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Cardiac potassium inward rectifier Kir2: Review of structure, regulation, pharmacology, and arrhythmogenesis

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Abstract

Potassium inward rectifier channel Kir2 is an important component of terminal cardiac repolarization and resting membrane stability. This functionality is part of balanced cardiac excitability and is a defining feature of excitable cardiac membranes. “Gain-of-function” or “loss-of-function” mutations in KCNJ2, the gene encoding Kir2.1, cause genetic sudden cardiac death syndromes, and loss of the Kir2 current I_{K1} is a major contributing factor to arrhythmogenesis in failing human hearts. Here we provide a contemporary review of the functional structure, physiology, and pharmacology of Kir2 channels. Beyond the structure and functional relationships, we will focus on the elements of clinically used drugs that block the channel and the implications for treatment of atrial fibrillation with I_{K1}-blocking agents. We will also review the clinical disease entities associated with KCNJ2 mutations and the growing area of research into associated arrhythmia mechanisms. Lastly, the presence of Kir2 channels has become a tipping point for electrical maturity in induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) and highlights the significance of understanding why Kir2 in iPS-CMs is important to consider for Comprehensive In Vitro Proarrhythmia Assay and drug safety testing.

Keywords

KCNJ2; KCNJ2 mutation; Kir2; Macromolecular complex; Pharmacology; Potassium inward rectifier

Introduction

Potassium inward rectifier channel Kir2 is an important component of terminal cardiac repolarization and resting membrane stability. This functionality is part of balanced cardiac excitability and is a defining feature of excitable cardiac membranes. “Gain”- or “loss-of-function” mutations in KCNJ2, the gene encoding Kir2.1, cause genetic sudden cardiac death syndromes, and loss of the Kir2 current I_{K1} is a major contributing factor to arrhythmogenesis in failing human hearts. Here we provide a contemporary review of the functional structure, physiology, and pharmacology of Kir2 channels. Beyond the structure

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and functional relationships, we will focus on the elements of clinically used drugs that block the channel and the implications for treatment of atrial fibrillation with I_{K1}-blocking agents. We will also review the clinical disease entities associated with *KCNJ2* mutations and the growing area of research into associated arrhythmia mechanisms. Lastly, the presence of Kir2 channels has become a tipping point for electrical maturity in induced pluripotent stem cell–derived cardiomyocytes (iPS-CMs) and highlights the significance of understanding why Kir2 is important to consider for Comprehensive In Vitro Proarrhythmia Assay (CiPA) and drug safety testing.

Methods

The research reviewed in this article adheres to the PRISMA guidelines as outlined by Moher et al.[1] Literature cited and research mentioned all have been derived from public domain sources from PubMed. We used the following search terms: Kir2, Potassium inward rectifier 2, *KCNJ2, I_{K1},* Andersen-Tawil syndrome (ATS), long QT syndrome 7, short QT syndrome, pharmacologic agents, Kir2 block, and Kir2 pore blockers. Additional search criteria included full-text articles, English language, and some electrophysiological-related concepts in induced pluripotent stem cells (iPSCs). A focus on cardiac electrophysiology was attempted, but when studies involved only neural or other myocytes and described key associations, noncardiac studies were included. Methodical cross-checking of available studies was used, but it is possible that nonpublic domain research has been performed that we could not include.

Structure, function, and cardiac isoforms

The Kir2.x channels are classified within a larger superfamily of potassium inward rectifiers consisting of Kir1–6 (Figure 1).[2] The Kir superfamily contains some parallels in structure and function, but their physiology and distribution are wide-ranging. Kir2, Kir3, and Kir6 subfamilies are known to be important in human cardiac electrophysiology. In concert with SUR subunits, Kir6 channels make up ligand-activated K_{ATP}, essential for the cellular response to ischemic preconditioning. Classic strong inward rectification is noted with Kir2 and Kir3 subfamilies. In mammalian hearts, the strong inward rectifiers Kir3.1 and Kir3.4 are the molecular correlates for I_{KACh}, an important receptor-activated current prevalent in the atria and nodal tissue.[3] The G-protein–gated atrial K^+ channel I_{KACh} is a heteromultimer of 2 inwardly rectifying K^+ channel proteins.[3]

In the mammalian heart, 3 isoforms of Kir2.x are present: Kir2.1, Kir2.2, Kir2.3, and they are encoded by KCNJ2, KCNJ12, and KCNJ4, respectively.[4] Kir2.1 (mouse) was the first to be cloned in 1993 and was found to have a structure similar to other known K^+ channels but with 2 transmembrane domains (not 6 like other voltage-gated K^+ channels), with an inner core for ionic passage and a large C-terminal domain.[5] Functional channels consist of 4 subunits that can assemble as homotetramers (single isoform) or heterotetramers (combination of isoforms).[6] Important tetrameric interaction of N- and C-terminus of separate subunits is necessary for proper membrane trafficking[7] and is important for channel regulation (discussed in the section on Regulatory molecules and binding partners).

