Kv11.1 (hERG1) Channels and Cardiac Arrhythmia

11

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Abstract

The rapid delayed rectifier K^+ current (I_{Kr}) is one of several outward currents that mediate repolarization of cardiac action potentials in humans and other vertebrates. In humans, I_{Kr} is conducted by Kv11.1 (hERG1) channels encoded by the gene *KCNH2*. Loss of function mutations in *KCNH2* cause inheritable long QT syndrome, characterized by a prolonged QT interval and an increased risk of ventricular arrhythmia. Gain of function mutations in *KCNH2* cause short QT syndrome and associated arrhythmia. In clinical practice, unintended drug-induced QT prolongation and associated ventricular arrhythmia are most often caused by unintended block of Kv11.1 channels. By contrast, compounds that activate Kv11.1 can shorten the QT interval and thereby have the potential to prevent arrhythmia associated with long QT syndrome. This chapter provides a brief overview of the biophysical properties and physiological and pathophysiological roles of Kv11.1 and a more in-depth description of the structural basis for binding and molecular mechanisms of action of compounds that inhibit or activate these channels.

Keywords

Antiarrhythmic drug · Class III · hERG · I_{Kr} · *KCNH2* · Kv11.1 · Kv11.1 activator · Kv11.1 blocker · Long QT syndrome · Potassium channel · QT interval · Short QT syndrome

11.1 Introduction

Cardiac myocytes express many different types of K^+ channels that conduct currents that either maintain the resting potential (e.g., inward rectifier K^+ current, I_{K1}) or mediate repolarization of the action potential (e.g., I_{to}, I_{Kur}, I_{Kr}, and I_{Ks}). The relative magnitudes of these outward currents and the two major inward currents that determine the action potential waveform of a typical human ventricular myocyte are depicted in Fig. 11.1. Loss of function mutations in the genes that encode channels that conduct I_{Kr} or I_{Ks} slow the rate of ventricular repolarization and cause inheritable long QT syndrome (LQTS), a cardiac disorder characterized by a prolonged QT interval measured on the body surface ECG and an increased risk of cardiac arrhythmia. LQTS is specifically associated with torsades de pointes (TdP), a ventricular arrhythmia that can degenerate into ventricular fibrillation (VF) and cause sudden cardiac death.

Prolonged QT interval and arrhythmia can also be induced by Class III antiarrhythmic agents that inhibit repolarizing K^+ currents with the intention

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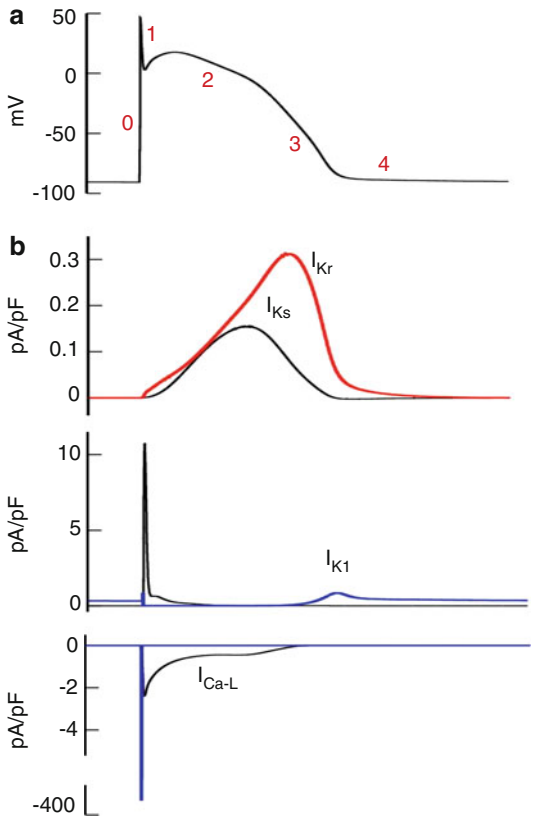

Fig. 11.1 Model of action potential and major ionic currents for a human ventricular myocyte. (**a**) Single action potential. Action potential duration for this cell type is ~280 ms at 1 Hz pacing rate. Numbers refer to different phases of the action potential waveform, including the initial upstroke (0), repolarization (1–3), and the resting membrane potential (4). (**b**) Major ionic currents that underlie action potential shown in panel A. Outward (repolarizing) K^+ currents include the rapid delayed rectifier (I_{Kr}), slow delayed rectifier (I_{Ks}), transient outward (I_{to}) and inward rectifier (I_{K1}). Inward currents include the L-type Ca current (I_{CaL}) and sodium current (I_{Na})

of uniformly prolonging the electrical refractory period of the ventricles. Most potent Class III agents inhibit I_{Kr} as their primary mechanism of action and can prevent ventricular tachycardia (VT) when drug dosing is carefully controlled to prevent excessive QT prolongation. However, excessive inhibition of I_{Kr} by these drugs is associated with a marked dispersion of ventricular repolarization that sometimes leads to TdP and VF. The risk of TdP/VF is especially pronounced when Class III drugs are administered in the setting of other risk factors such as hypokalemia or undiagnosed LQTS. Routine monitoring of adverse drug reactions in the past few decades has also revealed that many noncardiac medications (e.g., some antihistaminic and antibacterial drugs) also prolong the QT interval and are associated with an increased risk of VF.

Drug-induced QT prolongation and arrhythmia, often inappropriately referred to as acquired "long QT syndrome," by noncardiac medications, is most commonly caused by unintended inhibition of I_{Kr}. In the human heart, I_{Kr} channel α-subunits are encoded by a gene that was initially discovered using a human hippocampus cDNA library to screen for genes homologous to the *Drosophila* K^+ channel gene *eag* ("*ether-a-go-go*") [1]. The novel cloned gene was named "human *ether-a-go-go-related gene*" (*hERG*), although now the more accepted gene name is *KCNH2* and the International Union of Basic and Clinical Pharmacology (IUPHAR) preferred name for the encoded channel α-subunit protein is Kv11.1. Once inhibition of I_{Kr} was widely recognized as a common mechanism underlying drug-induced arrhythmia, the safety screening of compounds for undesired block of I_{Kr} in isolated cardiomyocytes, or Kv11.1 channels heterologously expressed in mammalian cell lines, became a routine practice early in the drug development process [2]. An unexpected consequence of these safety screening efforts was the discovery of several compounds that activate rather than inhibit Kv11.1 channel activity [3]. Kv11.1 activators shorten the QT interval and thereby have the potential to prevent arrhythmia associated with inherited LQTS or to counteract unintended I_{Kr} inhibition detected in many otherwise useful noncardiac medications. This chapter provides a brief overview of the physiological and pathophysiological roles of Kv11.1 channels, and a more in-depth description of the biophysical properties and the molecular mechanisms of action of compounds that inhibit or activate these channels.

