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K^+ Channel Structure-Activity Relationships and Mechanisms of Drug-Induced QT Prolongation

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■ **Abstract** Pharmacological intervention, often for the purpose of treating syndromes unrelated to cardiac disease, can increase the vulnerability of some patients to life-threatening rhythm disturbances. This may be due to an underlying propensity stemming from genetic defects or polymorphisms, or structural abnormalities that provide a substrate allowing for the initiation of arrhythmic triggers. A number of pharmacological agents that have proven useful in the treatment of allergic reactions, gastrointestinal disorders, and psychotic disorders, among others, have been shown to reduce repolarizing K^+ currents and prolong the QT interval on the electrocardiogram. Understanding the structural determinants of K^+ channel blockade may provide new insights into the mechanism and rate-dependent effects of drugs on cellular physiology. Drug-induced disruption of cellular repolarization underlies electrocardiographic abnormalities that are diagnostic indicators of arrhythmia susceptibility.

INTRODUCTION

The delicate synchronization of cardiac ion channels underlies the spread of cardiac electrical excitation that is coupled to continuous rhythmic contraction of the heart. The disruption of the precise ionic balance resulting from administration of pharmacological agents or congenital defects may undermine the cardiac electrical syncytium, which can undermine coordinated contraction and lead to insufficient pressure for blood circulation.

Cardiac excitation originates in the sino-atrial node and propagates through the atria into the atrial-ventricular node. The impulse then enters the Purkinje conduction system, which delivers the excitatory wave to the ventricles. Ventricular excitation spreads from the endocardium to the epicardium and is coupled to the

contraction of the ventricles that generates systolic blood pressure. The wave of excitation that spreads over the heart reflects membrane depolarization of cardiac myocytes, due primarily to activation of fast voltage-dependent Na^+ channels that underlie the action potential upstroke. Activation is followed by a long depolarized plateau phase that permits Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum, binding of Ca^{2+} to contractile proteins on the sarcomeres, and coordinated contraction (systole). Repolarization follows due to the activation of time- and voltage-dependent activation of repolarizing potassium currents. Relaxation of contraction is coupled to the electrical repolarization phase, which allows filling of the ventricles (diastole) prior to the next excitation. Each of these electrical processes can be detected on the body surface electrocardiogram (ECG) as a signal average of the temporal and spatial gradients (∇Vm) generated during each phase (1–3) (ECG, Figure 1*A*). Electrical excitation gradients in the atria (atrial depolarization) manifest on the ECG as P waves, whereas gradients of ventricular depolarization are seen as the QRS complex. Gradients in ventricular repolarization are reflected in the T wave (Figure 1).

Electrocardiographic abnormalities are related to changes in cellular action potential morphologies, which may be due to altered cell-to-cell coupling, congenital ion channel abnormalities, drug intervention, or electrolyte imbalance (1–3). Conduction abnormalities can be detected as changes in the QRS complex. Widening of the QRS reflects reduced conduction velocity, which typically

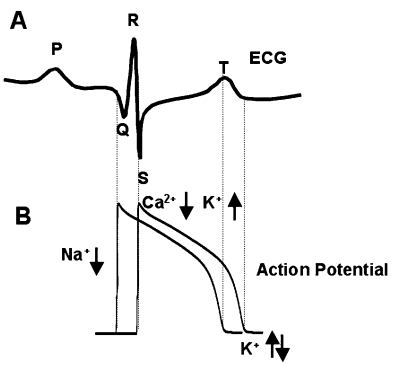

Figure 1 Electrical gradients in the myocardium can be detected on the body surface ECG. (*A*) An illustrative example of a single cardiac cycle detected as spatial and temporal electrical gradients on the ECG. The P wave is generated by the spread of excitation through the atria. The QRS complex represents ventricular activation and is followed by the T wave reflecting ventricular repolarization gradients. (*B*) Schematic representation of cellular electrical activity underlying the ECG [see text and (1) for details]. Arrows indicate the direction of ion flow during each phase of the action potential.

stems from altered Na^+ channel function (4). ST segment elevation reflects transmural voltage gradients during the action potential plateau, a hallmark of congenital forms or drug-provoked Brugada syndrome (5–7). Prolongation of the action potential duration (APD) (delayed repolarization) results in long QT intervals and may result in morphological changes in the T wave that can provide insight as to the underlying cellular mechanism of APD prolongation (1, 8–10).

Individuals displaying ECG abnormalities may be at higher risk of lethal arrhythmias associated with syncope and sudden death. Many such arrhythmic events are rate dependent and may be linked to sudden changes in heart rate due to exercise or auditory stimulation that may trigger life-threatening arrhythmias (10–14).

