Inherited and Acquired Vulnerability to Ventricular Arrhythmias: Cardiac Na\(^+\) and K\(^+\) Channels

COLLEEN E. CLANCY AND ROBERT S. KASS

Department of Physiology and Biophysics, Institute for Computational Biomedicine, Weill Medical College of Cornell University, and Department of Pharmacology, College of Physicians and Surgeons of Columbia University, New York, New York

I. INTRODUCTION

Meticulously timed opening and closing of cardiac ion channels results in cardiac electrical excitation and relaxation that is coupled to rhythmic contraction of the heart (Fig. 1). Abnormalities resulting from pharmacological agents or congenital defects can undermine the cardiac electrical syncytium, resulting in the development of debilitating arrhythmias that act to destabilize coordinated contraction and lead to the failure of sufficient pressure for blood circulation (85).

Cardiac excitation originates in the sinoatrial 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 (Fig. 1), due primarily to the activation of fast voltage-dependent Na\(^+\) channels that underlie the action potential (AP) 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 time- and voltage-dependent activation of K\(^+\) currents. Relaxation of con-
traction is coupled to the electrical repolarization phase, which allows filling of the ventricles (diastole) before 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 generated during each phase (Fig. 1) (9, 40, 85). Electrical excitation gradients in the atria (atrial depolarization) manifest on the ECG as P waves, while gradients of ventricular depolarization are seen as the QRS complex. Gradients in ventricular repolarization are reflected in the T wave.

Electrocardiographic abnormalities are related to changes in cellular AP morphologies, which may be due to altered cell-to-cell coupling, heart disease, congenital ion channel abnormalities, drug intervention, or electrolyte imbalance (9, 40, 85). Conduction abnormalities can be detected as changes in the QRS complex. Widening of the QRS reflects reduced conduction velocity, which typically stems from altered Na\(^+\) channel function (121). ST segment elevation reflects transmural voltage gradients during the AP plateau, a hallmark of congenital forms or drug-provoked Brugada syndrome (29, 40, 125, 140). 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 (40, 139, 142).

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 (99, 142).

II. LONG QT SYNDROME

Long QT syndrome (LQTS) is a collection of cardiac disorders characterized by a prolongation of the QT interval on the ECG and can be divided into two primary clinical categories: inherited and acquired. The acquired form of LQTS generally results from pharmacological therapeutic intervention, often for the purpose of treating disorders unrelated to cardiac dysfunction. Many medications, including antihistamines, antipsychotics, antibiotics, and antiarrhythmics, can predispose patients to lethal arrhythmias (26). Acquired forms of LQTS may also result from electrical or structural abnormalities arising from other rhythm disorders, cardiac ischemia, and a range of cardiomyopathies. Inherited forms of LQTS are predominantly autosomal dominant, although less common autosomal recessive forms exist and typically result in more severe phenotypes. Romano-Ward syndrome is typically autosomal dominant and predominantly heterozygous, although some more severe homozygous forms exist (131). Romano-Ward is distinguishable from Jervell Lange-Nielsen syndrome, since the latter is autosomal recessive and always accompanied by hearing loss (22). The inherited, or congenital, LQTS results from mutations in genes coding for cardiac proteins including ion channels, accessory subunits, and associated modulatory proteins, responsible for orchestrating the AP in the heart. To date, five genes have been implicated in Na\(^+\) and K\(^+\) channel linked LQTS (Table 1).

III. Na\(^+\) CHANNELOPATHIES: \(I_{Na}\), THE FAST VOLTAGE-GATED Na\(^+\) CHANNEL

Voltage-gated Na\(^+\) channels cause the rapid depolarization that marks the rising phase of APs in the majority of excitable cells. At negative membrane potentials, channels typically reside in closed and available resting states that represent a nonconducting conformation. Depolarization results in activation of the voltage sensors and channel opening, allowing for ion passage. Subsequent to channel activation, channels enter inactivated states that

FIG. 1. Electrical gradients in the myocardium can be detected on the body surface electrocardiogram (ECG) and reflect underlying cellular ionic current gradients. A: illustration of a single cardiac cycle ECG detected as electrical gradients on the body surface. B: schematic representation of the ventricular action potential gradients detected on the body surface ECG. C: ionic currents responsible for the different phases of the action potential and the genes that encode for them. Darkened shapes are indicative of relative current amplitude, duration, and direction. The shape of the current is also aligned with its approximate time of action during the ECG (A) and the cardiac ventricular action potential (B). (Modified from Clancy CE and Kass RS. J Clin Invest 110: 1075–1077, 2002; and The Sicilian Gambit. Circulation 84: 1831–1851, 1991.)
are nonconducting and refractory. Repolarization is required to alleviate inactivation with isoform-specific time and voltage dependence.