11.2 Physiological Roles of Kv11 Channels

After the initial discovery of the *hERG* gene, further cloning efforts culminated in the description of three families and eight *eag*-related Kv channel α-subunits, including EAG (Kv10.1, Kv10.2), ERG (Kv11.1-Kv11.3) and ELK (Kv12.1-Kv12.3). In the heart, the most prominently expressed *eag*-related Kv channel is Kv11.1, encoded by the gene *KCNH2*. Two closely related genes (*KCNH6* and *KCNH7*) encode hERG2 (Kv11.2) and hERG3 (Kv11.3) channel subunits, respectively, that are expressed in the nervous system but not in cardiac myocytes [4]. Outside the heart, Kv11 channels display a diversity of functions [5], including maintenance of K^+ homeostasis in hippocampal astrocytes [6], regulating spike frequency in glomus cells of the carotid body [7] and cerebellar Purkinje neurons [8], regulation of hormone secretion in anterior pituitary lactotrophs and pancreatic β-cells [9–11], and setting the resting membrane potential of smooth muscle cells in the gastrointestinal tract [12–14]. Kv11 channels are also implicated in cell cycle regulation and apoptosis [15, 16] and the pathogenesis of several cancers [17–19].

In the heart, Kv11.1 channel activity is one of the primary determinants of repolarization during phases 2 (plateau) and 3 (late repolarization) of the action potential waveform (Fig. 11.1a). Similar to other Kv channels, the open probability of Kv11.1 is highly voltage-dependent and thus varies throughout the cardiac cycle. In ventricular myocytes, channels are closed (nonconducting) during diastole (phase 4) when the membrane potential is at its most negative (−90 mV). Although channels open in response to membrane depolarization during the upstroke (phase 0) and initial repolarization phases of the action potential, most channels then rapidly transition to an inactivated (nonconducting) state. This results in a very small net outward current during the onset of the plateau phase. The open probability of Kv11.1 channels (and thus I_{Kr}) progressively increases during the later portion of the plateau phase and even more so during initial phase 3 repolarization, as channels recover from inactivation and re-enter their open state. As the rate of membrane repolarization increases during phase 3, open channels deactivate into a closed state, and I_{Kr} magnitude is reduced. Together, the voltage and time-dependent properties of activation, inactivation, and deactivation gating of Kv11.1 channels results in the whole myocyte I_{Kr} shown in Fig. 11.1b.

11.3 Pathophysiological Roles of Kv11.1 Channels in the Heart

11.3.1 Kv11.1 and Long QT Syndrome

Inherited loss of function mutations in *KCNH2* cause LQTS [20], characterized by a prolonged QT interval and reduced I_{Kr} (Fig. 11.2), an increased risk of TdP, VF and sudden cardiac death. This type of LQTS is called "LQT2" to distinguish it from the many other types of LQTS that are numbered based on the chronology of the gene discovered to underlie the particular LQTS type. LQT1 (*KCNQ1* mutations), LQT2 (*KCNH2* mutations), and LQT3 (*SCN5A* mutations) account for the vast majority of the autosomal dominant inherited LQTS (Romano-Ward syndrome) with an estimated prevalence of 1:2500 live births [21]. In one large study, it was estimated that LQT2 accounts for ~35% of all inherited LQTS [22]. LQT2 is characterized by a prolonged QT_c interval, defined as >440 ms for men and > 460 ms for women, and either a biphasic or notched T wave [23] (Fig. 11.2b). The most common trigger for VT/VF in LQTS is emotional stress, with triggering by sudden auditory stimuli often reported for LQT2 [24].

Multiple mechanisms have been shown to be responsible for the loss of Kv11.1 channel function caused by mutations in *KCNH2*. The most common mechanism is a misfolding and reduced trafficking of channels to the cell membrane [25]. Less common are problems with channel gating that reduce conductance such as a slower rate of activation, faster rate of deactivation or

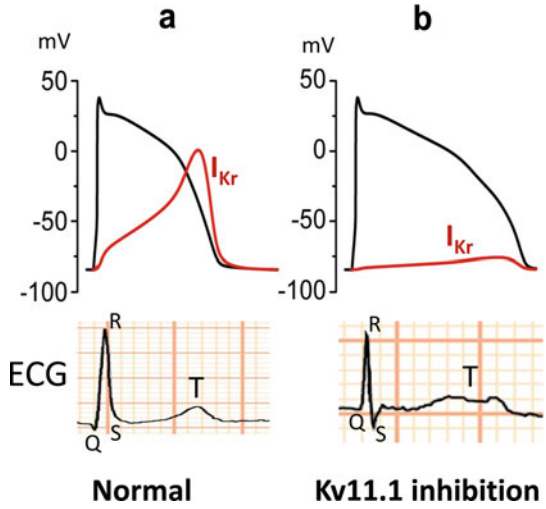

Fig. 11.2 Inhibition of I_{Kr} causes prolongation of action potential in cardiac myocytes and lengthening of QT interval measured on body surface ECG. (**a**) Normal action potential and I_{Kr} (top panel) and normal ECG (bottom panel). (**b**) Action potential duration is lengthened, QT interval is lengthened, and T-wave is typically notched when I_{Kr} is reduced. I_{Kr} reduction can result from the block of Kv11.1 channels by drugs or by a loss of function mutation in the *KCNH2* gene

enhanced inactivation [26]. Transgenic LQT2 rabbits have provided important insights into the cellular and tissue mechanisms of arrhythmia that result from a loss of function mutation (Gly628Ser) in Kv11.1. Isolated cardiac myocytes from LQT2 rabbits have prolonged APD (action potential duration), no measurable I_{Kr} and reduced I_{Ks} [27]. These rabbits have prolonged QTc intervals, exhibit discordant APD alternans, marked dispersion in ventricular effective refractory periods, and when anesthetized with isoflurane develop impaired conduction in the His bundle, block in the infra-His bundle and intermittent, spontaneous second-degree AV (atrioventricular) block [28, 29]. The multiple cardiac defects in LQT2 rabbits result in spontaneous polymorphic VT/VF and a high rate of sudden cardiac death [27].

11.3.2 Kv11.1 and Short QT Syndrome

Gain of function mutations in Kv11.1 channels cause short QT syndrome (SQTS), a rare disorder that hastens cardiac repolarization, increases the risk of VT/VF and atrial fibrillation (AF) that may lead to SCD. SQTS is diagnosed when QTc $\leq$ 330 ms [30]. The first two point mutations in *KCNH2* reported to cause SQTS (Asn588Lys, Thr618Ile) were shown to increase outward Kv11.1 current ($I_{Kv11.1}$) by shifting the voltage dependence of inactivation gating to more positive potentials. The voltage required to cause 50% of channels to inactivate is dramatically shifted by these mutations, i.e., +102 mV by Asn588Lys [31] and + 50 mV by T618I [32]. Reduced inactivation may increase I_{Kr} more in the ventricle than in Purkinje fibers, and the resulting disparity in APD could create the arrhythmogenic substrate responsible for VF [31]. A transgenic rabbit model of SQTS (with Asn588Lys Kv11.1 mutation) mimics the human phenotype, including reduced atrial and ventricular refractoriness and increased inducibility of VT/VF/AF [33].