Pharmacological intervention, often for the purpose of treating syndromes unrelated to cardiac disease, can increase the susceptibility of some patients to life-threatening rhythm disturbances (15–17). This may be due to underlying vulnerability stemming from genetic defects or polymorphisms, gender, or structural abnormalities that provide a substrate allowing for the initiation of arrhythmic triggers (16–19). Indeed, a number of histamine-blocking drugs, including antihistamines astemizole and terfenadine and more recently loratadine, have been shown to block HERG (human ether-a-go-go-related gene) channels as an adverse side effect and prolong the QT interval of the ECG (20). Cisapride (Propulsid®) is a gastrointestinal agent used to treat nocturnal heartburn as well as a variety of other gastrointestinal disorders, which also as a side effect blocks HERG K^+ channels and is associated with acquired long QT syndrome and ventricular arrhythmias, such as torsades de pointes (21, 22). In a study of patients with paranoid psychosis, changes in the morphology of the ECG T wave were observed in more than 85% of traces when the plasma concentration of the antipsychotic drug thioridazine was greater than 1 μM (23) due to blockade of I_{Kr} (IC_{50}, 1.25 μM) and I_{Ks} (IC_{50}, 14 μM). Unfortunately, examples of inadvertent side effects of pharmacological agents on cardiac K^+ channels are plentiful (Table 1). As a result, screening affinity of new therapeutic agents for cardiac K^+ channels is becoming commonplace in the drug development process.

Supraventricular tachyarrhythmias are generally treated with pharmacological intervention. K^+ channel blockers are one such therapy that acts to increase action potential duration and the effective refractory period to prevent premature re-excitation. Although these interventions can be useful in targeting tachyarrhythmias, they may predispose some patients to the development of other types of arrhythmias (24). However, the proarrhythmic effects of some drugs may actually prove useful as diagnostic indicators of underlying vulnerability to arrhythmia. HERG K^+ channels and to a lesser extent I_{Ks} are of considerable pharmaceutical interest as potential therapeutic targets for antiarrhythmic agents and as the molecular targets responsible for the cardiac toxicity of a wide range of pharmaceutical agents (25, 26).

TABLE 1 Classes of drugs with QT-prolonging potential (16, 17, 123)

Drug class	Drug (trade name)
Class IA antiarrhythmic agents	qunidine procainamide (Procan, Procanbid) disopyramide (Norpace)
Class III antiarrhythimic agents	sotalol (Betapace) dofetilide (Tikosyn) ibutilide (Covert)
Antianginal agents	bepridil (Vascor)
Hypocholesterolemic agents	pubucol (Lorelco)
Catecholaminergic agents	epinephrine (Adrenaline)
Antihistamines	terfenadine (Seldane) astemizole (Hismanol) loratadine (Claritin) diphenhydramine (Benadryl)
Antibiotics	erythromycin (E-Mycin, EES, EryPeds, PCE) pentamidine (Pentam) grepafloxacin (Raxar) sparfloxacin (Zagam) moxifloxacin (Avelox)
Antimalarials	halofantrine mefloquine (Lariam) chloroquine
Antifungal agents	ketoconazole (Nizoral) fluconazole (Diflucan) itraconazole (Sporanox)
Antidepressants	desipramine (Norpramin) nortriptyline amitriptyline (Elavil)
Antipsychotics	chlorpromazine (Thorazine) haloperidol (Haldol) thioridazine (Mellaril) droperidol (Inapsine) pimozide (Orap) risperidone (Risperdal) sertindole (Serdolect)
Gastrointestinal	cisapride (Propulsid)
Diuretics	indapamide (Lozol)
Impotence	sildenafil (Viagra)
Miscellaneous	ketanserin, tacrolimus (FK506) (Prograf) tamoxifen (Nolvacex) indapamide (Lozol) terodiline, potassium loss

I_{Kr}, THE RAPIDLY ACTIVATING COMPONENT OF THE DELAYED RECTIFIER K^+ CURRENT

The Physiological Role of I_{Kr} in the Heart

Two components of I_K (delayed rectifier K^+ current) have been separated on the basis of the activation kinetics: a rapidly activating component (I_{Kr}, encoded by HERG) and slowly activating component (I_{Ks}) (27). I_{Kr} can also be revealed pharmacologically by its sensitivity to block by class III antiarrhythmic drugs, such as E-4031 (28, 29) and dofetilide (30–32). HERG K^+ channels exhibit strong inward rectification (33) due to rapid voltage-dependent C-type inactivation (34, 35). Unlike C-type inactivation in many other channels, HERG inactivation appears to be unique in that it possesses intrinsic voltage dependence (34, 35).

HERG channels activate from closed to open states ($C \rightarrow O$) upon depolarization, but pass very little outward current because they rapidly inactivate ($O \rightarrow I$). HERG channels can also inactivate from closed states ($C \rightarrow I$) (36). Inactivation from both pathways results in the accumulation of HERG channels in inactivated states during depolarization. Channels then reopen, or open for the first time, during repolarization as they recover from inactivation through the open state ($I \rightarrow O$) (Figure 2*A*). HERG has unique channel properties that give rise to I_{Kr} current during repolarization of the cardiac action potential (Figure 2*B*) (36–38).