The Kir2.x channels are the molecular correlates to I_{K1} (Figure 1), which in the heart functions to complete phase 3 repolarization and maintain resting membrane potential on a beat-to-beat basis to rapidly achieve membrane polarization and establish potassium and sodium gradients. Inward rectification is the strongly voltage-dependent decrease in K^+ conductance with membrane depolarization. As shown in the current/voltage relationship in Figure 2, at physiologic voltages the channels conduct outward K^+ current with a peak between −60 to −40 mV, which diminishes at more positive voltages.[5] The current reverses direction to inward close to E_K at −80 mV. The original description of inward rectification focused on the large inward current component[8] and has been emphasized as the primary characteristic of Kir2 channels historically.[6] However, physiologically voltages below −80 mV are not achieved in the heart; therefore, the dominant component of I_{K1} for cardiac electrical stability is the outward current. It is the outward current that plays a critical role in phase 3 repolarization to reset the cardiac membrane and allows Na^+ channel recovery from inactivation.[9] Kir2 channels depend on binding of phosphatidylinositol 4,5-bisphosphate (PIP_2),[10] and réintroduction of PIP_2 to inside-out patches allows for recovery of rundown.[11] Binding of PIP_2 initiates a conformational change in the tetrameric channel to allow ionic conductance. Resolution of Kir2.2 crystal structure has revealed key conserved amino acid residues from interacting separate monomers are necessary for PIP_2 binding and stabilization of the PIP_2 binding site.[12] It is not surprising that mutations of key PIP_2 residues on Kir2.1 have been found in patients with the *KCNJ2* mutation-associated disease ATS[13,14] (discussed in detail in the section on Clinical disease association and arrhythmogenesis related to *KCNJ2* mutations).

Homotetrameric Kir2.x channels have distinct biophysical properties, including single channel conductance, rectification, and pH sensitivity.[6] Kir2.1 single channel conductance is approximately 20–31 pS compared to Kir2.2 34–42 pS and Kir2.3 10–14 pS and have differing sensitivity to divalent cation block.[15] Kir2.2 passes less outward current (“steep” rectification) than Kir2.1 and Kir2.3, and Kir2.3 passes more current at more positive voltages compared to the other two (“shallow rectification”). Kir2.3 is pH sensitive[6] and interestingly can “transfer” pH sensitivity in a heterotetramer formation.[16] Heterotetramers can also exert a dominant negative effect on the current of other subunits.[17,18] From the standpoint of isoform density, Kir2.1 contributes the majority of I_{K1}, with a lesser degree of Kir2.2 in the ventricle and Kir2.3 in the atrium[19,20]; yet all 3 isoforms can be found in atrium or ventricle with unique subcellular locations.[18] I_{K1} variance between species may be explained at least in part by the ratios of Kir2 isoforms,[21] and the gradient variance of intraventricular distribution may contribute to arrhythmogenesis. The exact impact of isoforms in human physiology, pathophysiology, and arrhythmogenesis is still a subject of investigation.[18]

Regulatory molecules and binding partners PIP_2 regulation

It is well known that lipid molecules are integral for regulation of ion channel activity. PIP_2, a dynamic lipid component of cell membranes, is known to regulate a number of ion channels, including Kir2 channels. Pioneering work from Huang et al[10] in 1998 revealed that PIP_2 prevented rundown of the channel during patch-clamp experiments. Application of PIP_2 to Kir2.1 expressed in *Xenopus* oocytes resulted in activation of the channel. Indeed,

competition with PIP_2 antibodies resulted in potent inhibition of channel activity. Consistent with direct binding of PIP_2 to the channel, GST-fusion proteins of the C-terminus of Kir2.1 revealed strong binding affinity to PIP_2.[10] The crystal structure of Kir2.2 confirmed that PIP_2 binds to Kir2.2 at the interface between the transmembrane domain and the cytoplasmic domain.[12] In addition to directly modulating Kir2.x activity, PIP_2 has been shown to affect other properties of Kir2.x channels. The pH sensitivity of Kir2.x channels, for example, is dependent on PIP_2 binding affinity.[22] Similarly, PIP_2 is required for Mg^{2+} inhibition of Kir2.x, as in the absence of PIP_2, Mg^{2+} was able to inhibit the channel irreversibly.

Protein kinase A and protein kinase C regulation

Protein kinase A (PKA) regulates Kir2 channels, with evidence in heterologous systems and native cells. In heterologous expression systems, evidence seems clear that activation of PKA via isoproterenol or “PKA cocktail” (for-skolin + 3-isobutyl-1-methylxanthine [IBMX]) results in an increase in outward current.[23–26] Mutation of the putative PKA phosphorylation site at Kir2.1 Ser425 results in lack of response to PKA activation. The 3 members of the Kir2.x family—Kir2.1, Kir2.2, and Kir2.3—are expressed in human heart[27,28] and underpin cardiac I_{K1}[29]; therefore, direct comparison with heterologous expression systems expressing only 1 isoform may not recapitulate the effect of PKA activation on I_{K1}. Older studies of PKA regulation in canine Purkinje myocytes and guinea pig ventricular myocytes suggest that activation of PKA by isoproterenol inhibits Kir2.1.[30–32] However, it is worth noting that these studies focus on the inward component of I_{K1}, with little resolution of physiologic voltages. Recent work by our group has characterized mouse,[33] rat (unpublished data), and human (unpublished data) isolated ventricular myocyte response to isoproterenol. In addition, the concentration of isoproterenol used in myocyte studies is high (1 μM), which could result in saturation of the response and a large increase in intracellular calcium, which is known to inhibit I_{K1}. Therefore, a submaximal dose and more physiologically relevant concentrations revealed that I_{K1} is, indeed, increased.[33]