11.4 Biophysical and Structural Properties of Kv11.1 Channels

11.4.1 Kv11.1 Channel Subtypes

Like other Kv channels, functional Kv11.1 channels are formed by the coassembly of four α-subunits into a tetramer. Two different Kv11.1 proteins are expressed in the heart – a full-length protein (hERG1a or Kv11.1a) and an N-terminal truncated protein (hERG1b or Kv11.1b) produced by alternative splicing [34, 35]. Homomeric Kv11.1a channels and heteromultimeric Kv11.1a/1b channels are readily trafficked to the plasma membrane, unlike Kv11.1b homotetramers that can be retained in the endoplasmic reticulum [36]. Kv11.1a channels deactivate much slower than Kv11.1b channels. Slow deactivation of Kv11.1a is dependent on an interaction between the cytoplasmic N-terminus of one subunit with the cytoplasmic C-terminus of an adjacent subunit [37–39]. Channels containing Kv11.1b subunits (with a truncated N-terminus) lack the N- to C-terminal interactions and thus deactivate faster than Kv11.1a homotetramers and in fact more closely match the kinetics of native I_{Kr} in the heart. The biophysical properties of heterologously expressed hERG1 channels can be altered when coexpressed with β-subunits such as MinK or MiRP1 [40–42]; however, the physiological relevance of this modulation in the heart is disputed [43].

11.4.2 Biophysical Properties of Kv11.1 Channels

Kv11.1 channels have biophysical properties that are intermediate between a typical Kv channel and an inward rectifier K^+ (Kir) channel. Kv11.1 channels are voltage-gated; i.e., the open probability and rates of activation, inactivation, and deactivation of channels are highly dependent on the transmembrane potential. In contrast, Kir channels lack a voltage sensor, and thus, their open probability is intrinsically voltage-independent. The inward rectification of Kir (outward currents are much smaller than inward currents) results from a voltage-dependent block of outward K^+ flux by intracellular polyamines [44]. Similar to Kir channels, outward $I_{Kv11.1}$ is also diminished at positive transmembrane potentials, but this reduction results from a gating process (inactivation) rather than from pore block by polyamines [45]. Although $I_{Kv11.1}$ (I_{Kr}) is often described as inward rectifying, the more proper biophysical description would be that the fully-activated current-voltage (I-V) relationship for the current exhibits a negative slope conductance at potentials positive to E_K, the equilibrium potential for K^+. An example of whole-cell $I_{Kv11.1}$ elicited at physiologically relevant test potentials (−70 to +40 mV) is shown in Fig. 11.3a. At the most negative potentials examined, the currents activate slowly and do not reach a steady-state value during the 2-s pulse. The magnitude of the outward current reaches a peak at about −10 mV. At pulses to more positive potentials, the rate of current activation increases and the current size is decreased. At the end of each 2-s pulse, the membrane potential is returned to −70 mV and the resulting "tail" current exhibits a transient increase in amplitude as channels recover from an inactivated state into an open state. After this initial outward surge, the "tail" current slowly decays in amplitude as channels deactivate (transition from an open to a closed state).

The I-V relationship for peak outward currents, measured at the end of each 2-s test pulse, is bell-shaped and peaks near −10 mV (Fig. 11.3b). The shape of this I-V relationship is determined by the relative dominance of voltage-dependent activation (Fig. 11.3c) at negative voltages versus inactivation (Fig. 11.3d) that dominates at positive voltages. The single channel behavior that underlies the reduced outward $I_{Kv11.1}$ at positive membrane potentials is illustrated in Fig. 11.4. At +40 mV, the probability of a Kv11.1 channel being in the inactivated (nonconducting) state is high and channel openings are rare and brief in duration. Repolarization of the membrane from +40 to −120 mV induces the channel to rapidly recover from its inactivated state into the open state whereupon it

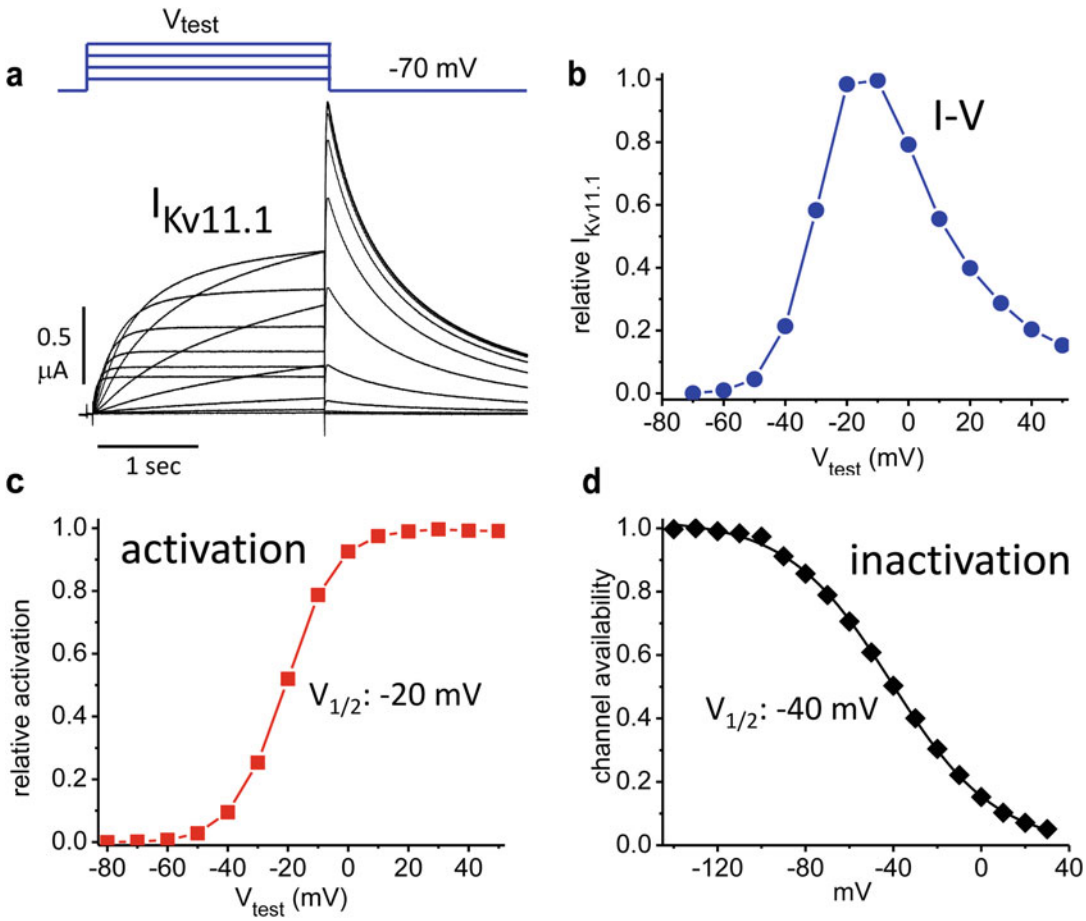

Fig. 11.3 Whole cell $I_{Kv11.1}$ recorded in a *Xenopus* oocyte. (**a**) Voltage clamp protocol (upper panel) and ionic currents were recorded at test potentials applied in 10-mV increments ranging from −70 to +40 mV (lower panel). Tail currents were elicited by repolarization of the membrane potential to −70 mV. (**b**) Current-voltage (I-V) relationship for $I_{Kv11.1}$ was measured at the end of the 2-s test pulse. (**c**) Voltage dependence of $I_{Kv11.1}$ activation was determined by plotting the relative value of tail current amplitude as a function of test potential. The half-point ($V_{1/2}$) for activation is −20 mV. (**d**) Voltage dependence of $I_{Kv11.1}$ inactivation ($V_{1/2} = -40$ mV)

more slowly enters its closed ("resting," nonconducting) state.