The Molecular Basis of HERG and I_{Kr} Current

The α-subunit of I_{Kr} is encoded by HERG (33, 39, 40). The topology of HERG channels is similar to many voltage-gated channels in that they are homo-tetramers of identical six *trans*-membrane spanning domains (S1–S6). A cluster of positive charges is localized in the S4 domain and acts as the putative activation voltage sensor (41).

There are, however, marked differences between native I_{Kr} current and HERG-induced currents in heterologous expression systems in terms of gating (42, 43), regulation by external K^+ (44–46), and sensitivity to antiarrhythmics (33). These data suggest the presence of a modulating subunit that co-assembles with HERG in order to reconstitute native I_{Kr} currents. A likely candidate is the minK-related protein 1 (MiRP1 = KCNE2), which when co-expressed with HERG, results in currents similar to native I_{Kr} (42). Coexpression with MiRP1 causes a $+5 \rightarrow 10$ mV depolarizing shift in steady-state activation, accelerates the rate of deactivation, and causes a decrease in single channel conductance from 13 to 8 pS (42). However, a specific and selective interaction of HERG and MiRP1 in the myocardium has not yet been demonstrated (47, 48), and other factors may contribute to the functional differences between native I_{Kr} current and HERG-induced currents in heterologous expression systems. Several alternatively spliced ERG1 variants have been demonstrated in the heart (49–51) and there is evidence for posttranslational modification of HERG proteins (52, 53).

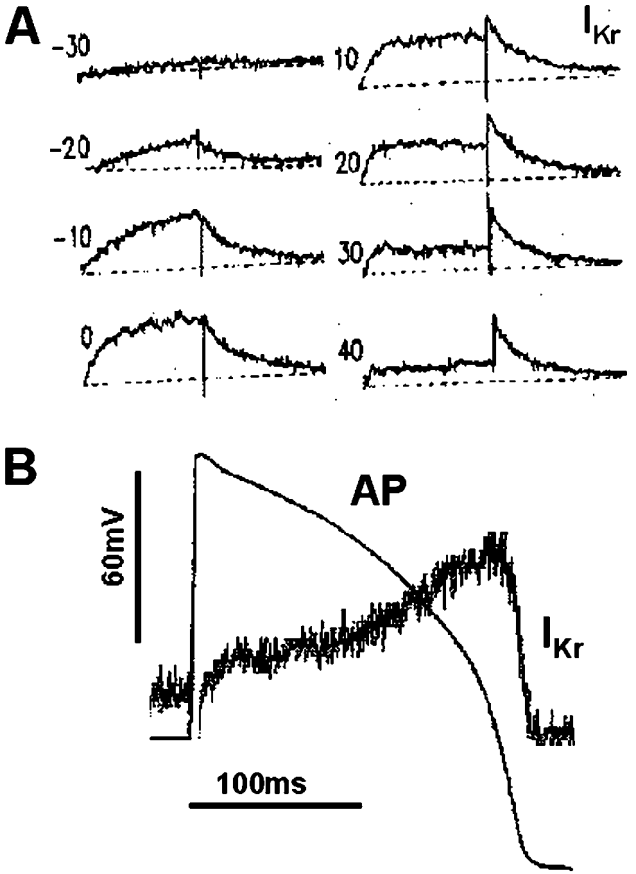

Figure 2 I_{Kr}, the fast component of the delayed rectifier current. (*A*) Time course of I_{Kr} at physiological temperature and ion concentrations (27). The cell is depolarized to the indicated test potential for 250 ms from a holding potential of −40 mV and then repolarized to −40 mV. The hook in the tail current reflects recovery from inactivation through the open state. (*B*) I_{Kr} recorded during an action potential clamp (121). Because recovery from inactivation (I → O) is very fast compared to deactivation (O → C), a large outward current appears during repolarization as channels slowly return to the closed resting states.

Structural Basis of I_{Kr}/HERG Blockade

Recent studies have revealed the molecular basis of the promiscuity of HERG K^+ channel drug binding and have provided further insight into the structure and function of HERG K^+ channels. I_{Kr} is the primary target of methanesulfonanilides (dofetilide, E-4031, ibutilide, and MK-499), a group of potent and specific class III antiarrhythmic drugs that prolong APD (27, 54). HERG channels can also be