SCN5A encodes the cardiac isoform (NaV1.5) of the voltage-gated Na\(^{+}\)/H\(^{+}\) channel, which is a heteromultimeric protein complex consisting of four heterologous domains, each containing six transmembrane spanning segments. Positive residues are clustered in the S4 segments and comprise the voltage sensor (52, 119) (Fig. 2). The intra-cellular linker between domains three and four, DIII/DIV, includes a hydrophobic isoleucine-phenylalanine-methionine (IFM) motif, which acts as a blocking inactivation particle and occludes the channel pore, resulting in channel inactivation subsequent to channel opening (119, 134). Recent studies also suggest a role for the COOH terminus in channel inactivation in brain and cardiac isoforms (NaV1.1 and NaV1.5, respectively) (31, 66, 74). The S5 and S6 transmembrane segments of each domain comprise the putative channel pore and associated ion selectivity filter (120, 138).

There has been renewed interest in the study of voltage-gated Na\(^{+}\) channels since the recent realization that genetic defects in Na\(^{+}\)/H\(^{+}\) channels can underlie several clinical syndromes including the LQTS type 3 (LQT3), Brugada syndrome (BrS), and isolated cardiac conduction disease (ICCD). Mutations underlying these clinical syndromes are scattered throughout the channel (Fig. 2).

In general, Na\(^{+}\) channel-linked LQTS stems from mutation-induced disruption of channel inactivation, as was originally identified in the ΔKPQ mutation, a three-amino acid deletion in the intracellular linker between domains III and IV of NaV1.5. This motif is known to be critical for fast inactivation of the channel, and the mutation results in persistent noninactivating current (11, 20). The noninactivating component of \(I_{Na}\) acts to prolong the plateau of the AP and may allow for the development of arrhythmogenic triggered activity, referred to as early afterdepolarizations (EADs) (27) (Fig. 3).

While ΔKPQ is one example of altered gating, several recent studies suggest that mutation-induced gain of function in cardiac \(I_{Na}\) can exist in at least three distinct forms (Fig. 4). The most common is due to transient inactivation failure as in ΔKPQ, which underlies sustained Na\(^{+}\) channel activity over the plateau voltage range (9, 19). A second is due to steady-state channel reopening called window current (20), because reopening occurs over voltage ranges for which steady-state inactivation and activation overlap. A third original mechanism was demonstrated in channels containing the I1768V mutation, which

Many mutations in the cardiac voltage-gated Na\(^{+}\) channel isoform Na\(_{v}\)1.5 have been shown to underlie several disease phenotypes including the LQTS type 3 (LQT3), Brugada syndrome (BrS), and isolated cardiac conduction disease (ICCD). Mutations underlying these clinical syndromes are scattered throughout the channel (Fig. 2).

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### TABLE 1. Na\(^{+}\) and K\(^{+}\) channel LQT-associated genes and proteins

<table>
<thead>
<tr>
<th>Type</th>
<th>LQT1</th>
<th>LQT2</th>
<th>LQT3</th>
<th>LQT5</th>
<th>LQT6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene</td>
<td>KCNQ1</td>
<td>KCNH2 (hERG)</td>
<td>SCN5A</td>
<td>KCNE1</td>
<td>KCNE2</td>
</tr>
<tr>
<td>Protein</td>
<td>KCNQ1, KvLQT1, α-subunit of (I_{Ks})</td>
<td>HERG or α-subunit of (I_{Ks})</td>
<td>Na(<em>{v})1.5 or α-subunit of (I</em>{Na})</td>
<td>KCNE1, MinK or β-subunit of (I_{Ks})</td>
<td>MIR1P or β-subunit of (I_{Ks})</td>
</tr>
</tbody>
</table>

\(I_{Na}\) fast voltage-gated Na\(^{+}\) channel; \(I_{Ks}\) slowly activating component of the delayed rectifier K\(^{+}\) current; \(I_{Kr}\) rapidly activating component of the delayed rectifier K\(^{+}\) current.

**Fig. 2.** The cardiac Na\(^{+}\) channel is involved in multiple arrhythmogenic syndromes. Shown is a schematic representation of the voltage-gated cardiac Na\(^{+}\) channel (Na\(_{v}\)1.5), in which mutations can lead to the LQT3 form of long QT syndrome (LQTS), Brugada syndrome (BrS), and isolated cardiac conduction disorder (ICCD) or mixed combinations of disorders.
does not result in an obvious gain of channel function (30, 44, 83). However, under nonequilibrium conditions during repolarization, channel reopening results from faster recovery from inactivation at membrane potentials that facilitate the activation transition (30). Mutation-induced faster recovery from inactivation results in channels that reopen during repolarization, and the resulting current amplitude rivals that of bursting channels. Simulations have demonstrated that late current due to channel reopening causes severe prolongation of the AP plateau and arrhythmic triggers (30).