The effect of protein kinase C (PKC) on I_{K1} is controversial. Activation of β3-adrenoreceptors with isoproterenol enhanced Kir2.1 and Kir2.2 currents with no effect on Kir2.3 channels.[34] Inhibition of PKA and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) had no effect on regulation of Kir2.1; however, PKC inhibition suppressed activation of Kir2.1. In contrast, PKA inhibition abolished activation of Kir2.2. This suggests that different isoforms of Kir2.x are differentially regulated by PKC, an effect that was observed when expressed as heteromeric channels and resulted in all channels being activated following isoproterenol.[34] Interestingly, Kir2.3 activity can be inhibited by PKC-mediated phosphorylation at threonine53.[35] When the N-terminus of Kir2.3 was replaced with that of Kir2.1, the chimeric channel lost sensitivity to inhibition via PKC activation. In contrast, treatment of monocytes with phorbol 12-myristate 13-acetate (PMA) activated Kir2.1 and Kir2.2 channels and seemed to be dependent on conversion of PIP_2 to PIP_3.[36] The majority of these studies are performed using *Xenopus* oocytes, without the full complement of cellular machinery, so it can be argued that in native tissues, the observed

effects could be different, resulting in the different effects of both PKA and PKC regulation observed on Kir2.x channels.

Nitric oxide

Nitric oxide plays an important regulatory role within cardiac cells. At physiological levels of nitric oxide, I_{K1} increased in atrial myocytes and in Chinese hamster ovary (CHO) cells.[37] Direct nitrosylation at Kir2.1 Cys76 increases channel open probability as well as frequency of channel opening. The effect is membrane hyperpolarization and action potential (AP) shortening in mouse and human atria.

Subcellular localization

Kir2.x channels are regionally distributed in the heart. I_{K1} is prominent in ventricular myocytes and Purkinje fibers, but is significantly smaller in atrial myocytes (with the exception of mouse atria). Real-time reverse transcriptase polymerase chain reaction of Kir2.x transcripts from human heart revealed the following expression profiles for Kir2.x isoforms: Purkinje fibers: Kir2.1>Kir2.3>Kir2.2; right ventricle: Kir2.1>Kir2.2>Kir2.3; and right atria: Kir2.3>Kir2.2>Kir2.1.[38] Immunostaining techniques show a distinct t-tubular staining pattern for Kir2.1 and Kir2.2 in human left ventricle sections, with Kir2.3 found only at the intercalated disc.[18,39] Disruption of t-tubules via osmotic shock in ventricular myocytes leads to decrease in I_{K1}, supporting their localization to t-tubular membranes. In addition, co-staining with caveolin-3 (Cav3), critical for the formation of caveolae, revealed colocalization with Kir2.1 at the sarcolemma; with Kir2.1 and Kir2.2 in t-tubules; and with Kir2.1 and Kir2.3 at the intercalated disc.[18] This suggests that Kir2.x isoforms may localize to caveolar domains within cardiac myocytes, which are important for clustering of ion channels and molecular partners required for appropriate excitation–contraction coupling.[40] The interaction between Kir2.x and Cav3 is discussed in the section on Cav3.

Synapse-associated protein-97

Kir2.x channels in macromolecular complexes are known to interact with the membrane-associated guanylate kinase (MAGUK) scaffolding protein family.[16,41,42] Synapse-associated protein-97 (SAP-97) belongs to the MAGUK family and is expressed in heart. Kir2.x proteins bind MAGUK proteins through a PDZ binding motif in the C-terminus, except Kir2.4. This binding motif may overlap with the putative PKA phosphorylation site, and phosphorylation of this site results in loss of SAP-97 binding.[43] Immunostaining also demonstrated colocalization of Kir2.x and SAP-97 in cardiac myocytes.[43] Heterologous expression of Kir2.3 and SAP-97 resulted in cellular relocalization and increased cell surface expression.[41] Reported single channel conductance for Kir2.3 were variable when coexpressed with SAP-97 without altering the open probability. Interestingly, knockdown of SAP-97 in rat ventricular myocytes decreased I_{K1} and blunted the response to isoproterenol. Additionally, loss of SAP-97 seemed to be due to a loss of Kir2.x channel abundance, perhaps related to cell surface expression.[16] Coimmunoprecipitation studies show that SAP-97 interacts with PKA and b_1-adrenergic receptors in cardiac myocytes, suggesting that SAP-97 also regulates the signaling complex involved in regulation of Kir2.x.[16]

Cav3

We have previously shown that Kir2.x channels associate with Cav3, encoded by CAV3 in the heart.[18,39,44] Fluorescent resonant energy transfer and immunoprecipitation demonstrated an association between Cav3 and Kir2.1. In addition, residues required for the association with Cav3 are conserved in the N-terminal sequence, containing a caveolin-binding motif (CBM) QxQxxxxQ where, Q is an aromatic amino acid (tyrosine, tryptophan, and phenylalanine)[45–47] and x represents other amino acids. CBM of Kir2.x is required for coimmunoprecipitation, as deletion results in loss of association. Cav3 scaffolding and membrane domains associate with Kir2.x. Disruption of these domains or CBM could influence Kir2.x localization to caveolar domains. Mutations in *CAV3* modulate Kir2.x channel activity and localization.[39] The long QT syndrome 9–associated Cav3 mutation F97C, when expressed with homomeric Kir2.1 and Kir2.2, decreased current density by 50%–60% but had no effect on Kir2.3. Heteromeric channels coexpressed with F97C-Cav3 decreased current density of Kir2.1-Kir2.2 and Kir2.2-Kir2.3 heteromers and seems to be related to decreased channel trafficking.[18,39,44]