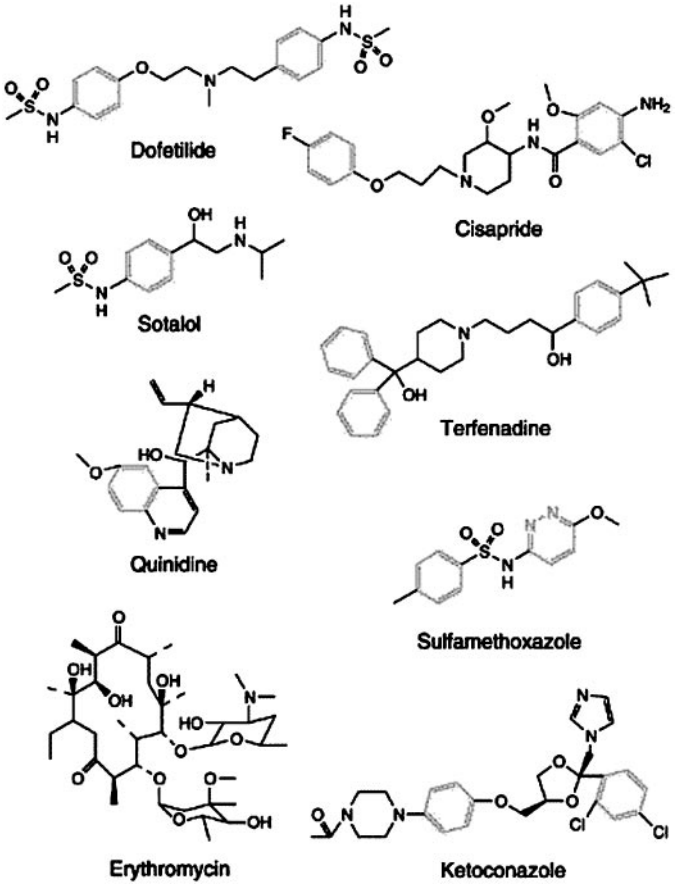

Figure 3 Promiscuous HERG channels are blocked by structurally diverse molecules [from (122)]. In addition to compounds developed for K^+ channel blockade (Dofetilide and Sotalol), a wide range of compounds, from antihistamines (Terfenadine) to antibiotics (Erythromycin), block HERG as an adverse side effect.

blocked by an array of other pharmacological agents with diverse chemical structures (55) (Figure 3). Recent studies have suggested the involvement of aromatic residues in the S6 domain (Y652 and F656) unique to eag/erg K^+ channels that may underlie the structural mechanism of preferential block of HERG by a number of commonly prescribed drugs (56) (Figure 4).

Initial investigation of the HERG antagonist binding site was carried out via site-directed mutagenesis techniques. One study (32) revealed that a single residue, Ser620 in the H5 domain of the S5-S6 linker of HERG, altered the sensitivity of the channel to dofetilide. The altered residue was believed to affect drug binding via an allosteric effect related to loss of inactivation. A more recent study (57) reported

that Phe656 in S6 was necessary, although not sufficient, for high-affinity binding of dofetilide and quinidine, but did not affect binding of tetraethyl ammonium (TEA) and did not disrupt inactivation.

Homology modeling based on crystallographic structure of the bacterial K^+ channel KcsA (58) predicts that Phe656 falls within the HERG pore region. This result was confirmed and extended in an elegant study by Mitcheson et al. (56) who identified four residues in addition to Phe656 that were crucial for high-affinity binding by methanesulfonanilides, namely Tyr652, Gly648, Val625, and Thr623. Using similar homology modeling of HERG channels, the authors showed that the aromatic rings of methanesulfonanilides are likely to interact with the aromatic rings of Tyr652 and Phe656 [Figure 4; from (56)] The crucial role of Tyr652 and Phe656 was confirmed by studies using cisapride and terfenadine, whereas Gly648, Val625, and Thr623 were found to be more specific for methanesulfonanilides (56).

The importance of residues Tyr652 and Phe656 was also demonstrated for the low affinity ligand choloroquine, an antimalarial agent that appears to preferentially block open HERG channels. Blockade of HERG by chloroquine requires channel opening followed by interactions of the drug with the aromatic residues in the S6 domain that face the central cavity of the HERG channel pore (59).

State-Specific Block of I_{Kr}

The biophysical properties of HERG blockade are consistent with a discrete state-dependent blocking mechanism (60, 61). Initial HERG channel studies demonstrated that methanesulfanonilides require channel opening for access to a presumptive intracellular binding site (54). Mutations that result in loss of inactivation act to reduce affinity for methanesulfonanilides, suggesting that inactivation may be required for drug binding. However, methanesulfanonilides are less effective at inhibiting HERG K^+ channels during strong depolarization (e.g., +60 mV), which promotes inactivation (60–62) and would therefore be expected to favor drug binding if the drugs bind to the inactivated state. A possible explanation for this apparent discrepancy may be that channel opening is required for a drug to contact its binding site, which becomes accessible as channels inactivate. At positive voltages, extremely rapid inactivation may reduce the channel open time sufficiently to prevent the drug from accessing the binding site. This idea has recently been proposed as a mechanism of flecainide binding to Na^+ channels, where channel opening is required for flecainide to bind to inactivated channels (63).

Recovery from HERG blockade by methanesulfonanilides is extremely slow, even at negative holding potentials when most channels are in closed states. Using a mutant HERG (D540K) channel that has the unusual property of opening in response to hyperpolarization (64), it was shown that methanesulfonanilides are trapped in the inner vestibule by closure of the activation gate. Opening of the channel in response to hyperpolarization allowed release of the drug from its receptor.