Recently, several studies have focused on the role of the intracellular COOH terminus of the Na⁺/H¹⁺ channel in voltage-gated Na⁺/H¹⁺ channel inactivation (31, 66). Notably, gene defects associated with LQT3 located in this region disrupt inactivation in a manner similar to mutations that affect the DIII/DIV linker inactivation gate. LQT3 mutations in this region (E1784K, 1795insD, Y1795C) evoke small, sustained currents similar to ΔKPQ (8, 82, 125).

Unlike LQTS that is associated with a gain of Na⁺ channel function, loss of Na⁺ channel function underlies the BrS phenotype. BrS is an arrhythmic syndrome characterized by right bundle branch block, ST segment elevation on the ECG, and ventricular tachyarrhythmias often resulting in sudden death (14). Mutations in Naᵥ1.5 have been linked to BrS and characteristically cause a reduction in I_Na (21, 42). This reduction in I_Na has been shown to occur by several mechanisms in BrS, including reduced rates of recovery from inactivation, faster inactivation subsequent to channel opening, and protein trafficking defects (29, 35, 82, 125, 129).

ICCD is observed on the ECG in a widening of the QRS complex, indicating delays in ventricular excitation.
A. Mutations Can Result in Multiple Phenotypes

The relationship between genetic mutations and clinical syndromes is becoming increasingly complex as the revelation of novel mutations suggests paradoxical phenotypic overlap or exclusivity. Recently, at least three loci in the cardiac sodium channel have been identified where the same mutation can result in different disease phenotypes. An insertion of an aspartic acid residue (1795insD) in the COOH terminus of NaV1.5 can result in either BrS or ICCD in several families (56). Expression studies resulted in no current, although no trafficking errors were detected, suggesting a potential modifier gene or genes affecting NaV1.5 function.

The deletion of a lysine (ΔK1500) in the III-IV linker of NaV1.5 is associated with BrS, LQTS, and ICCD (42). LQTS is typically associated with gain-of-function Na+ channel mutations while BrS and ICCD are typically associated with loss-of-function resulting in reduced I_{Na}. The fact that single mutations can underlie disparate phenotypes begs the question of underlying mechanisms. How can a single mutation simultaneously result in seemingly paradoxical syndromes (i.e., gain-of-function LQTSs and loss-of-function BrS)?

B. The Heterogeneous Myocardial Substrate

One explanation may stem from the intrinsic heterogeneity of the underlying myocardial substrate with which mutant Na+ channels interact (Fig. 5). The ventricular myocardium is comprised of at least three distinct cell types referred to as epicardial, midmyocardial (M), and endocardial cells, which exhibit distinct electrophysiological properties (60).

C. Mutations and/or Polymorphisms May Increase Susceptibility to Drug-Induced Arrhythmias

Within the context of arrhythmia, pharmacogenomic considerations are important to determine the potential for genetic heterogeneity to directly affect drug targets and interfere with drug interactions. Mutations or polymorphisms may directly interfere with drug binding (62) or can result in a physiological substrate that increases predisposition to drug-induced arrhythmia (113). Use-dependent block of voltage-gated Na+ channels results in preferential reduction of current at fast pacing rates (62). This property is potentially useful in reducing
runaway excitation by reducing Na\(^+\) current and thereby decreasing the likelihood of reexcitation. The unpredictable outcomes of pharmacological intervention with mutant channels must be investigated to develop appropriate treatments, since a Na\(^+\) channel blocker may be ineffective, or overly effective, in interacting with mutant channels.

Genetic mutations or polymorphisms may affect drug binding by altering the length of time that a channel resides in a particular state. For example, the epilepsy associated R1648H mutation in Na\(_v\)1.1 reduces the likelihood that a mutant channel will inactivate and increases the channel open probability (64). Hence, an anticonvulsant that interacts with open channels will have increased efficacy, while one that interacts with inactivation states may have reduced efficacy. However, even this type of analysis may not predict actual drug-receptor interactions (61, 62). The II768V mutation increases the cardiac Na\(^+\) channel isoform propensity for opening, suggesting that an open channel blocker would be more effective, but in fact, the mutation is in close proximity to the drug-binding site, which may render open channel blockers nontherapeutic (61, 62).