Kir2.1 and $Na_V1.5$ macromolecular complex

We and others have demonstrated that Kir2.1 and $Na_V1.5$ colocalize in ventricular myocytes in human, rat, and mouse.[18,48] Both Kir2.1 and $Na_V1.5$ interact independently with distinct PDZ domains within SAP97 (discussed in the section on Synapse-associated protein-97) and α_1-syntrophin. Functional changes in Kir2.1 were shown to modulate $Na_V1.5$ and vice versa, suggesting that they are functionally linked.[48] This interaction is a crucial determinant of cardiac excitability and AP duration, as overexpression of both in neonatal rat ventricular myocyte (NRVM) monolayers resulted in hyperpolarization of the resting membrane potential, shortening of the AP, and an increase in conduction velocity due to $Na_V1.5$ availability. Additionally, an increase in the frequency and persistence of reentrant rotor activity was observed in NRVM monolayers when both $Na_V1.5$ and Kir2.1 were overexpressed.[48] Interestingly, overexpression of the N-terminal domain of $Na_V1.5$ resulted in increased I_{Na} and I_{K1} via increasing expression of $Na_V1.5$, Kir2.1, and Kir2.2, which seems to mediated by α_1-syntrophin.[49] When chimeras of Kir2.1 and Kir2.2 were generated in which PDZ domains were replaced with PDZ domains that bind α_1-syntrophin but not SAP-97, cotransfection with $Na_V1.5$ increased I_{K1} inward and outward current. Knockdown of α_1-syntrophin in CHO cells and adult rat ventricular myocytes had decreased I_{K1} and I_{Na}.[49] The reciprocal modulation of $Na_V1.5$ and Kir2.1 is due in part to cotrafficking of the channels. Trafficking-deficient Kir2.1$^{\Delta 314-15}$ mutation reduced expression of $Na_V1.5$ at the surface membrane.[50] It has also been shown that endoplasmic reticulum (ER) trafficking-deficient $Na_V1.5$ channels associated with Brugada syndrome significantly decreased I_{K1}.[51] Similarly, Golgi trafficking-deficient $Na_V1.5$ channels had a dominant-negative effect on I_{K1}, resulting in additional decreases in I_{K1}. However, the effect of ER trafficking-deficient $Na_V1.5$ on Kir2.x channels can be partially rescued through an unconventional secretory route that involved Golgi reassembly stacking proteins.[51]

Pharmacology of Kir2.x

A number of pharmacologic agents affect Kir2.x current. As the contribution of I_{K1} in arrhythmic disease is better understood, the basis for block or enhancement of I_{K1} is important for both antiarrhythmic and proarrhythmic effects.

Pore blockers

It is well established that Kir2.x channels conduct K^+ ions inwardly through the cell membrane more efficiently than outwardly. Cations have been widely used to investigate the permeability and gating mechanisms of potassium channels.[52] Kir2.x channels are particularly sensitive to blockade by divalent cations and have been studied in native tissues and heterologous systems.[53–57] These studies identified 2 distinct binding sites: a shallow site that does not sense the membrane electrical field, and a deeper site that is approximately halfway within the membrane electrical field.[54–56,58,59] A single ion is all that is required to block the channel at either site. Removal of divalent cations from the external solution decreases the extent of inactivation of Kir2.x channels andofKirl.1 channels.[53,60] Increasing extracellular K^+ can decrease the extent to which external Mg^{2+} and Ca^{2+} can block Kir channels.[60–62] K^+ antagonizes the effect of Mg^{2+} in a manner that suggests they compete for an external inactivation site. Mg^{2+} was identified to reduce inward currents in Kir2.2 in a voltage-dependent manner.[63] Molecular dynamic simulations demonstrated that Mg^{2+} blocks the channel by staying at the selectivity filter and causes a reduction in current as a result. Mutagenesis of key negatively charged residues at the outer mouth of the pore in Kir2.2 decreased the voltage-dependent blockade of inward currents by Mg^{2+} via electrostatic repulsion.[63]

Ca^{2+} also produces voltage-dependent block of Kir2.x channels, but with less potency than Mg^{2+}.[64] Despite reduced potency and low average concentrations of $[Ca^{2+}]_i$, there is some evidence that Ca^{2+} can modulate I_{K1}. Recordings of I_{K1} during the AP in guinea pig ventricular myocytes, transient increases in Ca^{2+} lead to inhibition of the current.[65] It is proposed that this reduction in current flow is due to a decrease in outward current[66] by decreasing the open probability of the main open channel state. The concentrations of Ca^{2+} that generate changes in rectification are in the range of those observed during Ca^{2+} transients and may affect Kir2.1 conductance during the cardiac AP.