11.4.3 Structural Features of Kv11.1 Channels

Each Kv11.1 subunit has six α-helical transmembrane segments (S1-S6) that are divided into two domains, a voltage-sensing domain (VSD, S1-S4 segments) and a pore domain (S5-S6 segments). The long N-terminus of Kv11.1a is cytosolic and contains a Per Arnt Sim (PAS) domain (absent in Kv11.1b); both α-subunits have a long cytoplasmic C-terminus that contains a cyclic nucleotide-binding homology domain (CNBHD). In 2017, the cryo-EM structure of Kv11.1 (hERG1) channels in an open state was determined (Fig. 11.5a) [46]. Based on the comparison of Kv10 and Kv11.1 channel structures [46], the gating hinge in the S6 segment that mediates the opening and closing of channels is Gly648 (Fig. 11.5b). However, the critical or unique importance of the flexibility afforded to S6 by a Gly in this position seems unlikely since substitution with Ala (Gly648Ala) has little effect

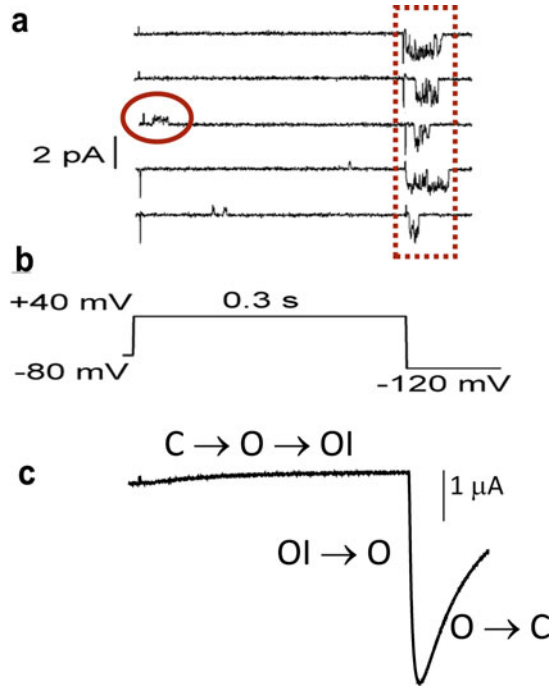

Fig. 11.4 Single channel and whole cell $I_{Kv11.1}$ measured in *Xenopus* oocytes. (**a**) Traces of single Kv11.1 channel activity in a cell-attached patch were recorded during five separate test pulses. At +40 mV, the channel is usually inactivated (nonconducting) and openings are rare (oval). Repolarization of the membrane patch to −120 mV induces the channel to open (dashed box) for a brief time before deactivating into the rested, closed state. $[K^+]_i$ and $[K^+]_o = 140$ mM. (**b**) Voltage pulse protocol used to elicit currents shown in panels a and c. (**c**) Whole-cell $I_{Kv11.1}$. Labeling indicates dominant transitions between different single channel states (*C* closed, *O* open, *OI* open/inactivated) that underlie whole-cell current

on channel gating [47]. Similar to Kv10 channels, the VSD of Kv11.1 channels is packed up against its own pore domain (i.e., not domain-swapped as in many other Kv channels), and each S4 segment connects directly with its adjacent S5 segment, without a long S4-S5 α-helical linker that is commonly found in other Kv channels and previously proposed to play an important role in Kv11.1 channel gating [48–51]. In contrast to the S4-S5 linker acting as a lever that couples movement of the VSD to the gating of the pore domain, the cryo-EM structures suggest instead that the outward movement of the VSD (mainly S4) transmits force required for channel opening via the S5-S6 interface and that inward S4 movement closes the channel by compressing the S5 helices [46].

11.4.4 Structural Basis of Kv11.1 Channel Inactivation

Inactivation of Kv11.1 is normally extremely rapid in onset, but similar to "C-type" inactivation of other Kv channels, can be slowed in the presence of extracellular tetraethylammonium or elevated $[K^+]_e$, and nearly eliminated by point mutations in regions of the pore near the selectivity filter [45, 52–54]. One of these point mutations (Ser631Ala) was found to induce a subtle change in the position of Phe627, a residue in the middle of the Gly-Phe-Gly motif of the K^+ selectivity filter. The altered position of Phe627 closely resembles the position of the residue in the selectivity filter motif of Kv channels that do not exhibit C-type inactivation [46]. The structural basis of C-type inactivation in Kv11.1 is

