Pharmacological Activation of Normal and Arrhythmia-Associated Mutant KCNQ1 Potassium Channels

Guiscard Seebohm, Michael Pusch, Jun Chen, Michael C. Sanguinetti

Abstract—KCNQ1 α-subunits coassemble with KCNE1 β-subunits to form channels that conduct the slow delayed rectifier K\(^+\) current (I\(_{\text{Ks}}\)) important for repolarization of the cardiac action potential. Mutations in KCNQ1 reduce I\(_{\text{Ks}}\) and cause long-QT syndrome, a disorder of ventricular repolarization that predisposes affected individuals to arrhythmia and sudden death. Current therapy for long-QT syndrome is inadequate. R-L3 is a benzodiazepine that activates I\(_{\text{Ks}}\) and has the potential to provide gene-specific therapy. In the present study, we characterize the molecular determinants of R-L3 interaction with KCNQ1 channels, use computer modeling to propose a mechanism for drug-induced changes in channel gating, and determine its effect on several long-QT syndrome–associated mutant KCNQ1 channels heterologously expressed in Xenopus oocytes. Scanning mutagenesis combined with voltage-clamp analysis indicated that R-L3 interacts with specific residues located in the 5th and 6th transmembrane domains of KCNQ1 subunits. Most KCNQ1 mutant channels responded to R-L3 similarly to wild-type channels, but one mutant channel (G306R) was insensitive to R-L3 possibly because it disrupted a key component of the drug-binding site. (Circ Res. 2003;93:941-947.)

Key Words: antiarrhythmic drugs ■ ion channel ■ arrhythmia

Normally the human heart beats with remarkable fidelity at an optimized rhythm to maintain normal blood pressure and adequate perfusion of the brain and other vital organs. However, if the heart rhythm is too rapid, adequate perfusion of the brain cannot be maintained, leading to syncope and sudden death. Long-QT syndrome (LQTS) is a disorder of ventricular repolarization that predisposes affected individuals to cardiac arrhythmias and sudden death. The most common form of LQTS is acquired, caused by medications that block cardiac K\(^+\) channels or as a secondary consequence of heart failure and is exacerbated by bradycardia and hypokalemia.\(^1\) Severely affected individuals can have intermittent syncope caused by a self-terminating arrhythmia called torsade de pointes, characterized by a sinusoidal twisting of the QRS axis around the isoelectric line of the ECG.\(^2\) Sudden cardiac death can occur if torsade de pointes arrhythmia degenerates into ventricular fibrillation. LQTS can also be inherited as an autosomal dominant (Romano-Ward syndrome) or recessive (Jervell and Lange-Nielsen syndrome) disorder due to loss-of-function mutations in cardiac K\(^+\) channel genes (HERG, KCNQ1, KCNE1, KCNE2) or gain-of-function mutations in the cardiac Na\(^+\) channel gene, SCN5A.\(^3\)

KCNQ1 and KCNE1 encode α- and β-subunits, respectively, that coassemble to form channels that conduct the slow delayed rectifier K\(^+\) current, I\(_{\text{Ks}}\).\(^4\) Mutations in either of these K\(^+\) channel subunits cause a decreased outward K\(^+\) current during the plateau phase of the cardiac action potential, delayed ventricular repolarization, and an increased QT interval.\(^5\) LQTS caused by mutations in KCNQ1 is often called LQT1 because this gene locus was the first to be identified with the disorder.\(^6\) Most common are missense mutations that result in change of a single amino acid\(^7\) and cause subunit misfolding that can disrupt the coassembly of subunits and usually leads to early degradation of the channel complex.