Polyamines have also been demonstrated to inhibit Kir2.x channels. Mice with altered polyamine biosynthesis, which results in increased spermidine levels, reduced I_{K1} by approximately 38%, with no effect on rectification.[67] Additionally, loss of spermine via disruption of spermine synthase gene resulted in I_{K1} with weakened rectification and no change in current density. This suggests a role of spermine in the rectification at potentials positive to E_K, with spermidine dominating at potentials around and negative to E_K. In all Kir2.x isoforms, blockade by spermine is characterized by 3 distinct components: 1 steep and 2 shallow.[68] These components are predicted to correspond to spermine blocking at 2 distinct sites in the pore.[69,70] The steep component is due to binding to negative residues in the cytoplasmic vestibule of the channel, whereas the slow component is due to binding to the “rectification controller” deep within the pore.[71–73] Kir2.x isoforms are differentially regulated by spermine at these 2 sites, with block at shallow site 1 being more potent in

Kir2.2 than in Kir2.3 as well as differences in the steep component of rectification.[68] It was demonstrated in heterologous experiments with Kir2.1 that polyamines regulate the amplitude of outward I_{K1} by modifying the proportion of channels with different sensitivities to blockade, and outward current is primarily generated by channels with lower affinity to polyamines.[74]

Barium is a widely used pore blocker of Kir2.x channels. It blocks Kir2.x channels in a voltage-dependent manner,[75] blocking 50% of Kir2.1 current at 3–10 μM when the membrane potential was −80 mV. Barium impairs Kir2.1 rectification via a noncompetitive mechanism. Binding of divalent cations to Kir2.x channels is thought to occur via 2 distinct binding sites. Additionally, 2 mutations in the pore region (E125N and T141A) impaired Ba^{2+} entry and binding to the channel.[75] Ba^{2+} was found to bind to the deeper site within the membrane electrical field.[54–56,58,59,76] Expression of Kir2.1 and Kir2.3 homomeric channels in *Xenopus* oocytes resulted in similar Ba^{2+} sensitivities, but they were less sensitive than native cardiac I_{K1}.[77] Kir2.2 showed similar sensitivity to cardiac I_{K1}, but blocking kinetics were faster than native currents. Coexpression of Kir2.x subunits had similar Ba^{2+} sensitivities and blocking kinetics to native I_{K1}. However, Ba^{2+} is known to inhibit other types of K^+ channels, including the delayed inward rectifier, native inward K rectifier of starfish eggs and frog skeletal muscle, and Ca^{2+}-activated K^+ channels.[59] Additionally, Ba^{2+} is a potent blocker of the BK channel pore.[78] Ba^{2+} also acts as a charge carrier for L-type Ca^{2+} channels.[79] Therefore, although Ba^{2+} is a useful pharmacologic tool for studying I_{K1}, in certain cell systems, its effect on other ion channels may confound results.

Chloroquine is an important therapeutic treatment of malaria as well as adjunct therapy for systemic inflammatory disorders. However, despite its use clinically, it has a narrow safety margin. It is known to cause prolongation of QT and QRS on surface electrocardiograms (ECGs).[80,81] At higher concentrations, chloroquine can lead to ventricular ectopy and ventricular arrhythmias.[82] These clinical outcomes are the result of prolongation of the cardiac AP duration, enhancement of automaticity, and a decrease in maximum diastolic potential at the cellular level.[83,84] These cellular changes are due, in part, to blockade of I_{K1} and I_{Kr}.[84,85] Like many of the pore blockers discussed here, chloroquine blocks Kir2.x channels from the cytoplasmic surface in a voltage- and K^+-dependent manner.[81] Interestingly, even when Kir2.1 channels were pre-blocked with polyamines, chloroquine was still able to reach its binding site, suggesting that it binds at a site distinct from polyamines. Despite the potentially fatal effects of chloroquine, there may be some therapeutic potential for certain cardiac conditions. In patients with persistent atrial fibrillation, a 14-day regimen of chloroquine decreased burden of atrial fibrillation,[86] which may be related to blocking I_{KACh}[87] and I_{K1} to prolong atrial APs.

PIP_2 interference

PIP_2 is required for Kir2.x channel function (discussed previously in the section on PIP_2 regulation). Several pharmacologic agents exert their effects via interference with PIP_2–Kir2.x channel interaction. Quinacrine, which originally was developed as an antimalarial drug, has been demonstrated to inhibit Kir2.x channels in guinea pig neurons.[88] Quinacrine has high lipophilicity and interacts with membrane phospholipids directly. It differentially

inhibits Kir2.3 to a greater degree than Kir2.1 channels. It is proposed that this occurs via direct pore blockade and disruption of PIP_2–Kir channel interaction. Evidence for disruption of this interaction was due to the slow onset of blockade by quinacrine, in addition to increasing or decreasing the affinity of the channel for PIP_2, respectively, which resulted in either decreased or increased sensitivity to blockade. In addition, application of PIP_2 with quinacrine resulted in decreased inhibition of Kir2.x channels.

Carvedilol is a commonly used β- and α-adrenoreceptor antagonist used in the treatment of congestive heart failure, hypertension, and myocardial infarction.[89,90] In addition to its effects on adrenoreceptors, it acts as a multichannel blocker that can inhibit I_{kr}, I_{ks}, I_{to}, K_{atp}, $I_{ca\text{-}L}$, $I_{ca\text{-}T}$, and I_{Na} with variable potency.[91–94] Studies in HEK293 cells demonstrated that carvedilol inhibits K_{ATP} and K_{ACh} channels, with no direct effect on I_{K1}.[95] The high lipophilicity and alpha-hydroxyl secondary amine functional group of carvedilol may insert into the membrane and interfere with PIP_2-channel interaction and thereby may inhibit Kir2 channels.[96] Kir2.3 has lower affinity for PIP_2 compared to Kir2.1. It is inhibited by carvedilol with IC_{50} = 0.49 μM, which is 100-fold higher than the IC_{50} of Kir2.1 (>50 μM). Inhibition was concentration- and voltage-dependent. Increasing the affinity of Kir2.3 for PIP_2 resulted in decreased inhibition by carvedilol.[96] Additionally, addition of exogenous PIP_2 decreased the inhibitory effect.