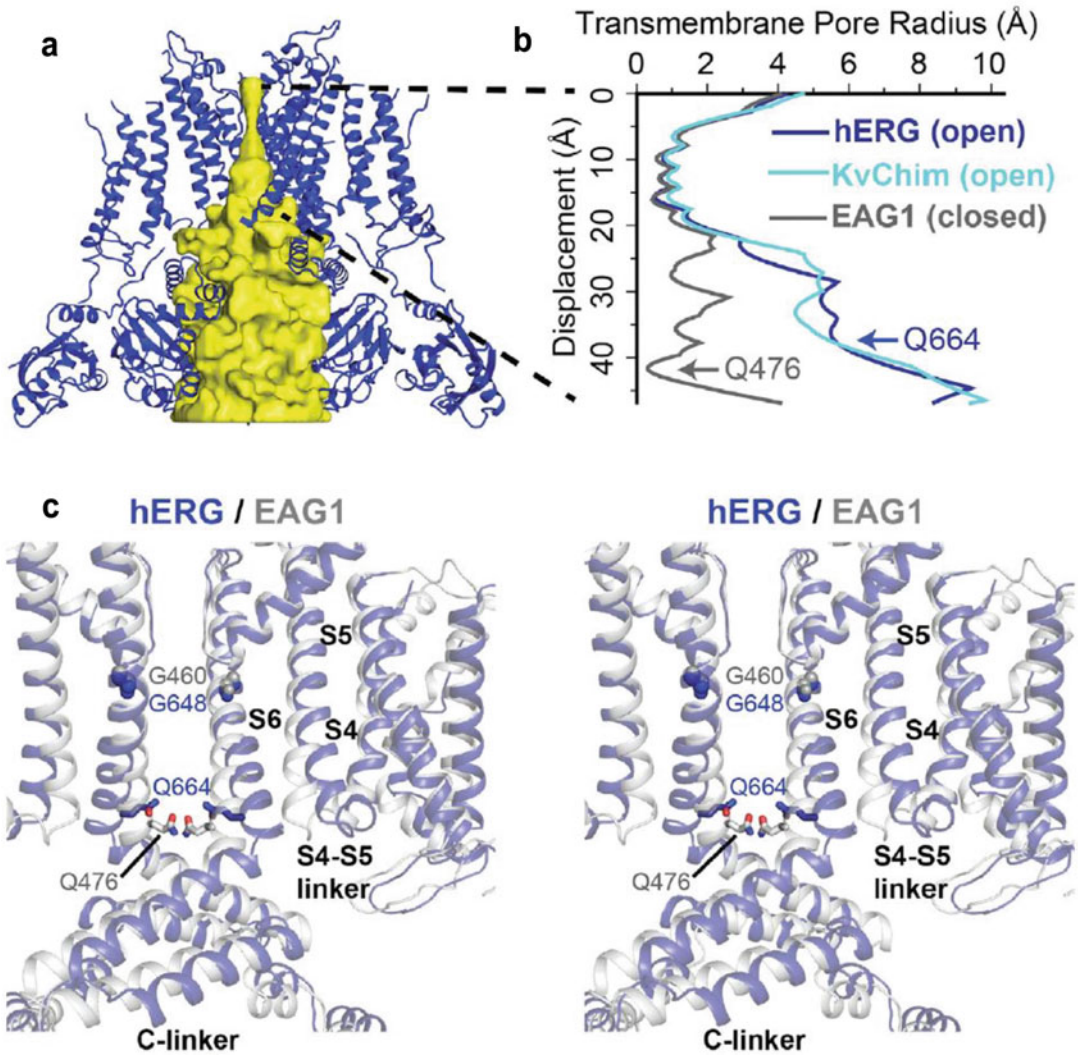

Fig. 11.5 Kv11.1 channel structure determined by cryo-EM. (**a**) Side view of the channel in ribbon mode. Yellow surface represents the central pore. (**b**) Pore radius was measured along the S6 segment of Kv11.1 (hERG) in an open state and Kv10.1 (EAG1) in a closed state. (**c**) Comparison of Kv11.1 and Kv10.1 channel structures (two opposing subunits for each channel) in stereo-view. Reprinted from Wang and MacKinnon, 2017 [46] with permission from Elsevier

likely to be quite complex and has been suggested to involve multiple regions of the channel and occur in sequential steps that culminate in a subtle change in the conformation of the selectivity filter [55, 56], perhaps similar to that described for Ser631Ala channels. Inactivation has been studied using concatenated (all four subunits covalently linked) Kv11.1 channels, and findings suggest that the final step in the gating process is mediated by a concerted, all-or-none cooperative interaction [57].

11.5 Drug-Induced QT Prolongation and TdP

Many drugs have been associated with QT prolongation and an increase in the risk of

ventricular arrhythmia. Accordingly, the list of drugs that are contraindicated in LQTS is vast [58, 59]. Although the reduction in either I_{Kr} or I_{Ks} can lengthen the cardiac repolarization time, extensive study has led to the general consensus that inhibition of I_{Kr} is by far the more common cause of drug-induced QT prolongation and arrhythmia.

Class III antiarrhythmic drugs were purposely developed for their ability to inhibit I_{Kr}, prolong cardiac refractoriness, and provide protection against arrhythmia initiation mediated by premature excitation. However, because drug-induced changes in the refractory period are not uniform throughout the heart, these drugs can also be proarrhythmic and induce TdP. The proarrhythmic risk of a Class III agent is greater when administered at a high dose or when combined with other conditions that prolong repolarization such as hypokalemia, bradycardia, impaired hepatic or renal function (that can slow metabolism or elimination of drug), or concomitant administration of other drugs that inhibit I_{Kr} [60]. Quinidine was reported to induce TdP in 2–9% of treated patients [61].

Noncardiac medications that block I_{Kr} and prolong the QT interval as an unintended side effect are structurally diverse [62–64] and span a wide spectrum of therapeutic drug classes, including psychiatric, antimicrobial, and antihistaminic compounds [65, 66]. Drug-induced TdP by these compounds is usually a relatively rare event. For example, TdP associated with terfenadine or cisapride treatment is estimated to have occurred in ~1 of 100,000 patients [67]. Of course, this incidence is unacceptable for drugs that are prescribed for the treatment of nonlife-threatening disorders [59]. Cisapride, sertindole, grepafloxacin, terfenadine, and astemizole were removed from the US market, or their use was severely restricted by drug enforcement agencies once a clear association was confirmed between their use and significant QTc prolongation or arrhythmia [65].

11.6 Mechanism of Drug-Induced Kv11.1 Channel Inhibition

The molecular basis of the Kv11.1 channel block has been extensively studied. The main findings can be summarized as follows: (1) Kv11.1 channels are preferentially blocked by most compounds when the channel is in an open or open/inactivated state. (2) Site-directed mutagenesis and functional analysis studies have identified specific amino acid residues in the pore domain that are critical to drug interaction with the channel. Finally, (3) the cryo-EM structure of Kv11.1 provides new clues into the structural basis of drug binding sites, but definitive identification of these site(s) awaits the structural determination of channels bound by specific compounds.

A large number of computational models to predict Kv11.1 channel block has been developed [68–70], including quantitative structure–activity relationship models. These studies have culminated in several pharmacophore models that are summarized elsewhere [71]. While the potency of Kv11.1 channel block by drugs is most commonly defined by an IC_{50} value derived from a fixed voltage clamp protocol, this measure does not always accurately predict the risk of QT prolongation associated with the clinical use of a specific compound. An important component of the disconnect between drug potency and QTc prolongation/arrhythmic risk is the kinetics of drug binding to the channel [72, 73], and it has been demonstrated that dynamic modeling of drug-channel interaction can improve risk assessment of compounds [74].

11.6.1 Open State Channel Block

Voltage clamp studies have provided unequivocal evidence that positively charged drugs require Kv11.1 channels to be in an open state to allow access to their binding site. Even at a high concentration of 10 μM, prolonged incubation with MK-499 (a methanesulfonanilide class III antiarrhythmic drug) does not result in block of Kv11.1

channels in cells that are voltage clamped at a negative membrane potential to ensure that all channels are in the closed state [75]. When the cell is depolarized to a positive potential to activate channels, the initial current magnitude is identical to control (predrug) current, and block develops slowly over a few seconds. Thus, MK-499 only blocks activated Kv11.1 channels, implying that a binding site within a region of the pore (the central cavity) is made accessible only after the activation gate is opened. Slow recovery from channel block by MK-499 appears to be caused by physical trapping of the compound inside the central cavity as the channel deactivates. Recovery from block can be strikingly accelerated when Kv11.1 channels contain a specific mutation (Asp540Lys) that allows the activation gate to reopen in response to membrane hyperpolarization [76]. These characteristics of onset and recovery from pore block are common to several other potent blockers of Kv11.1, including dofetilide, E-4031, and bepridil [77, 78].