Treatment for inherited LQTS includes β-adrenergic receptor blockade, high thoracic left sympathectomy, and implantation of a cardioverter-defibrillator.\(^8\) While these therapies have considerably reduced mortality, there is a well-recognized need for improved treatments in LQTS, preferably tailored to specific genetic mechanisms. A drug that activates K\(^+\) channels that mediate ventricular repolarization might be useful for treatment of both acquired and inherited forms of LQTS. R-L3 ([(3-R)-1,3-dihydro-5-(2-fluorophenyl)-3-(1H-indol-3-ylmethyl)-1-methyl-2H-1,4-benzodiazepin-2-one]) is the first and only K\(^+\) activator to be identified.\(^9\) L3 shortens action potential duration\(^a\) and suppresses early afterdepolarizations in ventricular myocytes isolated from hypertrophied rabbit hearts, induced by renal artery banding.\(^10\) L3 also reverses action potential lengthening and suppresses early afterdepolarizations in rabbit myocytes treated with dofetilide, a drug that blocks the rapid delayed rectifier K\(^+\) current, I\(_{\text{Kr}}\), and thus

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mimics LQT2, a form of inherited LQTS caused by loss-of-function mutations in HERG.\(^9\) The effects of R-L3 on KCNQ1 and \(I_{Ks}\) channels are complex. The drug increases the magnitude of KCNQ1 currents recorded before (control) and after modification by 1 \(\mu\)mol/L R-L3. Currents were elicited with 2-second pulses to potentials of \(-80\) to \(+60\) mV, applied in 10-mV increments from a holding potential of \(-90\) mV. Tail currents were recorded at \(-60\) mV. \(D\) and \(E\) show the age dependence of current activation determined from peak tail currents measured at \(-60\) mV. Currents were normalized to the peak tail currents elicited after a pulse to \(+40\) mV.

The effects of R-L3 on KCNQ1 and \(I_{Ks}\) channels are complex. The drug increases the magnitude of KCNQ1 current, shifts the voltage dependence of activation to more negative potentials, and slows the rate of channel activation and deactivation.\(^9\) When KCNQ1 subunits are coexpressed with KCNE1 subunits (to mimic \(I_{Ks}\)), these effects are reduced. If an excess of KCNE1 is used in coexpression experiments, then R-L3 has no effect, suggesting that the presence of KCNE1 in the heteromultimeric channel complex prevents R-L3 binding to KCNQ1 subunits. To complicate matters further, at concentrations \(\geq 10 \mu\)mol/L, R-L3 reduces \(I_{Ks}\)\(^9\) similar to the effect of L7, a structurally related compound and pure antagonist that interacts with residues located in the S6 domain of KCNQ1. However, unlike R-L3, the effect of L7 is greater on \(I_{Ks}\) (IC\(_{50}\)=40 nmol/L) than KCNQ1 (IC\(_{50}\)=200 nmol/L).\(^11\) The mechanisms of R-L3 binding and modulation of KCNQ1 channels are not understood. In the present study, we use a scanning mutagenesis approach to localize the putative binding site for R-L3 to residues on the S5 and S6 domains of KCNQ1.

A drug with properties like R-L3 could be used for treatment of LQT1 caused by loss-of-function mutations in KCNQ1. A potential problem with use of an activator is that the disease-causing mutation in the channel protein could also prevent drug action. Therefore, we determined the effects of R-L3 on channels formed by heteromultimeric assembly of KCNE1 subunits plus wild-type and LQTS-associated mutant forms of KCNQ1 subunits. Most responded to R-L3 similarly to wild-type channels, suggesting the potential utility of a pharmacological approach to treatment of LQT1. Only one mutant subunit (G306R KCNQ1) rendered the channels insensitive to R-L3, possibly because it disrupted a key component of the drug-binding site. Together, our experimental studies and modeling provide a molecular mechanism for channel modulation by R-L3, a prototypic compound for gene-specific treatment of LQT1.

Materials and Methods

Molecular Biology

Site-directed mutagenesis of KCNQ1 cDNA subcloned into the pSGEM vector\(^12\) was performed by PCR using the megaprimer method\(^13\) and a 5:1 Pfu/Taq-polymerase mix. Constructs were confirmed by automated DNA sequencing. The constructs were linearized with NheI, and cRNA was transcribed in vitro with Capscribe (Roche Applied Science, Indianapolis, Ind) and T7 polymerase. KCNE1 cDNA was subcloned into the pSP64 vector. This construct was linearized with EcoRI, and cRNA was transcribed in vitro with Capscribe and SP6 polymerase.

