and slow time constants of deactivation (upper panel) and the relative amplitude of the fast component of deactivation (lower panel) at -70 mV for WT and mutant hERG1 channels are plotted as a function of the fold change in I_{tail} induced by 10 μ M RPR. A similar plot for WT and mutant rERG2 channels is presented in Fig. 11B. These data indicate that the reduced response of WT rERG2 and some of the mutant hERG1 and rERG2 channels cannot be simply attributed to a slower rate of deactivation under control conditions.

Fig. 8. Effect of RPR on rERG2 channels containing mutations in the proximal region of the C-terminal domain to match the sequence of hERG1. (A–E) Representative current traces recorded before (control) and after 10 μ M RPR for rERG2 channels containing the indicated amino acid substitutions introduced to match residues in homologous positions in hERG1. Pulse protocol is shown as the upper inset of (A). Tail currents were recorded at -60 mV (black traces) and -70 mV (red traces). Small horizontal arrows indicate the 0 current level.

Discussion

ERG channels differ in their response to the agonist effects of RPR. We previously found that the reduced sensitivity to RPR of rERG3 compared with hERG1 channels could be explained by a single residue in the S5 segment located within the putative RPR binding pocket. Substitution of Ile558 in rERG3 with a Thr, the homologous residue in hERG1, conferred hERG1-like sensitivity to RPR. The converse substitution had the opposite effect, making hERG1 insensitive to RPR (Perry and Sanguinetti, 2008). However, because the residues that compose the binding pocket for RPR are fully conserved between rERG2 and hERG1, it was clear that the mechanism of reduced response of rERG2 to RPR differed from that previously described for rERG3. Analysis of hERG1-rERG2 chimeric channels, followed by multiple amino acid swaps in hERG1 and rERG2 to match the other channel sequence revealed that differential sensitivity to RPR could be fully accounted for by a few residues in the C-linker and the initial region of the adjoining CNBHD. Based on the effects achieved by cumulative amino acid substitutions, the C-linker appears to be more important than the CNBHD as a structural determinant of the efficacy of RPR. Swapping the five nonconserved residues in the C-linker reduced the sensitivity of hERG1 to that of rERG2, whereas the equivalent substitutions in rERG2 made this channel almost as sensitive to RPR as hERG1. There were two anomalies associated with these observations. First, substitution of Arg681 with Lys (R681K) in the α A' helix of the C-linker had an unexpectedly

Fig. 9. Effect of RPR on modified rERG2 channels. (A) Representative current traces recorded before (control) and after 50 μ M RPR for rERG2 channels plus seven amino acid substitutions (aa subs) introduced to match residues in homologous positions in hERG1. Currents were elicited with a 1-second pulse to $+40$ mV, followed by repolarization to a V_{ret} of -60 (larger currents) or -70 mV. Small horizontal arrow indicates the 0 current level. (B) Fold change in $\int I_{\text{tail}}$ at -60 mV for indicated mutant rERG2 channels ($n = 5$). Data for rERG2 + seven aa subs were fitted with a logistic equation (smooth curve) to determine EC_{50} ($11.6 \pm 0.7 \mu\text{M}$) and the Hill coefficient (1.54 ± 0.07). The S.E. bars are smaller than the symbol size for some of the data points. (C) Representative current traces recorded before (control) and after 50 μ M RPR for the rERG2 channel containing eight aa substitutions (i.e., L404A rERG2 + seven aa subs). L404A mutation prevents effects of RPR on channel deactivation.

large effect given the conserved nature of this point mutation. RPR at 10 μ M induced a 2.4-fold increase in $\int I_{\text{tail}}$ at a V_{ret} of -70 mV, which is a greater reduction in efficacy than the 2.9-fold change that was obtained by swapping all four of the remaining nonconserved residues (Asn733, Ser735, Lys741, and Pro742) located in the C-linker. Second, addition of a seventh amino acid swap (S599T) in rERG2 reduced the response to RPR relative to the six substitutions. The reason why such conserved mutations resulted in relatively large changes in the pharmacological response is unclear, but suggests that even subtle changes in the C-linker can have significant effects on the ability of RPR to slow ERG channel deactivation.