11.6.2 Inactivated State Channel Block

Initial studies of state-dependent block of Kv11.1 indicated that compounds preferentially inhibit channels that are in an inactivated state [79–81], although exceptions such as halofantrine were notable [82]. The main evidence for inactivated state block is that inhibition is enhanced when a cell is depolarized to very positive membrane potentials where most channels are forced into an inactivated state and by the finding that inactivation-deficient mutant channels are usually far less susceptible to drug-induced inhibition. More recent studies using concatenated Kv11.1 tetramers, having four subunits (either wild-type or mutant) covalently linked together with defined stoichiometry, have led to a more nuanced interpretation of these experimental results. Kv11.1 channels with specific point mutations (either Ser620Thr or Ser631Ala) do not inactivate [83]. The link between inactivation gating and blocking potency of three compounds (cisapride, dofetilide, MK-499) was assessed using concatenated hERG1 tetramers containing a variable number of wild-type and Ser620Thr or Ser631Ala subunits. The presence of a single Ser620Thr subunit in a concatenated channel removed inactivation just as effectively as a Ser620Thr homotetramer [57], yet the heteromeric channels with only one mutant subunit were much more sensitive to block by the three compounds than the mutant homotetrameric channel [84]. Although Ser631Ala mutant subunits disrupt the inactivation of concatenated channels in a graded fashion, the blocking potency of cisapride was unaltered with up to three Ser631Ala subunits incorporated into the tetramer [84]. Together these findings indicate that inactivation facilitates, but is not an absolute requirement for, high-affinity block of Kv11.1 channels.

11.6.3 Structural Basis of Kv11.1 Blocker Binding Site

Based on the results of binding displacement studies using radiolabeled dofetilide or astemizole [85–87], structurally diverse drugs bind to a common or at least overlapping site on the Kv11.1 channel. Site-directed mutagenesis and functional analysis of mutant channels have provided insights into the structural basis of this binding site. Phe656 of the S6 segment was the first Kv11.1 residue reported to be a critical residue for the binding of dofetilide and quinidine [88]. Additional key amino acid residues that form the drug binding site were identified with more extensive site-directed mutagenesis and MK-499 as a probe [89]. Mutation to Ala of three residues near the pore helix (Thr623, Ser624, and Val625) and several residues in the S6 segment (Gly648, Tyr652, Phe656, and Val659) reduced channel sensitivity to block by MK-499. More recently, Phe557 in the S5 segment was implicated in the binding of Kv11.1 blockers [90]. Compounds that are structurally related to MK-499 such as E4031 and dofetilide have a nearly identical pattern of residue interactions [77]. The locations of these key

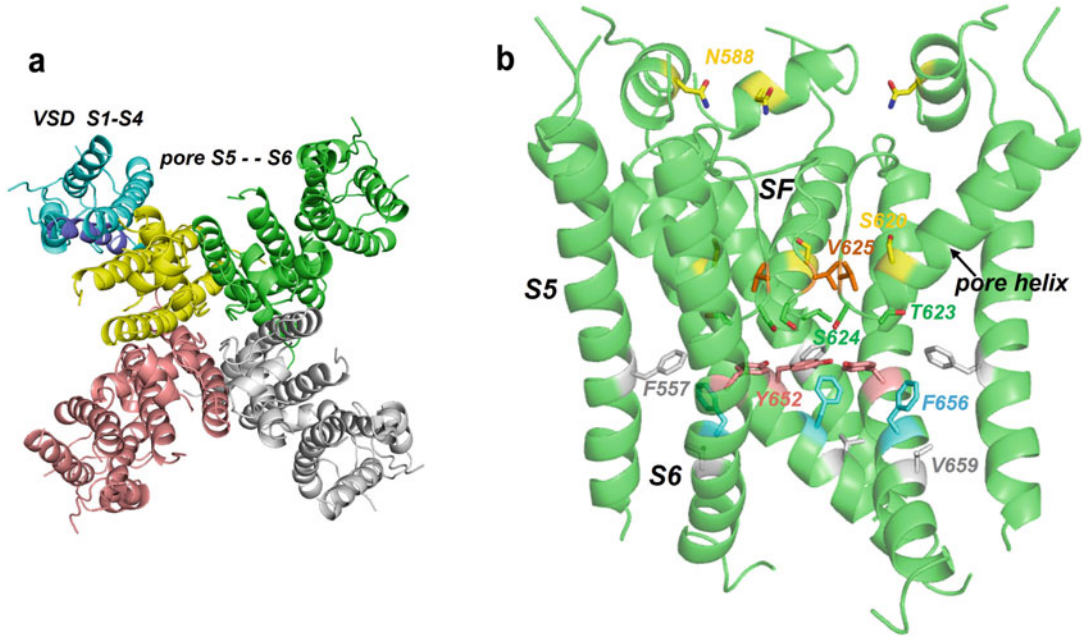

Fig. 11.6 Key residues in Kv11.1 that interact with blockers. (**a**) Top-down view of cryo-EM Kv11.1 structure [46] illustrating fourfold symmetry of the tetrameric channel. For one subunit, the VSD is colored in cyan (S1-S3 segments) and blue (S4 segment), and the pore domain is colored yellow. (**b**) Side view of Kv11.1 channel pore domain with the side chains of key amino acid residues (in stick mode) that comprise the putative blocker binding site indicated. *SF* selectivity filter. Also shown are N588 and S620 (in yellow); mutation of either one of these residues removes inactivation. Figure reproduced from Helliwell et al. 2018 [91]

residues in the Kv11.1 structure are illustrated in Fig. 11.6.

The side chains of Thr623, Ser624, Tyr652, and Phe656 are all orientated toward the central cavity of the closed channel [62], although in the open-state Phe656, sidechains are repositioned and instead face toward Phe557 of the S5 segment. The two-pore helix residues (Thr623 and Ser624) are highly conserved in Kv channels. In contrast, Tyr652 and Phe656 in the S6 segment are not conserved; most Kv channels have an Ile and a Val at these positions. These two aromatic residues have been shown to be of high importance for interaction with many drugs, including chloroquine [92], quinidine [93], halofantrine [82], terfenadine, and cisapride [94], lidoflazine [95], clofilium and ibutilide [96], and cocaine [97]. Further mutagenesis showed that potent Kv11.1 block by MK-499, cisapride, and terfenadine require an aromatic residue in position 652 (either Tyr or Phe), suggesting the possibility of a cation-π interaction. The potency for block by all three drugs was well correlated with the van der Waals hydrophobic surface area of the side chain of residue 656 [94]. Specific residue-drug interactions predicted from site-directed mutagenesis studies have been corroborated by several in silico molecular docking and dynamic simulation studies [98, 99].