Oocyte Isolation and Voltage Clamp

Oocytes were isolated from ovarian lobes of Xenopus laevis as previously described.\(^14\) To record KCNQ1 channel current, each oocyte was injected with 10 ng of KCNQ1 cRNA. To record \(I_{Ks}\), each oocyte was co-injected with 3 ng KCNQ1 cRNA plus 0.15 ng of KCNE1 cRNA. For voltage-clamp experiments, the oocytes were bathed in a nominal Cl\(^-\) saline solution containing (in mmol/L) NaOH 96, KOH 2, KOH 2, MgCl\(_2\) 1, Mes 101, and HEPES 5 (pH 7.6). A Dagan TEV-200 amplifier (Dagan Corp, Minneapolis, Minn) was used to record currents at 24°C in oocytes 2 to 3 days after injection with cRNA using standard two-electrode voltage-clamp techniques.\(^15\) Data acquisition was performed using a Pentium IV computer, a Digidata 1322 A/D interface, and pClamp 8 software (Axon Instruments, Union City, Calif).

The effects of R-L3 on WT and mutant KCNQ1 channel currents were determined by repetitive 5-second pulses to +10 mV applied every 20 seconds. Heteromeric KCNQ1/KCNE1 channel currents (\(I_{Ks}\)) were elicited every 30 seconds with 7-second depolarizing pulses to 0 mV. All currents were elicited from a holding potential of \(-80\) mV. R-L3 (Merck Research Laboratories, West Point, Pa) was prepared daily from a frozen 10 mmol/L DMSO stock solution.

Data Analysis

Data were analyzed with pCLAMP 8 (Axon Instruments, Union City, Calif) and Origin 7 software (OriginLab Corp, Northhampton, Mass). Data are reported as mean±SEM (n indicates the number of experiments), and Student’s t test was used to test for statistical significance between groups.

Modeling

The KcsA channel structure\(^16\) was retrieved from the Protein Data Bank (1BL8). A three-dimensional structural model of the S5-S6 domains of KCNQ1 was constructed based on the crystal structure of the corresponding domains of KcsA. The S5-S6 sequence of KCNQ1 shares 46% homology and 36% identity with KcsA. The KCNQ1 model was generated using the MODELER module of INSIGHTII version 98 (Accelrys, Burlington, Mass). Docking of an energy-optimized R-L3 conformer (ACD/Chemsketch, Advanced Chemistry Development, Inc, Ontario, Canada) in the KCNQ1 homology models was performed using a hydrophobic docking approach with the Global Range Molecular Matching program (Gramm v1.03 program).\(^17,18\) The noncovalent bond finder\(^19\) and CASTp\(^20\) were
changed to facilitate block by L7 in oocytes pretreated with 1 μmol/L R-L3 (●, KCNQ1 alone; ○, KCNQ1 plus R-L3; †, KCNQ1+KCNE1; ◀, KCNQ1+KCNE1 plus R-L3) (n=3 to 7). B, Concentration-response relationship for R-L3 activation of F340Y KCNQ1 channel current (n=3).

The averaged effect of 1 μmol/L R-L3 slowed the rate of activation, and slowed the rate of deactivation of channel activation by about 7-fold (Figure 1B). The averaged effect of 1 μmol/L R-L3 on the peak current-voltage relationship is shown in Figure 1C. In addition to increasing current magnitude, R-L3 shifted the voltage dependence of channel activation by about −10 mV (Figure 1D).

To gain insights into the mechanisms of action of R-L3, we first determined if this compound binds to the same site previously characterized for L7, a benzodiazepine that blocks KCNQ1 channels.11 If the compounds bind to the same site, then we predict that pretreatment with R-L3 would diminish channel block by L7. However, the block of KCNQ1 (or IC50) by concentrations of L7 ranging from 1 to 100 nmol/L was facilitated by pretreatment of oocytes with 1 μmol/L R-L3 on the peak current-voltage relationship is shown in Figure 1C. In addition to increasing current magnitude, R-L3 shifted the voltage dependence of channel activation by about −10 mV (Figure 1D).

The effects of R-L3 on KCNQ1 current amplitude and kinetics are illustrated in Figure 1. Similar to a previous report,9 R-L3 increased current amplitude at all potentials, slowed the rate of activation, and slowed the rate of deactivation (Figure 1B). The averaged effect of 1 μmol/L R-L3 on the peak current-voltage relationship is shown in Figure 1C. In addition to increasing current magnitude, R-L3 shifted the voltage dependence of channel activation by about −10 mV (Figure 1D).