Local anesthetic molecules such as lidocaine and flecainide block Na\(^+\) channels and have been used therapeutically to manage cardiac arrhythmias (89, 90, 136). Despite the prospective therapeutic value of the inherent voltage- and use-dependent properties of channel block by these drugs in the treatment of tachyarrhythmias, their potential has been overshadowed by toxic side effects (91, 133). However, Na\(^+\) channel blockers have proven useful as a diagnostic tool and in treatment of BrS and LQT3 (15). Na\(^+\) channel blockade by flecainide is of particular interest as it had been shown to reduce QT prolongation in carriers of some LQT3 mutations and to evoke ST segment elevation, a hallmark of the BrS, in patients with a predisposition to the disease (15). Thus, in the case of LQT3, flecainide has a potential therapeutic application, whereas for BrS it has proven useful as a diagnostic tool. However, in some cases, flecainide has been reported to provoke BrS symptoms (ST segment elevation) in patients carrying LQT3 mutations (81). Furthermore, flecainide preferentially blocks some LQT3- or BrS-linked mutant Na\(^+\) channels (5, 43, 62, 126). Investigation of the drug interaction with these and other LQT3 and BrS linked mutations may indicate the usefulness of flecainide in the detection and management of these disorders and determine whether or not it is reasonable to use this drug to identify potential disease-specific mutations.

Recent findings revealed the differential properties of certain drugs on mutant and wild-type cardiac Na\(^+\) channels. One such example is the preferential blockade by flecainide of persistent \(I_{ks}\) in the \(\Delta\)KPQ Na\(^+\) channel mutant (75). It was also shown that some LQT-associated mutations were more sensitive to blockade by mexilitene, a drug with similar properties to lidocaine, than wild-type channels (130). In three mutations, \(\Delta\)KPQ, N1325S, and R1644H, mexilitene displayed a higher potency for blocking late Na\(^+\) current than peak Na\(^+\) current (130).

We found that flecainide, but not lidocaine, showed a more potent interaction with a COOH-terminal D1790G LQT3 mutant than with wild-type channels and a correction of the disease phenotype (4, 62). The precise mechanism underlying these differences is unclear. Lidocaine has a \(pK_s\) of 7.6–8.0 and thus may be up to 50% neutral at physiological pH. In contrast, flecainide has a \(pK_s\) of ~9.3, leaving <1% neutral at pH 7.4 (46, 100, 118). Thus one possibility underlying differences in the voltage dependence of flecainide- and lidocaine-induced modulation of cardiac Na\(^+\) channels is restricted access to a common site that is caused by the ionized group of flecainide. Another possibility is that distinctive inactivation gating defects in the D1790G channel may underlie these selective pharmacological effects. Indeed, we recently found mutations that promote inactivation (shift channel availability in the hyperpolarizing direction) enhance flecainide block. Interestingly, our data also showed that flecainide sensitivity is mutation, but not disease, specific (62).

These studies are important in the demonstration that effects of flecainide segregate in a mutation-specific manner that is not correlated with disease phenotype, suggesting that it may not be an effective agent for diagnosing or treating genetically based disease. The nature of the interaction between pharmacological agents and wild-type cardiac Na\(^+\) channels has been extensively investigated. However, the new findings of drug action on mutant channels in LQTS and BrS have stimulated a renewed interest in a more detailed understanding of the molecular determinants of drug action, with the specific aim of developing precise, disease-specific therapy for patients with inherited arrhythmias.

**IV. \(K^+\) CHANNELOPATHIES: \(I_{ks}\), THE SLOWLY ACTIVATING COMPONENT OF THE DELAYED RECTIFIER \(K^+\) CURRENT**

\(I_{ks}\), the slowly activating component of the delayed rectifier \(K^+\) current, is a major contributor to repolarization of the cardiac AP (49). Moreover, \(I_{ks}\) is a dominant determinant of the physiological heart rate-dependent shortening of APD (37, 117, 127, 141). Sympathetic stimulation and resulting fast pacing results in short diastolic (recovery) intervals that prevent complete deactivation of \(I_{ks}\) resulting in the build-up of instantaneous \(I_{ks}\) repolarizing current at the AP onset (37, 117, 127, 141). At slower rates, less repolarizing current exists during each AP due to sufficient time between beats to allow for complete deactivation of \(I_{ks}\) (37, 117, 127, 141).
A. Mutations in KCNQ1 or KCNE1 Can Underlie Long QT Syndrome

The first LQTS locus (LQT1) was linked to mutations in KCNQ1, a gene coding for a voltage-gated K⁺ channel α-subunit. I\(_{Ks}\) results from coassembly of KCNQ1 (Kv-LQT1) and KCNE1 (minK) (10, 95). KCNQ1 was identified by positional cloning and mapped by linkage analysis to chromosome 11 (131). KCNQ1 shares topological homology with other voltage-gated K⁺ channels with 676 amino acids that form six transmembrane domains and a pore-forming region. Four identical α-subunits form a tetramer with a central ion-conduction pore. KCNQ1 and its ancillary subunit, KCNE1, have been shown to recapitulate the gating properties of native I\(_{Ks}\) (55).