Interestingly, gambogic acid, which is an anticancer agent, showed both PIP_2 interference and pore-block effects on homomeric and heteromeric channels.[97] Gambogic acid showed slow inhibition of monomeric and heteromeric channels at low micromolar concentrations; however, it did not reach saturation during the course of experiments in *Xenopus* oocytes and was irreversible. Site-directed mutation of amino acids involved in polyamine block resulted in reduced inhibition in both homomeric and heteromeric channels, suggesting a direct pore-block mechanism. In addition, mutation of residues to alter the affinity for PIP_2 resulted in increased inhibition when affinity was increased (I214L Kir2.3).[97] This effect was more pronounced in Kir2.3 and Kir2.2 channels than in Kir2.1 channels, which may be explained by their differences in PIP_2 affinity.

Cross-reactivity

Dronedarone is a Class III antiarrhythmic drug used to treat atrial fibrillation.[98] It was shown in guinea pig ventricular myocytes that dronedarone is a multichannel blocker that inhibits I_{Na}, $I_{Ca\text{-}L}$, I_{Kr}, I_{Ks}, and I_{K1}.[99] Dronedarone inhibited I_{K1} in a dose-dependent manner, with incomplete block at 10 and 30 μM in guinea pig ventricular myocytes. Expression of heteromeric channels in *Xenopus* oocytes revealed inhibition of Kir2.1 but not Kir2.2 or Kir2.3.[100] The onset of block was slow and reversible upon washout. Blockade had no voltage- or frequency-dependence. Kir2.1 mutation of E224 within the cytoplasmic pore region of the channel resulted in loss of dronedarone inhibition, suggesting that this site is involved in binding the drug to mediate its effect.

Recently, a selective I_{K1} agonist was identified.[101] Zacopride is a potent 5-HT_3 receptor antagonist and 5-HT_4 receptor agonist, commonly used as a gastrointestinal prokinetic agent. Application of zacopride to isolated rat cardiomyocytes revealed dose-dependent activation, resulting in hyperpolarization of the resting membrane potential and shortening

of the AP. Interestingly, in a model of drug-induced arrhythmia, treatment with zacopride protected from ventricular arrhythmias.[101] This agonist effect on I_{K1} is specific to Kir2.1, as treatment of atrial myocytes resulted in no drug effect.[23] Zacopride treatment of homomeric Kir2.1 channels of HEK293 cells increased current density but not homomeric Kir2.2, Kir2.3, or Kir2.1/Kir2.2, and Kir2.1/Kir2.3 heteromeric channels. Mutation of the putative PKA phosphorylation site in Kir2.1 abolished zacopride-mediated increase in I_{K1}[23] and suggests that zacopride mediates its effect via PKA phosphorylation of Kir2.1 channels.

I_{K1} is susceptible to modulation by pharmacologic agents, which initially were identified as blocking other ion channels. Quinidine is used clinically to terminate atrial fibrillation as a Class IA antiarrhythmic drug. However, it is well documented to be a multichannel inhibitor.[102] It was demonstrated that quinidine inhibited Kir2.1 by acute pore block of subunits via interactions with E224, F254, and D259 residues.[103] Further investigation of other Kir2.x isoforms revealed Kir2.3 to have the highest affinity for quinidine.[104] Block is achieved in a voltage-dependent manner, with residues E224, F254, D259, and E299 essential for block in Kir2.1 and corresponding residues in Kir2.3; however, only D260 was essential in Kir2.2. Mutational analysis of PIP_2 sensitivity sites revealed that high PIP_2 affinity resulted in low inhibition by quinidine and vice versa.[104] Although Kir2.1 is the least sensitive to quinidine compared to Kir2.2 and Kir2.3, caution still should be used when prescribing this drug to avoid potential proarrhythmic effects.

Propafenone, another Vaughn-Williams Class IC antiarrhythmic drug, was also shown to inhibit Kir2 channels. It has greater efficacy for Kir2.3 compared to Kir2.2 and Kir2.1, most likely related to differences in PIP_2 affinity.[105] Interestingly, extracellular K^+ does not affect inhibition by propafenone; however, decreasing intracellular K^+ results in subconductance levels in channel gating and reduced affinity for PIP_2. Using mutagenesis, propafenone binds to a cytoplasmic domain located at the interface between subunits, with conserved arginine residues 228 and 260 being important for binding.[105]

Clinical disease association and arrhythmogenesi s related to *KCNJ2* mutati ons

The association of *KCNJ2* mutations in arrhythmogenic disease has been recognized for more than 20 years. Clinical phenotypic disease association with *KCNJ2* mutations has been described for ATS,[106] short QT syndrome type 3 (SQT3),[107] catecholaminergic polymorphic ventricular tachycardia (CPVT),[24,108] and familial atrial fibrillation (FAF).[109] Here we review the various phenotypes, broadly classified as gain of function (SQT3 and FAF) vs loss of function (ATS and CPVT), and what is known about the arrhythmia mechanism(s) for different phenotypes.