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To determine residues of the KCNQ1 channel that affected interaction with R-L3, point mutations were introduced into regions of the channel (Figure 3A) previously shown to reduce block by a structurally related compound, L7.11 Based on preliminary findings, we also chose to mutate individual amino acids located in the S5 domain. Residues in S5 and S6 were individually mutated to Ala (or Ala to Cys). Mutation to Ala of residues in the pore helix of KCNQ1 resulted in several nonfunctional channels. Therefore, a portion of the pore loop (V307 to T312) was scanned by introducing relatively conserved mutations. Some mutant KCNQ1 subunits (F270A, L273A, T309S, P343A) either did not form
functional channels or were expressed too poorly to evaluate drug sensitivity. Wild-type (WT) and mutant KCNQ1 channels were expressed in Xenopus oocytes and tested for their sensitivity to 1 μmol/L R-L3, a maximally effective concentration. WT KCNQ1 channel current was increased 92±8% (n=14), whereas as shown in Figure 3B, some mutant channels were less sensitive (eg, Y267A, I268A) to the drug. Mutation of four residues in the S5 domain (Y267, I268, L271, G272) and two residues in the S6 domain (F335, I337) caused the most significant (P<0.001) decrease in drug sensitivity (Figure 3C). In addition, three residues in the pore helix and three residues in the S6 domain caused more modest decreases (P<0.05) in sensitivity to R-L3. Three mutant channels (I268A, F339A, F340A) inactivated more than WT channels; however, there was no obvious correlation between altered gating and sensitivity to R-L3. Mutant channel analysis cannot easily distinguish between direct and allosteric ligand interactions. However, because the important residues were clustered together (see below), it is likely that R-L3 binds to specific residues of the S5 and S6 domains.

Molecular Modeling of R-L3 Docked to KCNQ1

The putative binding site defined above by mutation analyses was corroborated with molecular modeling. We constructed a homology model of the S5-S6 domains of KCNQ1 based on the x-ray coordinates of the KcsA K⁺ channel crystal structure. Automated docking of the R-L3 molecule predicted interaction of the fluorophenyl-methyl-benzodiazepine with residues in S5, and positioning of the indole into a pocket of about 5 Å formed between the S5 and S6 domains (Figure 4). To achieve this preferred docking, the S6 domain was rotated 5 degrees counterclockwise along its longitudinal axis, a movement that may accompany K⁺ channel opening. Further analysis using the noncovalent bond finder module of Protein Explorer revealed potential interaction (<0.4 Å) with the following amino acids: Y267, I268, G269, L271, G272, V310, F332, F335, and A336. All amino acids predicted to interact with R-L3 by this analysis, except G269, were also identified by the Ala screen.

Model for Slow KCNQ1 Gating Caused by R-L3

R-L3 has multiple effects on KCNQ1 channel current. The drug increases the amplitude of current, especially those induced in response to weak depolarization. This effect arises in part from a leftward shift in the voltage dependence of channel activation. In addition, R-L3 slows the rate of current activation and deactivation. We made a limited number of changes to a kinetic model of KCNQ1 channel gating to simulate these changes.

A published KCNQ1 channel gating model was altered to include a third closed state to allow a more precise description of the current time course. The individual states and the transition rates were as follows:

\[
C_1 \xrightleftharpoons{r_1} C_2 \xrightleftharpoons{r_2} C_3 \xrightleftharpoons{r_3} C_4 \xrightleftharpoons{r_4} C_5 \xrightleftharpoons{r_5} O_1 \xrightleftharpoons{r_6} O_2 \xrightleftharpoons{r_7} O_3 \xrightleftharpoons{r_8} O_4 \xrightleftharpoons{r_9} O_5
\]

Each rate constant was assumed to be of the form:

\[
r = r(0)e^{-z_i \phi}
\]

with \(\phi = \frac{VF}{RT}\), \(r(0)\) = rate constant at 0 mV and \(z_i\) = gating valence. The model parameters were adjusted by a time-course fit of voltage-clamp data. The open channel \(iV\) was empirically approximated by \(i(E) = (E + 92 \text{ mV})[1 + \exp(E/140 \text{ mV})]\) to account for the single-channel rectification at positive voltages. The inclusion of the rectifying factor \(1/[1 + \exp(E/140 \text{ mV})]\) had little influence on the calculations.