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 (53, 54, 67, 128) (Fig. 6A). 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 protein kinase A (PKA) phosphorylation (53, 54, 67). A leucine zipper motif in the COOH 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 NH\(_3\) terminus of KCNQ1 (67). PKA phosphorylation of I\(_{Ks}\) considerably increases current amplitude, by increasing the rate of channel activation and reducing the rate of channel deactivation (53, 54, 67). Each of these outcomes acts to increase the channel open probability leading to increased current amplitude and faster cardiac repolarization.

Mutations in either KCNQ1 or KCNE1 can reduce I\(_{Ks}\) amplitude resulting in abnormal cardiac phenotypes and the development of lethal arrhythmias (Fig. 6B) (112). Reduction of I\(_{Ks}\) during the delicate plateau phase of the AP 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 electrocardiogram. Gene defects in KCNQ1 and KCNE1 are associated with distinct disease forms, LQT1 and LQT5, respectively (12, 13, 24, 76).

More than 30 mutations have been identified in KCNQ1 or KCNE1, and many act to reduce I\(_{Ks}\) through dominant negative effects (12, 13, 104), reduced responsiveness to β-AR signaling (1, 54), or alterations in channel gating (22, 86, 112, 137) (Fig. 6B). Mutations in KCNQ1 cause LQTS by a reduction in or loss of I\(_{Ks}\), resulting in reduced repolarizing current during the AP (77, 131). These mutations often act by dominant negative suppres-
sion of normal subunits, which results in a significant reduction in current. In other instances, there is a complete loss of current, which has been demonstrated to result from the assembly of nonfunctional channels or the failure of channels to traffic to the plasma membrane (12, 13, 33, 71). Mutations can also alter channel-gating properties, which typically manifest as either reduction in the rate of channel activation, such as R539W KCNQ1 (25), R555C KCNQ1 (23), or an increased rate of channel deactivation including S74L (114), V47F, W87R (13), KCNE1 and W248R KCNQ1 (39). An LQTS-associated KCNQ1 COOH-terminal mutation, G589D, disrupts the leucine zipper motif and prevents cAMP-dependent regulation of Ik,s (67). The reduction of sensitivity to sympathetic activity likely prevents appropriate shortening of the APD in response to increases in heart rate.

As with KCNQ1, homozygous mutations in KCNE1 can cause the more severe Jervell and Lange-Nielsen phenotype (114), while heterozygous mutations can manifest via dominant-negative suppression or changes in channel kinetics resulting in reduced outward K⁺ current (87). KCNE1 has recently been shown to be required for cAMP-dependent regulation of Ik,s (54). Even though KCNQ1 phosphorylation is independent of coassembly with KCNE1, transduction of the phosphorylated channel into the physiologically required increase in reserve channel activity requires the presence of KCNE1 (54). Several LQT-5 linked point mutations of KCNE1 can severely disrupt the important physiologically relevant functional consequences KCNQ1 phosphorylation including the D76N and W87R mutations (54). Both the D76N and W87R mutations reduce basal current density when expressed with WT KCNQ1 subunits (54). This effect would be expected to reduce repolarizing current, prolong cellular APs, and contribute to prolonged QT intervals in mutation carriers even in the absence of sympathetic nervous system (SNS) stimulation. However, in addition, the mutations would be expected to eliminate or reduce physiologically important reserve K⁺ channel activity in the face of SNS stimulation. The W87R mutation speeds channel deactivation kinetics, which then are not slowed by cAMP. Thus the data suggest that the W87R mutation eliminates the important cAMP-dependent accumulation of channel activity that is the normal response to the SNS. The D76N mutation ablates functional regulation of the channels by cAMP. The result is an expected delay in the onset of repolarization that is more pronounced in the face of SNS stimulation.

Despite their distinct origins, congenital and drug-induced forms of LQTS related to alterations in Ik,s are remarkably similar. In either case, reduction in Ik,s 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 (40). Reduced Ik,s leads to the 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.

B. Cellular Consequences of Ik,s Channel Blockade

Some pure class III compounds block both native and heterologously expressed Ik,s current. Chromanol 293B and the benzodiazepine L7, which are distinct in their chemical structures, as well as the diuretic agent indapamide were some of the first compounds discovered to selectively block Ik,s (17, 47, 65). The application of Chromanol 293B revealed that Ik,s inhibition appears to have rate-independent effects on human and guinea pig myocytes (17). Chromanol 293B exhibits slow binding kinetics to open channels and blocks Ik,s in a voltage-dependent manner, favoring positive potentials (65). It is possible that this type of voltage and time dependence of drug-induced Ik,s blockade might have less proarrhythmic potency compared with other compounds. Azimilide, a class III compound which blocks both Ik,r and Ik,s also appears to have rate-independent effects that are maintained under ischemic or hypoxic conditions, properties of potential clinical significance (48). Some evidence suggests that Ik,s blockers can prolong QT intervals in a dose-dependent manner, an effect that is exacerbated when administered in combination with isoproterenol (107).