The 2 clinical phenotypes associated with a gain of Kir2.1 function are SQT3 and FAF, both of which are exceedingly rare. SQT3 patients are known to have both atrial fibrillation and ventricular fibrillation, and the resting ECG has characteristic extreme abbreviation of repolarization, with QT <300 ms.[107] Due to the low prevalence of these disorders, little is known about the clinical course. It has been shown experimentally that *KCNJ2* gain of function dramatically shortens the AP and therefore the atrial and ventricular refractory period. Mechanistically, computer modeling has demonstrated this in turn supports the

initiation and stabilization of rotors of fibrillation.[110] Pharmacologic therapy logically rests on prolonging repolarization, and clinical use of hydroxychloroquine for that purpose has been reported.[111]

ATS is a rare (although probably underreported clinically and in the literature) disorder comprising a clinical triad of ventricular arrhythmia, periodic paralysis, and dysmorphic features.[106] Due to the ubiquitous presence of Kir2.1 in excitable tissue, it is not surprising that there is a notable overlap with neurologic symptoms and skeletal muscle abnormalities occurring in ATS patients with loss of normal function mutations in *KCNJ2.* The majority of ATS patients harbor mutations in *KCNJ2,* but not all patients manifest the full clinical triad.[112] This incomplete penetrance of clinical features does not seem to be mutation specific, as individual family members can present differently. Additionally, there may be some modification based on sex for some mutations.[113] ECG findings of ATS patients include frequent ventricular ectopy and prominent U waves with usually normal QT intervals but long QTu intervals.[114] In addition, more sustained ventricular arrhythmia associated with ATS includes polymorphic ventricular tachycardia and bidirectional ventricular tachycardia (BiVT).[112] The observation of BiVT in ATS patients is interesting, as this is the signature arrhythmia for CPVT. In contrast to patients with CPVT due to *RYR2* or *CASQ2* mutations (CPVT1 and CPVT2, respectively) who experience arrhythmia during adrenergic surges, ATS patients can have BiVT at rest and even during sleep. There is some nuance to this finding, as some ATS patients can present with stress-/exercise-induced BiVT and polymorphic ventricular tachycardia, in a manner similar to CPVT, so the clinical syndrome of CPVT has been associated with *KCNJ2* mutations. However, this phenotypic label probably is inconsistent with classically described CPVT1 or CPVT2 because the clinical course is much more benign for *KCNJ2* mutation–associated CPVT phenotypes. Compared to CPVT1/CPVT2, ATS patients have a very low incidence of sudden death, as recently reviewed by Pérez-Riera et al.[115] Moreover, ATS also has been categorized with long QT syndrome (LQTS) as LQT7.[116,117] Recent experimental work and ongoing clinical observations call this categorization of ATS as an LQTS into question.[118,119] These distinctions are important due to divergent clinical management strategies between CPVT and LQTS.

Experimental investigation has provided some insight into the mechanism for BiVT in patients with certain *KCNJ2* loss-of-function mutations or loss-of-function models. Loss of Kir2 function can lead to membrane instability and therefore promote or fail to inhibit triggered activity. In a rabbit heart failure model with barium initiated to block I_{K1}, loss of Kir2.1 current was linked to small increases in diastolic Ca^{2+} causing an imbalance in membrane voltage to intracellular Ca^{2+} (V_m/Ca_i imbalance) leading to delayed afterdepolarizations (DADs).[120] Using a canine ventricular wedge model, I_{K1} block predisposed to DADs, and this was proposed as a mechanism for both U waves and ventricular arrhythmia in ATS.[121] However, ATS has been categorized as a LQTS, and theoretically ventricular arrhythmia should be related to triggered activity of early afterdepolarizations (EADs). That said, as a strong rectifier, I_{K1} is negligible at AP plateau voltages, so loss of I_{K1} is unlikely to induce phase 2 EADs, which are classically associated with torsades de pointes from AP prolongation and phase 2 EAD vulnerability.[122] We sought to address this controversy by creating a transgenic mouse model with *KCNJ2*

mutation from a patient in our clinic associated with stress-induced BiVT.[25] Isolated myocytes demonstrated phase 3 but not phase 2 EADs in association with adrenergic-induced loss of I_{K1} and repolarization reserve (Figure 3).[33] DADs were noted but were much less frequent than phase 3 EADs. Our findings are supported by others who have shown using mathematical modeling that inhibition of I_{K1} underlies initiation for phase 3 EADs.[123]

We suspect that, at least in our transgenic model, a hybrid of EAD and DAD conditions is necessary for phase 3 EADs, as these require adrenergic stimulation and Ca^{2+} loading (DAD conditions) with adrenergic-dependent loss of I_{K1} AP prolongation. Thus, the clinical phenotypic syndrome, although categorized as an LQTS but mimics CPVT, actually is neither one. Important clinical implications arise from this observation because beta-blockers alone likely will not adequately suppress ATS-related ventricular arrhythmia,[124] and agents that block phase 3 EADs are likely to be more effective treatment methods.[125] Understanding the mechanism behind ATS arrhythmia and methods for arrhythmia suppression are active investigations in our laboratory.