HERG trapping of MK-499, despite its large size, suggests that the vestibule of the HERG channel is larger than the well-studied Shaker K^+ channel. Indeed,

homology modeling based on the KcsA structure revealed two unusual features of the HERG inner vestibule (the site of drug block) that are unique among potassium channels (56). Other voltage-gated K^+ channels contain a Pro-X-Pro sequence in the S6 domain that has been predicted to "kink" the S6 segment and therefore limit the size of the inner vestibule (65). In addition, HERG K^+ channels have two aromatic residues predicted to face the inner pore, whereas other K^+ channels lack these residues, or in the case of KCNQ1, contain only one. As shown in the molecular model of Mitcheson et al. (56), these residues (Y652 and F656) are crucial for electrostatic interactions between aromatic rings of Y652/F652 and the drug molecules.

Cellular Consequences of I_{Kr} Blockade

The HERG channel subunit was originally identified by genetic studies on patients with the congenital long QT syndrome. Incorporation of mutated HERG subunits in the channel tetramer generally cause a reduction of I_{Kr} current, which leads to prolongation of the ventricular action potential (Figure 5). A delay in ventricular repolarization predisposes the heart to arrhythmogenic early afterdepolarizations (38, 66, 67).

Both the cellular effects of these congenital defects and the resulting electrocardiographic abnormalities are analogous to those seen with inhibition of HERG channels by a variety of compounds. Reductions in I_{Kr} result in prolongation of APD and dispersion of repolarization across the wall of the ventricle, which manifests on the ECG as prolongation of the QT interval and widening of the T wave, respectively (1). The ECG alterations have been associated with an increased risk of arrhythmias and sudden cardiac death. Certain factors can increase the disruption of the repolarization balance (e.g., hypokalemia due to diuretics and sudden changes in pacing rate), and can exacerbate the arrhythmogenic effect of HERG-blocking drugs. These additional interventions may result in the appearance of notched T waves on the ECG (1, 2). The recognition of the fundamental role played by the K^+ channels encoded by HERG in cardiac pathophysiology has the potential to improve the understanding of mechanisms of arrhythmogenesis.

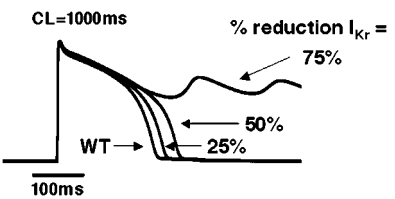

Figure 5 Reduction of I_{Kr} prolongs action potential duration. Here, the prolongation effects of 25%, 50%, and 75% reduction of I_{Kr} on APD are shown in a virtual cell model after 1000 paced beats at a cycle length (CL) = 1000 ms. A 75% current reduction results in the development of arrhythmogenic early afterdepolarizations (EADs). [Adapted from (38).]

I_{Ks}, THE SLOWLY ACTIVATING COMPONENT OF THE DELAYED RECTIFIER K^+ CURRENT

I_{Ks} in Cardiac Repolarization

I_{Ks}, the slowly activating component of I_K, is a major contributor to repolarization of the cardiac action potential (AP) (68). Moreover, I_{Ks} is a dominant determinant of the physiological heart rate–dependent shortening of APD (69). At fast rates, I_{Ks} underlies the rate-dependent adaptation of the APD (70). Fast pacing results in short diastolic (recovery) intervals that prevent complete deactivation ($O \rightarrow C$) of I_{Ks}, resulting in the build-up of instantaneous I_{Ks} repolarizing current at the AP onset [Figure 6, from (70)]. At slower rates, less repolarizing current exists during each action potential due to sufficient time between beats to allow for complete deactivation of I_{Ks} (30, 70, 71). In some species, I_{Ks} deactivation results in a reduction of outward current in pacemaker cells (sino-atrial node), which allows for the slow diastolic depolarization preceding the action potential upstroke (72, 73).

The Molecular Basis of I_{Ks}

I_{Ks} results from co-assembly of two subunits, KCNQ1 (KvLQT1) and KCNE1 (minK) (74, 75). KCNQ1 was identified by positional cloning and mapped by linkage analysis to chromosome 11 (76). KCNQ1, the α-subunit of I_{Ks}, shares topological homology with other voltage-gated K^+ channels in that its 676 amino