The sensitivity of Ik,s to blockade by chromanol 293B and XE991 is modulated by the presence of KCNE1 (16). KCNE1 is itself a distinct receptor for the Ik,s agonists stillbene and fenamate (16), which bind to an extracellular domain on KCNE1. Stillbene and fenamate have been shown to be useful in reversing dominant negative effects of some LQT5 COOH-terminal mutations and restoring Ik,s channel function (3). On the other hand, L364,373, a 1,4-benzodiazepine compound, is an effective agonistic on KCNQ1 currents only in the absence of KCNE1 (92). These types of studies illustrate the importance of accessory subunits in determining the pharmacological properties of Ik,s.

C. Blockade of Ik,s and β-Adrenergic Stimulation

In a model of acquired LQTS (Ik,s blockade by chromanol 293B), the addition of the β-adrenergic agonist isoproterenol induced the development of torsades de pointes (107). These results are consistent with the clinical findings that cardiac events are more likely to be associated with sympathetic nervous stimulation in LQT1 patients than either in LQT2 or LQT3 patients (99). Moreover, β-blockers are reported to reduce cardiac events...
dramatically in LQT1 patients (108). Under normal circumstances, \( \beta \)-adrenergic stimulation increases Ca\(^{2+} \) current, which acts to prolong APD, but a concomitant increase in \( I_{Ks} \) shortens APD. Hence, a controlled feedback system exists to prevent excessive APD prolongation (45). However, in the presence of mutations that reduce \( I_{Ks} \), appropriate APD shortening in response to \( \beta \)-adrenergic stimulation may fail to occur and increase arrhythmia susceptibility.

Investigation into the structural determinants of \( I_{Ks} \) block is not yet well understood, and recent studies demonstrate that genetic defects can disrupt drug block (102), which may aid in the revelation of the drug binding site. Studies revealed a common site for binding of \( I_{Ks} \) blockers including Chromanol 293B and L735821 (L7) in the S6 domain (F340) of KCNQ1 (101). Other putative interaction sites in the S6 domain (T312 and A344) and the pore helix (I337) may lend specificity to pharmacological interactions (101). 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 (55, 122, 123). Drug interaction sites for channel agonists stilbene and fenamate have been shown on extracellular domains in KCNE1 (3).

V. \( K^+ \) CHANNELOPATHIES: \( I_{Kr} \), THE RAPIDLY ACTIVATING COMPONENT OF THE DELAYED RECTIFIER

\( \text{HERG} \) (Human ether-a-go-go related gene) has been implicated in the chromosome 7-linked LQT2 syndrome (84, 112). \( \text{HERG} \) encodes a typical voltage-gated \( K^+ \) channel pore-forming \( \alpha \)-subunit, but with unique and physiologically significant biophysical and structural characteristics. \( \text{HERG} \) rapidly inactivates and slowly deactivates, resulting in the passage of little outward current but significant transient current upon repolarization that contributes to AP shortening (84, 85).

While \( \text{HERG} \) is widely accepted as the \( \alpha \)-subunit of \( I_{Kr} \), marked differences exist between native \( I_{Kr} \) current and \( \text{HERG} \)-induced currents in heterologous expression systems in terms of gating (2, 143), regulation by external \( K^+ \) (2, 68, 106), and sensitivity to antiarrhythmics (96, 97). These data suggest the presence of a modulating subunit that coassembles with \( \text{HERG} \) to reconstitute native \( I_{Kr} \) currents. A candidate is the minK-related protein 1 (MiiRP1 = KCNE2), which when coexpressed with \( \text{HERG} \), results in currents similar to native \( I_{Kr} \) (2) (Fig. 7). Coexpression with MiiRP1 causes a +5–10 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 (2). However, a specific and selective interaction of \( \text{HERG} \) and MiiRP1 in the myocardiun has not yet been demonstrated (132), and other factors may contribute to the functional differences between native \( I_{Kr} \) current and \( \text{HERG} \)-induced currents in heterologous expression systems. Several alternatively spliced ERG1 variants have been demonstrated in the heart (58, 63), and there is evidence for posttranslational modification of \( \text{HERG} \) proteins (79, 80).