A precise understanding of how compounds bind to the pore of Kv11.1 could facilitate design of new drugs devoid of this undesirable molecular interaction [100]. Initial molecular dynamic simulation and docking studies of Kv11.1 blockers such as MK-499 or dofetilide suggested that the orientation of bound compounds was parallel to the longitudinal axis of the central cavity, oftentimes with the basic amine making a cation-π interaction with Tyr652. However, subsequent studies found that compounds could also interact

with most of the residues identified by mutagenesis studies when docked perpendicular to this axis and tucked into hydrophobic pockets located beneath each of the pore helices (Fig. 11.7a). These hydrophobic pockets are not found in most other Kv channels and were not revealed until the cryo-EM open-state structure of Kv11.1 was solved [46]. Docking models based on the cryo-EM structure suggest that the basic amine of blockers is positioned in the ion permeation pathway, just below the selectivity filter in a specific location normally occupied by a potassium ion [101] (Fig. 11.7b and c). This mode of docking suggests a more specific mechanism of pore block, interference with ion permeation by inhibiting K^+ coordination at a specific site, rather than a more generalized action (physical plugging of the pore) as was suggested by the parallel orientation docking mode.

11.6.4 Inhibition of hERG Trafficking

Although drug-induced QT prolongation and TdP are most often caused by block of Kv11.1 channels, some drugs reduce I_{Kr} by interference with the process of channel trafficking from the Golgi apparatus to the cell surface. Drug-induced inhibition of Kv11.1 trafficking has been reported for arsenic trioxide [102], pentamidine [103], fluoxetine [104] celastrol [105], and cardiac glycosides [106]. The realization that hERG1 channel function can be reduced by mechanisms other than pore block prompted the development of simple assays to test for drug-induced effects on hERG1 channel trafficking [107, 108].

11.7 Kv11.1 Channel Activators

Pharmacotherapy for LQTS is very limited. β-Adrenergic receptor blockers are the only drugs used commonly to treat LQTS [109]. The most common cause of inherited LQTS is a loss of function mutation in either *KCNH2* or *KCNQ1*. Thus, activators of I_{Kr} or I_{Ks} have been proposed as a potentially useful therapeutic approach to prevent VT/VF associated with prolonged QT intervals [110, 111]. While several compounds with such activity have been discovered and characterized in vitro and in animal models [112–114], none are currently available for clinical use.

11.7.1 Mechanisms of Action of Kv11.1 Activators

Routine screening of compounds for off-target channel activity led to the serendipitous discovery of Kv11.1 channel activators. The first such compound to be discovered and characterized was RPR260243 ((3R,4R)-4-[3-(6-methoxyquinolin-4-yl)-3-oxo-propyl]-1-[3-(2,3,5-trifluorophenyl)-prop-2-ynyl]-piperidine-3-carboxylic acid) in 2005 [3]. A plethora of compounds that activate Kv11.1 by a variety of mechanisms has since been reported, including NS1643 [115, 116], PD-118057 [117], PD-307243 [118], ICA-105574 [119], ML-T531 [120], A-935142 [121], ginsenoside Rg3 [122], AZSMO-23 [123], LUF7244 [124], and HW-0168 [125]. Activation of Kv11.1 is mediated by allosteric modulation of one or more properties of channel gating. The mechanisms of action identified to date include: (1) slowed rate of channel deactivation, (2) attenuation (positive shift in voltage dependence) of C-type inactivation, (3) shift of the voltage dependence of channel activation to more negative potentials, and (4) an increase in channel open probability [111]. RPR260243 (RPR) primarily slows the rate of Kv11.1 deactivation [3], but it also slows the rate of channel activation and enhances current magnitude by attenuation of inactivation [126]. Two compounds, NS1643 [115, 116] and PD-118057 [117], increase the magnitude of outward $I_{Kv11.1}$ but have little or no effect on the rate of channel deactivation. PD-307243 has similar effects on channel gating [118]. Several $I_{K11.1}$ activators markedly inhibit channel inactivation, including ICA-105574 [119], ML-T531 [120], and AZSMO-23 [123]. PD-118057 increases the channel open probability of Kv11.1 and shifts its voltage dependence of inactivation to more positive potentials [127]. NS1643 increases both