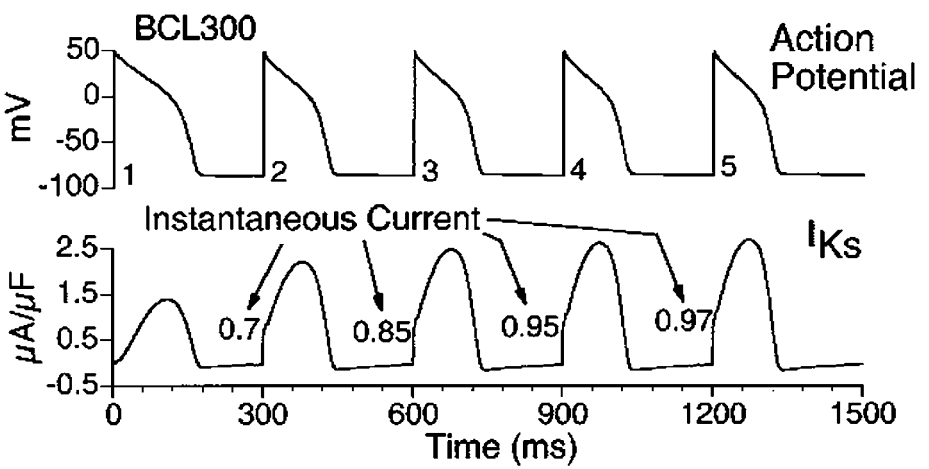

Figure 6 The role of I_{Ks} in APD shortening during rapid pacing (70). A simulated cell is paced from rest at a rate of 300 ms. Five action potentials and corresponding I_{Ks} currents are shown at the onset of rapid pacing. Rapid shortening of APD occurs due to an increase in the instantaneous I_{Ks} (*indicated by the arrows*) caused by incomplete deactivation of I_{Ks} between beats.

acids consist of six transmembrane domains and a pore-forming region. KCNE1, the β-subunit of I_{Ks}, was cloned from human cardiac tissue and encodes a protein containing 129–130 amino acids consisting of a single transmembrane spanning domain (77–80).

Autonomic Regulation of I_{Ks}

The contribution of I_{Ks} to regulation of APD is augmented by the sympathetic branch of the autonomic nervous system, which increases I_{Ks} through primary and secondary effects on channel gating kinetics (27, 81). β-adrenergic receptor (β-AR) stimulation acts to increase the heart rate, which results in rate-dependent shortening of the APD, resulting from the slow deactivation of I_{Ks} (as described above). I_{Ks} amplitude is also directly mediated by β-AR stimulation through PKA phosphorylation (81, 82) (Figure 7). PKA phosphorylation of I_{Ks} considerably increases current amplitude by increasing the rate of channel activation (C $\rightarrow$ O transition) and reducing the rate of channel deactivation (O $\rightarrow$ C transition) (81). Each of these outcomes acts to increase the channel open probability, leading to increased current amplitude and faster cardiac repolarization. β-AR stimulation also hastens

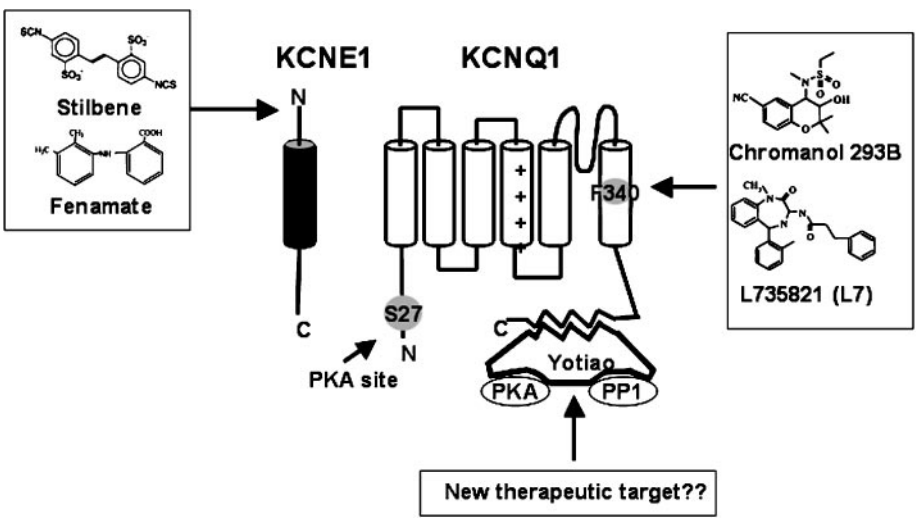

Figure 7 Sympathetic regulation of I_{Ks} requires a macromolecular signaling complex (82). KCNQ1 and KCNE1 co-assemble to form I_{Ks}. Sympathetic stimulation results in the activation of PKA, which is recruited to the channel C-terminus in conjunction with protein phosphatase 1 (PP1) by Yotiao (an AKAP scaffolding protein). PKA phosphorylation of serine 27 (*arrow*) ensues and I_{Ks} is upregulated, allowing for rate-dependent adaptation of the APD. Both KCNQ1 and KCNE1 are targets for pharmacological agents. Stilbene and Fenamate bind to the extracellular domain of KCNE1 and increase I_{Ks} (91). Chromanol 293B and L7 interact with the S6 domain of KCNQ1 and reduce I_{Ks} (87).

diastolic depolarization in the sinus node mainly by enhancing the L-type Ca^{2+} and I_f channels (83). Hypothetically, the simultaneous reduction in the rate of I_{Ks} deactivation during β-AR stimulation may act to regulate the slow diastolic depolarization, thereby adding another layer of regulation to the modulation of heart rate.