More than 50 mutations in \( \text{HERG} \) have been linked to the congenital LQTS (84, 112) (Fig. 7). Defects in the \( \text{HERG} \) pore have been shown to have heterogeneous cellular phenotypes. Mutations in \( \text{HERG} \) resulting in LQTS result in a reduction in \( K^+ \) current, either by dominant-negative suppression, nonfunctional channels, or trafficking errors (84, 112). Pore mutations may result in a loss of function, sometimes due to a trafficking defect (78) and may or may not coassemble with WT \( \text{HERG} \) subunits to exert dominant negative effects (94, 112). Other defects in the channel pore give rise to altered channel kinetics leading to decreased repolarizing current (38, 109). Nearby mutations in the S4-S5 linker have been shown to variably affect activation (98). Mutations in the Per-Arnt-Sim (PAS) domain located in the NH\(_3\)-terminal region of \( \text{HERG} \), which normally interacts with the channel and reduces the rate of deactivation, alter deactivation kine-
ics of the channel and tend to reduce the amount of time channels reside in open states, thereby reducing current (18). Mutations in this region presumably prevent proper association of the NH$_2$ terminus with the channel and act to increase the rate of channel deactivation. A particularly interesting mutation, N629D, results in a “gain-of-function” defect in HERG that leads to loss of C-type inactivation coupled with loss of K$^+$ selectivity (57). HERG contains a pore selectivity sequence that is changed by the mutation from GFGN to GFGD, allowing for nonspecific passage of monovalent cations.

Mutations in KCNE2 alter the biophysical properties of $I_{Kr}$ and act to reduce current (2, 112). KCNE2 polymorphisms have also been associated with increased affinity of $I_{Kr}$ to blockade by clarithromycin (103).

A. Cellular Consequences of $I_{Kr}$ Blockade by Mutations and/or Drugs

The HERG channel subunit was originally identified by genetic studies on patients with the congenital LQTS. Incorporation of mutant HERG subunits in the channel tetramer generally causes a reduction of $I_{Kr}$, which leads to prolongation of the ventricular AP (84). A delay in ventricular repolarization predisposes the heart to arrhythmogenic early afterdepolarizations (6, 7, 28, 36, 51).

Both the cellular effects of these congenital abnormalities 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 (40). 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 (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 (40). 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.

B. HERG Channels Are Promiscuous Drug Targets

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 (97, 111). HERG channels can also be blocked by an array of other pharmacological agents with diverse chemical structures (80). Experiments suggest 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 (69, 70).

Initial investigation of the HERG antagonist binding site was carried out via site-directed mutagenesis techniques. One study (38) revealed that a single residue, Ser-620, 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 Phe-656 in S6 was necessary, although not sufficient, for high-affinity binding of dofetilide and quinidine, but did not affect binding of tetraethylammonium and did not disrupt inactivation.

Homology modeling based on crystallographic structure of the bacterial K$^+$ channel KcsA (34) predicts that Phe-656 falls within the HERG pore region. This result was confirmed and extended in an elegant study by Mitcheson et al. (69), who identified four residues in addition to Phe-656 that were crucial for high-affinity binding by methanesulfonanilides, namely, Tyr-652, Gly-648, Val-625, and Thr-623. 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 Tyr-652 and Phe-656 (69). The crucial role of Tyr-652 and Phe-656 was confirmed by studies using cisapride and terfenadine, whereas Gly-648, Val-625, and Thr-623 were found to be more specific for methanesulfonanilides (69).

The importance of residues Tyr-652 and Phe-656 was also demonstrated for the low-affinity ligand chloroquine, an antimalarial agent that appears to preferentially block open HERG channels. Block 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 (93).

C. State-Specific Block of $I_{Kr}$

The biophysical properties of HERG blockade are consistent with a discrete state-dependent blocking mechanism (51, 110). Initial HERG channel studies demonstrated that methanesulfonanilides require channel opening for access to a presumptive intracellular binding site (111). Mutations that result in loss of inactivation act to reduce affinity for methanesulfonanilides, suggesting that inactivation may be required for drug binding. However, methanesulfonanilides are less effective at inhibiting HERG K$^+$ channels during strong depolarization (e.g.,
+60 mV), which promote inactivation (51, 110), 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 drug to contact its binding site, which then becomes accessible as channels inactivate. At positive voltages, extremely rapid inactivation might reduce the channel open time sufficiently to prevent drug access to 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 binding to inactivated channels (62).

Recovery of HERG from block 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 (98), 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 K⁺ channels (69). 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. (69), these residues (Y652 and F656) are crucial for electrostatic interactions between aromatic rings of Y652/F652 and the drug molecules.