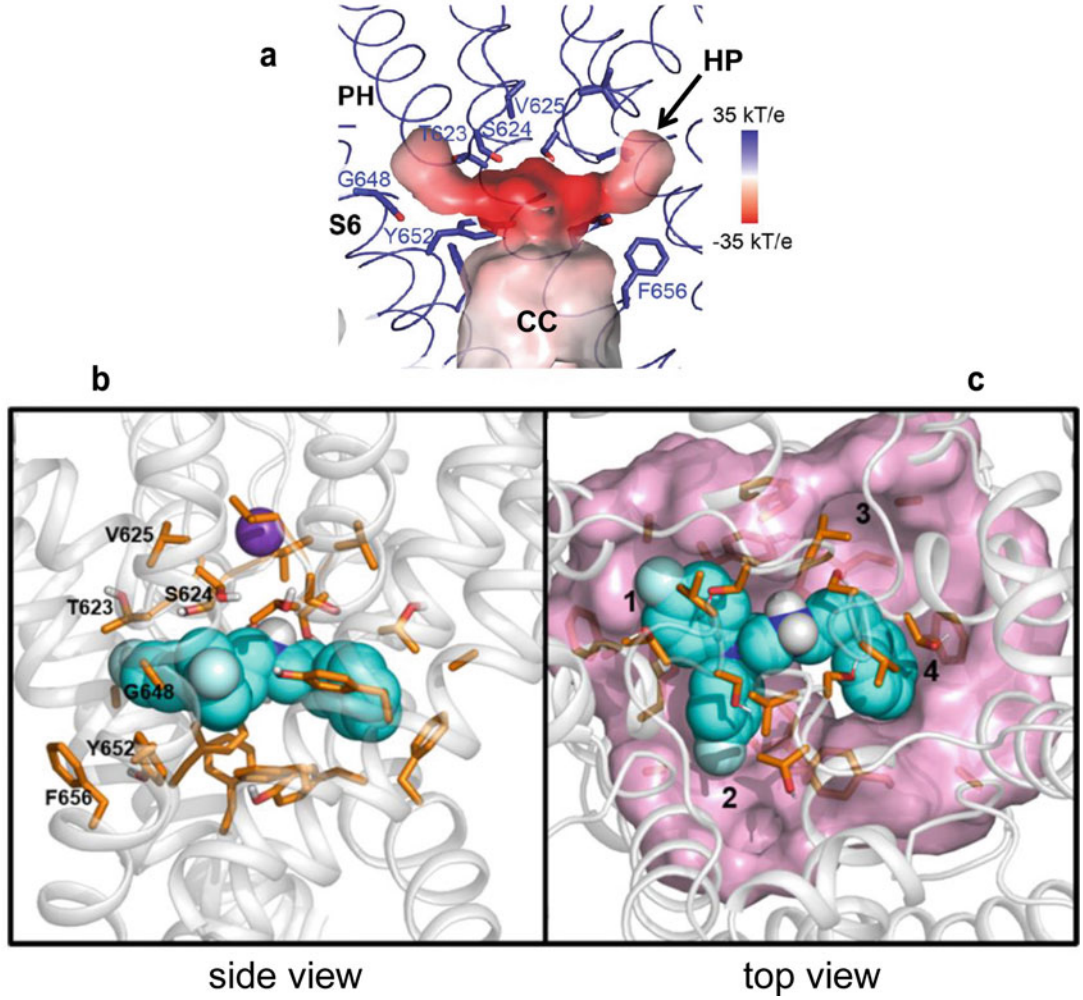

Fig. 11.7 Model of interaction between a potent blocker (Cavalli-6) and hydrophobic pockets located beneath pore helices of Kv11.1. (**a**) Side view of a region of the Kv11.1 channel cryo-EM structure that highlights the upper portion of the central cavity (CC) and lateral hydrophobic pockets (HP) located underneath each pore helix (PH). Structure is shown in ribbon mode, with key drug binding residues shown in stick mode. Figure modified with permission from Wang and MacKinnon [46]. (**b**) Cavalli-6 (in cyan) binds to hydrophobic pockets located beneath the pore helices and selectivity filter of Kv11.1. The key interacting channel residues identified by site-directed mutagenesis studies are labeled and shown in stick mode. (**c**) Top view of Cavalli-6 blocking the ion conduction pathway immediately below the selectivity filter, with its basic amine (in blue) in a location that is normally occupied by a coordinated potassium ion. The hydrophobic pockets illustrated in side view of panel A are numbered 1–4. Panels b and c were reprinted with permission from Dickson et al. 2020 [101]. Copyright 2020 American Chemical Society

$I_{Kv11.1}$ peak and tail currents [116]. Most, if not all, Kv11.1 activators also alter the gating or inhibit channels other than Kv11.1 and, thus, are not ideally suited for testing the specific efficacy or clinical utility of "pure" I_{Kr} activation. In addition, some compounds, especially those that dramatically inhibits inactivation, have the potential to cause excessive QT shortening, essentially mimicking SQTS.

11.7.2 Structural Basis of Kv11.1 Activator Binding Sites

The structural basis of Kv11.1 activation by compounds has been explored by functional analysis of drug response using mutant channels and by molecular modeling. Single residue mutations in the S6 segment (Asn658, Val659) and S5 segment (Leu553, Phe557) attenuate the effects of RPR on deactivation and inactivation, whereas other single mutations (e.g., of Val549, Leu550 in the S4-S5 linker and Ile662, Leu666, Tyr667 in the S6 segment) only affect deactivation [126]. These findings suggest that RPR binds to hydrophobic pockets located between the S5/S6 regions of adjacent subunits to partially inhibit inactivation and the coupling of VSD movement to channel opening. The stoichiometry of RPR activities was characterized using tetrameric Kv11.1 concatemers containing a variable combination of wild-type subunits and subunits containing a mutation (Leu553Ala) that eliminates the drug effect. The slowing of deactivation by RPR was found to be directly proportional to the number of wild-type subunits present in a tetramer, whereas inhibition of inactivation was half-maximal with a single wild-type subunit and maximal in channels with three wild-type subunits [128]. These findings suggest that there are four identical RPR binding sites per channel and that allosteric modulation of deactivation and inactivation are characterized by distinct subunit stoichiometry. Characterization of mutant and chimeric Kv11.1/11.2 channels indicate that the cytoplasmic region of the channel (C-linker and attached cyclic nucleotide-binding homology domain) is the key structural component that mediates the slowing of deactivation by RPR [129]. Based on mutational analyses, other activators, including PD-118057 [127] and ICA-105574 [130, 131] have also been proposed to bind to four symmetrical inter-subunit hydrophobic pockets of the Kv11.1 tetramer. Concatenated tetramer analysis indicates that the increased single-channel open probability induced by PD-118057 (a 2-(phenylamino)-benzoic acid) and the attenuated inactivation induced by ICA-105574 (a substituted benzamide) are mediated by cooperative subunit interactions and that occupancy of all four binding sites in a Kv11.1 channel is required for maximal channel activation by these compounds [132].

Based on analysis of multiple negatively charged activators (e.g., PD-118057) and potassium channel types (including K_2P, BK, and Kv11.1), it has been proposed that channel activators bind to a common site located below the selectivity filter and enhance channel conductance by increasing K^+ occupancy at specific ion coordination sites within the pore and selectivity filter [133]. This binding mode is somewhat analogous to that proposed for Kv11.1 blockers [101], but instead of inhibiting K^+ coordination, this model proposes that activators facilitate ion coordination. Molecular dynamic simulation studies utilizing long periods of sampling (100s of nanoseconds) of the open-state cryo-EM structure of Kv11.1 has revealed refined docking modes for activators, including PD-118057, that are consistent with earlier site-directed mutagenesis studies [101].

11.7.3 Kv11.1 Activators Can Be Proarrhythmic

Some Kv11.1 activators may cause excessively short action potential duration and refractoriness and, therefore, may be proarrhythmic. It is noteworthy that some activators are used to create drug-induced models of SQTS as a short electrical refractory period provides a prime substrate for re-entry-based arrhythmia. Attenuated inactivation by a compound such as ICA-105574 mimics the gain of function Kv11.1 mutations that cause inherited short QT syndrome. In coronary-perfused canine right atria, PD-118057 was reported to increase spatial dispersion of repolarization and a single premature stimulus-induced fibrillation [134]. PD-118057 was also reported to induce polymorphic VT in left ventricular wedge preparations [135]. NS1643 suppressed arrhythmias induced by infusion of dofetilide to methoxamine-sensitized rabbits or

by atrioventricular block with ventricular bradypacing in rabbits [136]. However, protection was not observed in a transgenic rabbit model of LQTS; instead, NS1643-induced shortening of QTc was accompanied by an increased incidence of arrhythmia [137].

11.8 Conclusions

Kv11.1 channels conduct a repolarizing K^+ current that is a major determinant of cardiac repolarization in humans. Loss of function mutations in *KCNH2* are a common cause of LQTS. Gain of function mutations in *KCNH2* are a very rare cause of SQTS. In clinical practice, drug-induced QT prolongation is most often caused by block of Kv11.1 channels. The cryo-EM structure of Kv11.1 has been solved, and the molecular determinants of Kv11.1 blockers and activators have been described. Extensive structure–activity relationships for a wide spectrum of compounds are now available and have guided the development of pharmacophore models that facilitate the rational design of medications that are devoid of Kv11.1 channel blocking activity. Further research is warranted to determine if novel Kv11.1 activators can safely prevent arrhythmia associated with congenital LQTS or drug-induced excessive QT prolongation.

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