The resulting parameters in the absence of the activator were (rates in s⁻¹):

\[
\begin{align*}
\alpha_1(0) &= 17.7, z\alpha_1 = 1.7; \beta_1(0) = 6.8 \times 10^{-5}, z\beta_1 = -2.5 \\
\alpha_3(0) &= 3.7, z\alpha_3 = 0.005; \beta_3(0) = 144, z\beta_3 = -1.7 \\
\alpha_0(0) &= 223, z\alpha_0 = 0.02; \beta_0(0) = 32, z\beta_0 = -0.01 \\
\delta(0) &= 13, z\delta = 0.005; \delta(0) = 1.0, z\delta = -1.26 \\
\lambda(0) &= 71, z\lambda = 0; \mu(0) = 26, z\mu = 0
\end{align*}
\]

To obtain a model that required minimal changes to describe the data in the presence of R-L3, the following strategy was used. First, it was assumed that the activator might easily change the absolute current magnitude by altering the fast flickery gating process that underlies the large increase of currents that occurs when K⁺ is replaced by Rb⁺ as a charge carrier. Rb⁺ increases the current magnitude by about a factor of three without gross changes of the activation, inactivation, or deactivation kinetics. Indeed, the current increase caused by R-L3 in steady state must either reflect a decrease of the flicker closed probability, an increase of the single-channel current amplitude, or a decreased probability of inactivation. The former two possibilities
cannot be distinguished using macroscopic recordings alone. The latter possibility was tested and falsified in the context of the model. However, we recently suggested23 that the inactivation and the fast flicker reflect the same basic mechanism of a flickery gating process. Thus, the increase in steady-state current caused by R-L3 suggests either a reduced flicker closed probability or an effective increase of the single-channel current.

More difficult to explain are the two major kinetic effects of R-L3: a simultaneous slowing of activation and deactivation. In a first attempt, it was assumed that a single kinetic parameter ($r$) is changed by the drug.

Thus, for all kinetic steps, either $r(0)$ was allowed to vary. None of these models could account for the kinetic effects. In a next step, combinations of two parameters were allowed to vary. To fit currents in the presence of R-L3, the value of $e(0)$ was increased by $\sim 150$-fold to 2287 s$^{-1}$. In addition, a modest change in $\alpha_3(0)$, from 223 s$^{-1}$ to 170 s$^{-1}$, improved the fit. Thus, the major effects of R-L3 can be modeled by an acceleration of the transition from $O_1$ to $O_2$. The results of the model are illustrated in Figure 5 and in the online data supplement available at http://www.circresaha.org.

**Activation of LQTS-Associated Mutant KCNQ1 Channels by R-L3**

Pharmacological activation of $I_{Ks}$ is a potentially useful and gene-specific treatment for LQT1. Therefore, we determined if R-L3 could activate KCNQ1/KCNE1 ($I_{Ks}$) channels harboring one of eight previously identified LQTS-associated mutations. Oocytes were coinjected with 3 ng WT KCNQ1 cRNA and 0.15 ng KCNE1 cRNA (a 3.8:1 molar ratio) to induce a current (WT $I_{Ks}$, Figure 6A) with activation kinetics similar to native cardiac $I_{Ks}$,24 and still sensitive to the activator. As we previously reported,9 WT $I_{Ks}$ was less sensitive than KCNQ1 current to activation by R-L3. The average increase of WT $I_{Ks}$ induced by 1 $\mu$mol/L R-L3 varied between the 4 batches of oocytes used in these experiments, but averaged 47±7% (n=20). To evaluate the effects of drug on LQT1-associated mutant channel current (LQT1 $I_{Ks}$), oocytes were injected with 1.5 ng of WT and 1.5 ng mutant KCNQ1 cRNA, plus 0.15 ng KCNE1 cRNA. As previously reported,7,25–31 each LQT1-associated mutation caused a reduction in current magnitude compared with WT $I_{Ks}$. Five of the KCNQ1 mutant channels were activated by 1 $\mu$mol/L R-L3 similarly to WT $I_{Ks}$, including channels containing R243C, W248R, L273F, A341V, or G345E KCNQ1 subunits (Figure 6D). For mutations that cause loss of function (eg, A341V), the effect of the drug is likely due to activation of channels formed by coassembly of WT KCNQ1 and KCNE1 subunits. However, LQT1 $I_{Ks}$ conducted by channels containing E261K KCNQ1 subunits was activated 3 to 7 times more than WT $I_{Ks}$ (Figure 6B). The reason for this supernormal response to R-L3 is unknown, but may result from activation of mutant channels that have an abnormally low open probability in the absence of drug. LQT1 $I_{Ks}$ conducted by channels containing G306R KCNQ1 subunits was not activated at all and in fact was slightly blocked by 1 $\mu$mol/L R-L3 (Figure 6C). The effects of R-L3 on WT $I_{Ks}$ and LQT1 $I_{Ks}$ are summarized in Figure 6D. These data illustrate the spectrum of response of LQT1 $I_{Ks}$ to R-L3.