Several recent studies have provided strong evidence for physical association between the cytoplasmic N- and C-termini and the role such an interaction has in determining the kinetics of transitions between the open and closed state of ERG channels (Gustina and Trudeau, 2011; Gianulis et al., 2013; Li et al., 2014; Ng et al., 2014). The N-terminal eag domain has also been proposed to modulate the deactivation rate by a direct interaction with the S4-S5 linker (Li et al., 2010; de la Pena et al., 2011; Ng et al., 2011); however, another study concluded that the eag domain associates with the C-linker/CNBHD, but not the S4-S5 linker (Gianulis et al., 2013). Interactions between specific residues in the eag domain and the C-linker have recently been investigated. Based on homology modeling and site-directed mutagenesis results, Ng et al. (2014) proposed that Arg56 of the PAS domain and Asp803 in the CNBHD form a stable interaction, whereas Arg4 and Arg5 of the PAS-cap domain transiently interact with Glu698 and Glu699 of the C-linker (yellow

Fig. 10. Incremental changes in RPR sensitivity associated with cumulative point mutations in the C-terminus of hERG1 and rERG2 channels. The fold change ($\pm$ S.E.M.) in I_{tail} at -70 mV induced by $10 \mu\text{M}$ RPR is plotted as a function of the number of amino acid substitutions (number of aa subs) introduced into the C-terminus of either hERG1 or rERG2 ($n = 5-7$). Zero substitutions indicate the WT channel.

colored side chains in Fig. 5B) in the open state, and this association slows the rate of deactivation. CNBHD-eag domain interactions have also been investigated using purified proteins and NMR spectroscopy. Thr747 (the seventh hERG1 amino acid substituted in our study) was among the many residues in the CNBHD identified as interacting with the eag domain (Li et al., 2014). Finally, a disulfide bridge can be formed between a Cys introduced into the PAS cap and either Cys723 in the C-linker (de la Pena et al., 2013, 2014) or an introduced Cys (Y542C) in the S4/S5 linker (de la Pena et al., 2011). Together these studies suggest that the PAS cap can interact with both the S4/S5 linker and the C-linker regions of the hERG1 channel and that these interactions alter the kinetics of channel deactivation.

We recently studied concatenated hERG1 tetramers to investigate the stoichiometry of altered channel gating induced by RPR. Concatemers were constructed to contain a variable number of WT subunits and subunits containing the L553A mutation that presumably diminishes RPR binding affinity (Wu et al., 2015). RPR-induced slowing of deactivation was directly proportional to the number of WT subunits (and by inference, the number of high affinity sites available to bind RPR) incorporated into a concatenated tetrameric channel. In contrast, normal slow deactivation is an all-or-none process with regard to the requirement of full-length N-terminal domains (Thomson et al., 2014). Although our current findings indicate that differential sensitivity to the allosteric effects of RPR on channel gating can be swapped between hERG1 and rERG2 by mutating the few key nonconserved residues in the C-linker, the structural basis of RPR-induced slowing of hERG1 deactivation remains elusive. We previously reported that RPR has no effect on the kinetics, magnitude, or voltage dependence of hERG1 gating currents (Abbruzzese et al., 2010), indicating that the compound dissociates movement of the voltage sensor domain from the opening and closing of the activation gate. RPR could directly or by an allosteric mechanism stabilize the association between the PAS cap and the C-linker (Ng et al., 2014) or interfere with the coupling between the S4/S5 linkers and the C-terminal region of the S6 segments (Ferrer et al., 2006). Further