Additionally, endothelin-1, a myocardial and endothelial peptide hormone, inhibits I_{Ks} currents presumably through inhibition of adenylate cyclase via a PTX-sensitive G protein (84), and results in APD prolongation. Because both β-AR signaling and ET_A receptor signaling result in PKA phosphorylation, the molecular mechanisms of phosphorylation and dephosphorylation of I_{Ks} are of major interest as potential therapeutic targets.

Recently, the requirement of a macromolecular signaling complex for PKA phosphorylation of I_{Ks} has been shown (82). A leucine zipper motif in the C-terminus of KCNQ1 coordinates the binding of a targeting protein yotiao (85, 86), which in turn binds to and recruits PKA and protein phosphatase 1 (PP1) to the channel. The complex then regulates the phosphorylation of Ser^{27} in the N-terminus of KCNQ1 (Figure 7).

Structural Basis of I_{Ks} Blockade

Investigation into the structural determinants of I_{Ks} blockade has only recently begun. Not unlike HERG drug interaction sites, preliminary studies revealed a common site for binding of I_{Ks} blockers, including chromanol 293B and L735821 (L7) in the S6-domain (F340) of the KCNQ1 subunit (Figure 7). Other putative interaction sites in the S6-domain (T312 and A344) and the pore-helix (I337) may lend specificity to pharmacological interactions (87). Interestingly, these binding sites are located near an aqueous crevice in KCNQ1 that is thought to be important for interactions with KCNE1 that allosterically affect pore geometry (88–90). Drug interaction sites for channel agonists stilbene and fenamate have also been elucidated on extracellular domains in KCNE1 (Figure 7) (91).

Cellular Consequences of I_{Ks} Channel Blockade

Although the efficacy of I_{Kr} blockade is reduced at fast pacing rates due to reverse use dependence (i.e., APD-prolonging effects are least pronounced at fast stimulation rates), I_{Ks} blockade may be expected to be more useful in prolonging APD at fast rates, whereas I_{Ks} accumulates due to slow deactivation (30, 92).

Some pure class III compounds block both native and heterologously expressed I_{Ks} currents. Chromanol 293B and the benzodiazepine L7, which are distinct in their chemical structures (Figure 7), as well as the diuretic agent indapamide were some of the first compounds discovered to selectively block I_{Ks} (93–96). The application of chromanol 293B revealed that I_{Ks} inhibition appears to have rate-independent effects on human and guinea pig myocytes (94). Chromanol 293B exhibits slow binding kinetics to open channels and blocks I_{Ks} in a voltage-dependent manner, favoring positive potentials (97). It is possible that this type of voltage and time dependence of drug-induced I_{Ks} blockade may have less proarrhythmic potency compared to other compounds. Azimilide, a class III compound that blocks both

I_{Kr} and I_{Ks}, also appears to have rate-independent effects that are maintained under ischemic or hypoxic conditions, properties of potential clinical significance (98).

Some evidence suggests that I_{Ks} blockers can prolong QT intervals in a dose-dependent manner, an effect that is exacerbated when administered in combination with isoproterenol (99). These studies in canine preparations may even underestimate the proarrhythmia potential of I_{Ks} blockade because canine repolarization appears to be less dependent upon I_{Ks} than other species (100), and chromanol 293B was shown to markedly prolong human and guinea pig APD (94).

The sensitivity of I_{Ks} to blockade by chromanol 293B (101) and XE991 (102) is modulated by the presence of KCNE1. KCNE1 is itself a distinct receptor for the I_{Ks} agonists stilbene and fenamate (101), which bind to an extracellular domain on KCNE1. Stilbene and fenamate have been shown to be useful in reversing dominant negative effects of some LQT5 C-terminal mutations and restoring I_{Ks} channel function (91). On the other hand, a 1,4-benzodiazepine compound, L364,373, was an effective agonistic on KCNQ1 currents only in the absence of KCNE1 (103). These types of studies illustrate the importance of accessory subunits in determining the pharmacological properties of I_{Ks}. Variable subunit expression may determine tissue selectivity or electrical heterogeneity of pharmacological action that could exacerbate dispersion of repolarization (71, 104).

Blockade of I_{Ks} and β-Adrenergic Stimulation

In a model of acquired LQTS (I_{Ks} blockade by chromanol 293B), the addition of the β-adrenergic agonist isopreterenol induced the development of torsades de pointes (99). These results are consistent with the clinical findings that cardiac events are more likely to be associated with sympathetic nervous system stimulation in LQT1 patients than in either LQT2 or LQT3 patients (11, 105, 106). Moreover, β-blockers were reported to reduce cardiac events dramatically in LQT1 patients (106–108). Indeed, clinical data indicate that sudden cardiac death is significantly associated with sympathetic nervous system stimulation (106).