**Discussion**

Substituted benzodiazepines have diverse pharmacology, including activation of $\gamma$-aminobutyric acid-A receptors, block of gastrin or cholecystokinin receptors,33,34 and block of KCNQ1 channels and cardiac $I_{Ks}$.35 In this study, another pharmacological action of a benzodiazepine was investigated. R-L3 is a benzodiazepine that is a partial agonist of KCNQ1 channels, causing activation at concentrations up to 1 $\mu$mol/L and block at 10 $\mu$mol/L.9 In addition to altering the amplitude of KCNQ1 current, R-L3 slows the rate of activation and deactivation of channels. The increase in steady-state current caused by R-L3 suggests either a reduced flicker closed probability or an increase in single-channel conductance. Our data cannot distinguish between these two possibilities, and direct measurement of single-channel activity is not possible because it is below the limit of reliable detection.36,37

A computer model was used to simulate channel gating and suggests that the effects of R-L3 can be simulated by slightly reducing the rate of transition from the last closed state to the first open state and by increasing the rate from $O_1$ to $O_2$ such that $O_1$ is seldom occupied. The structural basis of these changes in channel gating is unknown. However, we used a site-directed mutagenesis approach to identify residues in KCNQ1 subunits that interact with R-L3. The location of the putative docking site for R-L3 contrasts with that of a structurally related benzodiazepine, L7, that blocks KCNQ1 channels. Functional experiments also suggest separate binding sites for the two drugs; pretreatment of oocytes with R-L3 facilitated the block by L7, a compound that preferentially blocks the open state of KCNQ1 channels.11 Our modeling suggests that R-L3 stabilizes the open state of KCNQ1.

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**Figure 5.** Simulated effects of R-L3 on KCNQ1 currents. A, Currents recorded in a single oocyte (data) under control conditions and model currents (fit). B, Currents recorded from the same oocyte after treatment with 1 $\mu$mol/L R-L3 (data) and corresponding model currents (fit). Currents were elicited with 2-second pulses to test potentials ranging from −70 to +50 mV, applied in 20-mV increments. Holding potential was −80 mV and tail currents were recorded at −60 mV.

**Figure 6.** A, Activation of WT (−) and LQT1 (○) KCNQ1 channels by R-L3. B, Currents from WT (−) and LQT1 (○) KCNQ1 channels exposed to 1 $\mu$mol/L R-L3 for 15 sec. C, Currents from WT (−) and LQT1 (○) KCNQ1 channels exposed to 10 $\mu$mol/L R-L3 for 15 sec. D, Currents from WT (−) and LQT1 (○) KCNQ1 channels exposed to 1 $\mu$mol/L R-L3 for 15 sec.