Fig. 11. Altered sensitivity of mutant hERG1 and rERG2 channels is not correlated with the basal rate of current deactivation. (A) Plot of the fast and slow time constants of deactivation (upper panel) and the relative amplitude of the fast component of deactivation (lower panel) of WT and mutant hERG1 channels as a function of the fold change in I_{tail} at -70 mV induced by $10 \mu\text{M}$ RPR. Lines represent linear regression analysis with adjusted R^2 values (in parentheses) as follows: τ_f (0.25), τ_s (0.16), and $A_f/(A_f+A_s)$ (0.12). (B) Plot of the time constants of deactivation (upper panel) and the relative amplitude of the fast component of deactivation (lower panel) of WT and mutant rERG2 channels as a function of the fold change in I_{tail} at -70 mV induced by $10 \mu\text{M}$ RPR. Lines represent linear regression analysis with adjusted R^2 values (in parentheses) as follows: τ_f (0.06), τ_s (0.27), and $A_f/(A_f+A_s)$ (0.32). The numbers shown in parentheses adjacent to the data points in the upper panels indicate the number of mutations harbored by the hERG1 (A) or rERG2 (B) channels. The number 0 indicates the WT channel.

experimentation will be required to distinguish between these and other possible mechanisms of RPR action.

Our study has a few limitations. First, we did not examine the effects of RPR on inactivation of WT ERG2 or the many hERG1 and rERG2 mutant channels. However, the peak of I_{tail} of mutant rERG2 channels containing the seven amino acid substitutions in the C-terminus to match hERG1 was increased by high concentrations of RPR (Fig. 9), an effect previously shown to be associated with a positive shift in the voltage dependence of inactivation in WT hERG1 channels (Perry et al., 2007). Second, poor expression of mutant ERG2 channels coupled with interference from endogenous currents at negative potentials in oocytes prevented analysis of deactivation kinetics over a broad range of voltages. Third, a Hill coefficient >1 for the [RPR]-response relationships could indicate positive ligand-binding cooperativity or could result from cooperative subunit interactions, as we previously found for two other hERG1 activators, PD-118057 [2-[[4-[(3,4-dichlorophenyl)ethyl]phenyl]amino]benzoic acid] and ICA-105574 [3-nitro-*N*-(4-phenoxyphenyl)-benzamide]. In our previous study of the effects of PD-118057 and ICA-105574 on concatenated hERG1 tetramers (Wu et al., 2014), we found that although subunit cooperativity contributes to the effects of these two hERG1 agonists, there was no change in EC_{50} or the Hill coefficient as the number of drug binding sites was increased from one to four. This finding indicates that occupancy of one binding site did not lead to an enhancement of PD-118057 and ICA-105574 affinity of other sites. In the [RPR]-response relationships presented in Fig. 3, the fold change in I_{tail} was determined using 9-second pulses, which is an insufficient duration to measure the full effect of the drug on deactivation. Thus, EC_{50} values are underestimated and Hill coefficients cannot be used as an

indicator of the extent of either subunit or drug binding cooperativity.

In summary, together with previous findings (Perry and Sanguinetti, 2008), our studies indicate that differential sensitivity of ERG channels to the slowing of deactivation induced by RPR is not explained by a common mechanism. Reduced sensitivity of rERG3 channels to RPR is explained by a single residue in the putative RPR binding pocket, whereas differences in the C-linker can account for the reduced effect of RPR on rERG2 compared with hERG1 channels. Our findings may also have clinical implications as it is clear that activators that display substantial selectivity among the different ERG channels can be developed. Activation of ERG1 channels can be achieved (e.g., for the treatment of congenital long QT syndrome) in the relative absence of effects on the related ERG2 and ERG3 channels that are prominently expressed in the central nervous system.

Authorship Contributions

Participated in research design: Gardner, Sanguinetti.

Conducted experiments: Gardner.

Performed data analysis: Gardner, Sanguinetti.

Wrote or contributed to the writing of the manuscript: Gardner, Sanguinetti.

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