D. Gene-Specific Triggers and Treatments

Although all forms of inherited long-QT that stem from ion channel mutations act to disrupt repolarization, the specific genetic loci are indicators of likelihood of cardiac events stemming from different types of triggers. Patients harboring mutations in KCNQ1 (LQT1) and KCNE1 (LQT5) are prone to arrhythmia in response to β-adrenergic discharge as occurs during exercise, especially swimming (72, 135). In response to β-adrenergic stimulation, there is an accompanying increase in heart rate coupled to shortening of APD, which allows for sufficiently long diastolic intervals for filling of the ventricles. This shortening results, in large part, from PKA phosphorylation of Iₖₑₐ (as described in detail in sect. IV). A reduction in Iₖₑₐ due to dominant negative mutations, those that reduce the current through alterations in kinetics, or mutations that disrupt the transduction of PKA phosphorylation will prevent appropriate shortening of the AP and may lead to conduction block.

HERG mutations (LQT2), on the other hand, are associated with auditory triggers and are less heart rate dependent, with events occurring at both fast and slow heart rates. While the mechanisms of HERG-linked arrhythmia at slow heart rates have been investigated in detail (28), the mechanisms underlying auditory induction of events is elusive. HERG is regulated by cAMP via at least two distinct pathways (32, 50). Activation of PKA results in HERG current reduction and a rightward shift of the activation curve (implying a reduction in current). However, direct binding of cAMP to HERG has the opposite effect of hyperpolarization of the activation curve and a current increase (32, 50).

LQT3 resulting from mutations in Na⁺1.5 are bradycardia-dependent inducers of arrhythmia events. Studies have shown that mutations that result in a gain of Na⁺ channel function are sufficient to cause events at slow heart rates as a result of kinetic alterations (27). Current flowing during the AP plateau through defective Na⁺ channels during the AP plateau results in AP prolongation in the presence of reduced repolarizing currents and may trigger arrhythmogenic early afterdepolarizations (27). While persistent current in wild-type Na⁺ channels is not regulated by PKA, one study has shown significant regulation of a long QT-associated mutant by PKA (124).

The standard prophylactic therapy for all forms of LQTS is β-adrenergic blockade, although pacemakers, implantable cardioverter defibrillators, and surgical sympathetic ganglionectomy have been used in cases where patients are resistant to drug therapy (73). A study of the effectiveness of β-adrenergic blockade has shown that the therapy is gene specific, and although it does not act to reduce QT intervals per se, blockade does significantly reduce the likelihood of arrhythmic events in LQT1 and LQT2 patients (73). β-Blockers do not appear to aid in sudden cardiac death prevention for LQT3 forms of LQTS (73). Given the bradycardia association of arrhythmic events in this form of the disease, this result is perhaps not surprising, but raises the important issue of gene-specific therapy and the necessity for identification of genetic loci of disease.

VI. SUMMARY

In general, the broad diversity in genetic defects underlying arrhythmia syndromes and potential interactions with pharmacological interventions makes the prediction of treatment outcomes difficult. Variability in response may be due to the presence of gene defects in the drug targets themselves or influenced by other genetic heterogeneity. Clinical presentation is determined by complex interactions between pharmacology, causal genes, genetic background (modifier genes), and environmental factors. Although individual modifier genes are largely un-
known, potential modifiers of arrhythmia include, but are not limited to, gender, electrolyte disturbances, febrile states, receptor stimulation, channel-associated protein kinases, channel-associated protein phosphatases, and individual electrophysiological and morphological substrates. Identification of modifier genes will complement the current studies that increasingly identify and characterize causative genes, which may improve upon genetically based diagnosis, risk stratification, and implementation of preventive and therapeutic interventions in patients with arrhythmia.

Indeed, identification of genetic mutations underlying arrhythmia syndromes and other genetic modifiers that may identify pharmacological targets or affect treatment efficacy is important but may prove to be less than half the battle. Establishing a link between abnormalities or common polymorphisms and manifestation of disease is a major challenge. Even once the gap is bridged between genotype and phenotype, the challenge to appropriately and cost-effectively treat patients is difficult to even begin to address. Is it feasible to develop patient-specific treatments that target an individual’s distinct genetic makeup? Certainly not at this juncture, but identifying genetic markers that may identify pharmacological targets or affect treatment efficacy is important but may prove to be less than half the battle. Establishing a link between abnormalities or common polymorphisms and manifestation of disease is a major challenge. Even once the gap is bridged between genotype and phenotype, the challenge to appropriately and cost-effectively treat patients is difficult to even begin to address.

Address for reprint requests and other correspondence: C. E. Clancy, Dept. of Physiology and Biophysics, Institute for Computational Biomedicine, Weill Medical College of Cornell University, 1300 York Ave., LC-501E, New York, NY 10021 (E-mail: clc7003@med.cornell.edu).

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