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channels and could therefore enhance block by L7. The putative binding site for L7 determined by a similar Ala-scanning approach was located to residues of the S6 domain and residues at the base of the selectivity filter that face the central cavity.11 Models of drug docking have the obvious limitation that the homology model for KCNQ1 channels is based on the crystal structure of the bacterial channel KcsA that only includes transmembrane domains equivalent to S5 and S6 of KCNQ1. This limitation is of special concern for a drug like R-L3 that appears to interact with residues outside the central cavity where it is unknown how the remainder of the protein (S1-S4) may affect the pore domains (S5-S6). However, although our model for the binding of R-L3 must be viewed with caution, mutational analyses suggest that the binding site for benzodiazepine agonists and antagonists do not overlap. We previously reported that KCNQ1 channels containing indicated mutant KCNQ1 subunits before and after treatment of oocyte with 0.15 mol/L R-L3. Data are expressed relative to amplitude of current in oocytes expressing WT Iks in the absence of drug. Relative current magnitude before (cross-hatched bars) and after R-L3 (unfilled bars) are indicated (n=4 to 9). Error bars represent SEM. *P<0.05.

Figure 6. R-L3 activates most LQTS-associated Iks mutant channels. A, WT Iks elicited by 7-second pulses to 0 mV before and after (#) treatment of oocyte with 1 μmol/L R-L3. B and C, Iks currents of indicated mutant KCNQ1 subunits before and after treatment of oocyte with 1 μmol/L R-L3. D, Peak currents of Iks in oocytes injected with 0.15 ng KCNE1+1.5 ng each of WT and indicated KCNQ1 mutant cRNAs. Relative current magnitude before (cross-hatched bars) and after R-L3 (unfilled bars) are indicated (n=4 to 9). Error bars represent SEM. *Percent activation significantly different than WT Iks, P<0.05.

mutations were investigated. It is possible that mutation to other residues would have produced greater changes in apparent drug affinity. Third, we made no attempt to detect additive or synergistic effects due to mutation of more than a single residue at a time. Finally, altered drug sensitivity may have been caused by an allosteric effect not directly related to the specific amino acid that was mutated. Nevertheless, the mutated residues in S5 and S6 that affected R-L3 action were clustered together, consistent with a binding pocket, and were distinct from the binding site defined for L7, a structurally similar benzodiazepine that blocks the channel.

Genotype-specific pharmacological treatment for LQTS holds great promise for tailored therapy and has been evaluated for individuals with loss-of-function mutations in HERG (LQT2) or gain-of-function mutations in the cardiac Na+ channel gene SCN5A (LQT3). Elevation of serum [K+] activates HERG K+ channels39 and shortens QT interval in patients with LQT2.40 Mutations in SCN5A disrupt channel inactivation and induce a maintained Na+ current41 that can be blocked by mexiletine, an antiarrhythmic drug that shortens the QT interval in LQT3 patients.42 Our studies portents KCNQ1 channel activation as a promising gene-specific treatment for LQT1.

The spectrum of response to R-L3 (Figure 6) indicates that pharmacological therapy of LQT1 could be effective for most, but not all, KCNQ1 mutations. Introduction of an Arg residue at position 306 in the homology model suggests a plausible explanation for the lack of effect of R-L3 on G306R KCNQ1 Iks. The large side group of Arg (compared with Gly) occludes a space between S5 and S6 predicted to form part of the binding pocket for R-L3. An alternative explanation is that coexpression of WT and G306R subunits may result in the formation of functional Iks channels that contain a higher amount of minK subunits relative to KCNQ1 subunits, preventing activation of channels by R-L3.9

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References


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Supplemental Figure 1.
Effects of R-L3 on voltage dependence of KCNQ1 activation and kinetics of deactivation predicted by the model.
(a) Apparent $p_{\text{open}}(V)$ in control (filled symbols) and in the presence of 1 µM R-L3 (open symbols) and the respective predictions of the model (solid and dashed line, respectively). The values are normalized to that measured at +50 mV. The $V_{1/2}$ and slope factor ($k$) values of the fit were: -18 mV and 9.2 mV for control, and -32 mV and 8.9 mV in the presence of R-L3.
(b) Time constants of exponential fits to tail currents. In control (filled symbols) the sum of two exponentials was fitted. In the presence of R-L3 only one exponential could be extracted. For voltages > -100 mV the relaxation was so slow that no fit could be performed. The predictions were obtained by fitting predicted tail currents with 2 (control, solid lines) or 1 exponential (broken line).