Gene Defects in KCNQ1 or KCNE1 Can Disrupt Cellular Repolarization

Mutations in either KCNQ1 or KCNE1 can reduce I_{Ks} amplitude, resulting in abnormal cardiac phenotypes and the development of lethal arrhythmias. Reduction of I_{Ks} during the delicate plateau phase of the action potential disrupts the balance of inward and outward current, leading to delayed repolarization. Prolongation of APD manifests clinically as forms of LQTS that are characterized by extended QT intervals on the ECG. Gene defects in KCNQ1 and KCNE1 are associated with distinct disease forms, LQT1 and LQT5, respectively (109–114).

In general, mutations in KCNQ1 or KCNE1 act to reduce I_{Ks} through dominant negative effects (76, 109, 113, 115–118), reduced responsiveness to β-AR signaling (82, 106), or alterations in channel gating (112, 119, 120). The latter effects typically manifest as either reduction in the rate of channel activation, such as R539W KCNQ1 (109), R555C KCNQ1 (118), or an increased rate of channel

deactivation, including S74L (119), V47F, W87R (112), KCNE1, and W248R KCNQ1 (120). An LQTS-associated KCNQ1 C-terminal mutation, G589D, disrupts the leucine zipper motif and prevents cAMP-dependent regulation of I_{Ks} (82). The reduction of sensitivity to sympathetic activity likely prevents appropriate shortening of the action potential duration in response to increases in heart rate.

Despite their distinct origins, congenital and drug induced forms of ECG abnormalities related to alterations in I_{Ks} are remarkably similar. In either case, reduction in I_{Ks} results in prolongation of the QT interval on the ECG without an accompanying broadening of the T wave, as observed in other forms of LQTs (1). Reduced I_{Ks} leads to loss of rate-dependent adaptation in APD, which is consistent with the clinical manifestation of arrhythmias associated with LQT1 and LQT5, which tend to occur due to sudden increases in heart rate. This strongly suggests that investigation of congenital forms of electrical abnormalities may act as a paradigm for drug-induced forms of clinical syndromes that is simplified by the absence of accompanying structural heart disease.

SUMMARY

In general, the broad diversity in response to pharmacologic intervention in the presence or absence of gene defects among individuals prone to arrhythmia is likely influenced by other genetic traits separate from the primary disease locus. Clinical presentation is determined by complex interactions between pharmacology, causal genes, genetic background (modifier genes), and environmental factors. Although individual modifier genes for lethal arrhythmias remain largely unknown, potential modifiers of cardiac arrhythmias include, but are not limited to, gender, febrile states, adrenergic stimulation, signaling molecules, channel-associated protein kinases, channel-associated protein phosphatases, and individual electrophysiological and morphological substrates. Identification of modifier genes will complement the current studies that have identified and characterized causative genes, which may improve upon genetically based diagnosis, risk stratification, and implementation of preventive and therapeutic interventions in patients with drug-induced arrhythmias.

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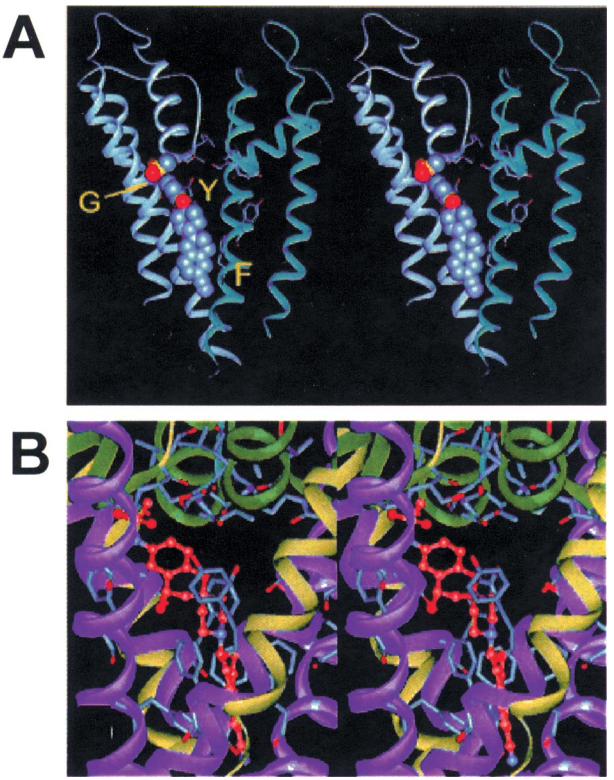

Figure 4 Drug docking in a homology model of the HERG K^+ channel based on the KcsA (58) channel structure. (*A*) Stereo view of the S5-S6 domains of two HERG subunits with docked molecule of MK-499 (shown as a space-filling model). T623, S624, V625, G648, Y652, and F656 are shown as sticks. (*B*) Close-up stereo view of MK-499 in a four-subunit model of the channel. T623, S624, and V625 of the pore helix (green), and G648, Y652, and F656 of the S6 domain (magenta) are shown as sticks; MK-499 is shown as a ball and stick model. Only two of the four S5 domains (yellow) are shown (from Reference 56).