iPSCs and Kir2.x

Features of iPS-CMs

The advances of using iPSCs has allowed for the investigation of many human diseases in a human context without the need for invasive biopsies.[126] Furthermore, development of patient-specific iPS-CMs enables the investigation of inherited arrhythmia disorders such as LQTS and CPVT.[127–131] Most iPS-CMs possess several features of immature cardiomyocytes, which limits their use for modeling cellular arrhythmia mechanisms. This is due in part to immature AP properties with a depolarized resting membrane potential and spontaneous automaticity due to small I_{K1}[132,133] and unchecked I_f. Spontaneous automaticity interferes with the ability to control the depolarization frequency, undermining attempts to model bradycardia- or pause-dependent arrhythmias such as torsades depointes, the arrhythmia characteristically associated with acquired or congenital LQTS. A variety of methodologies have been developed to generate more mature iPS-CMs. Most studies focus on structural maturity and calcium handling[134] and some structural developments,[135,136] and coculture may increase ion channel expression.[137,138] However, these developments and prolonged culture time can result in only a very modest increase in gene expression of I_{K1} that remains several levels below what is observed in adult cardiac myocytes.[136,139] Our group has demonstrated that an electrically native-like myocytes can be achieved with the creation of I_{K1}-enhanced iPS-CMs. We have shown that I_{K1} enhancement establishes the normal polarized membrane potential, allowing for recovery of inactivation for sodium channels, reflected in dV/dt values in the range of adult myocytes.[9] A polarized membrane also allows for larger calcium transients compared to controls, without changes in basal calcium levels or rate of decay of the transient. Normal excitability and calcium cycling are essential cardiomyocyte properties required for disease modeling. Studies without a normal polarized membrane with physiologic I_{K1} hinders the accurate evaluation of pharmaceutical agents.[140] These are important considerations when utilizing iPS-CMs for cardiac drug safety testing such as the Food and Drug Administration–initiated CiPA.[141]

Summary

Significant progress has been made toward understanding the role of Kir2.x in cardiac excitability, structure, function, and regulation, but there is still much to be learned from studying this channel. New arrhythmia mechanisms from novel mutations and new pharmaceutical agents are emerging, highlighting the importance of fully understanding the role of this channel in cardiac excitability, response to therapeutic drugs, and disease.

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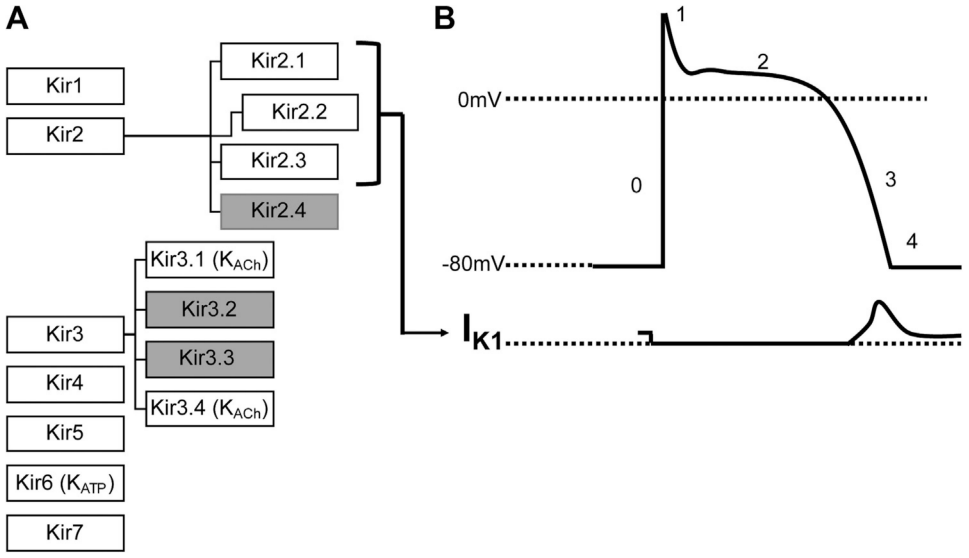

Figure 1.

Kir superfamily and role in cardiac action potential. **A:** Hierarchy plot showing different Kir family members. **B:** Ventricular cardiac action potential with I_{k1} current activity highlighted below.

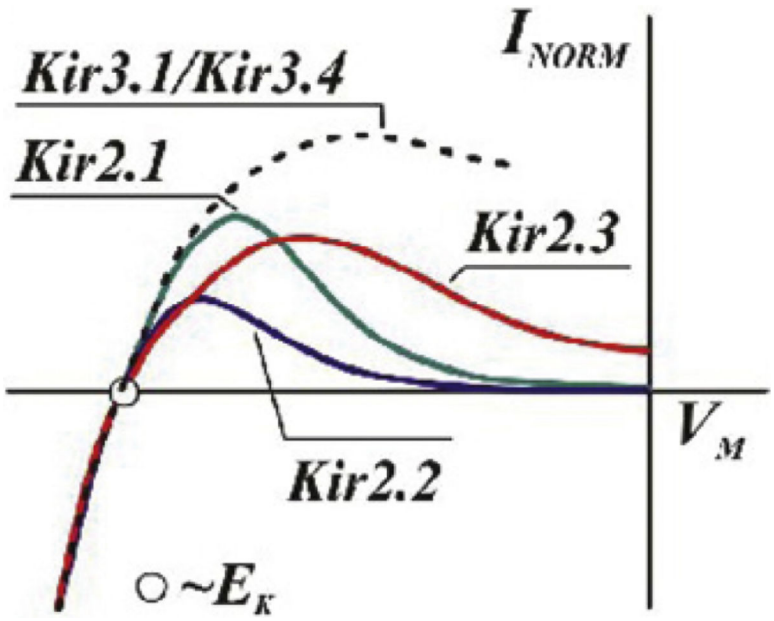

Figure 2.
Kir2 isoform current/voltage profiles shown in comparison with Kir3.1/3.4.

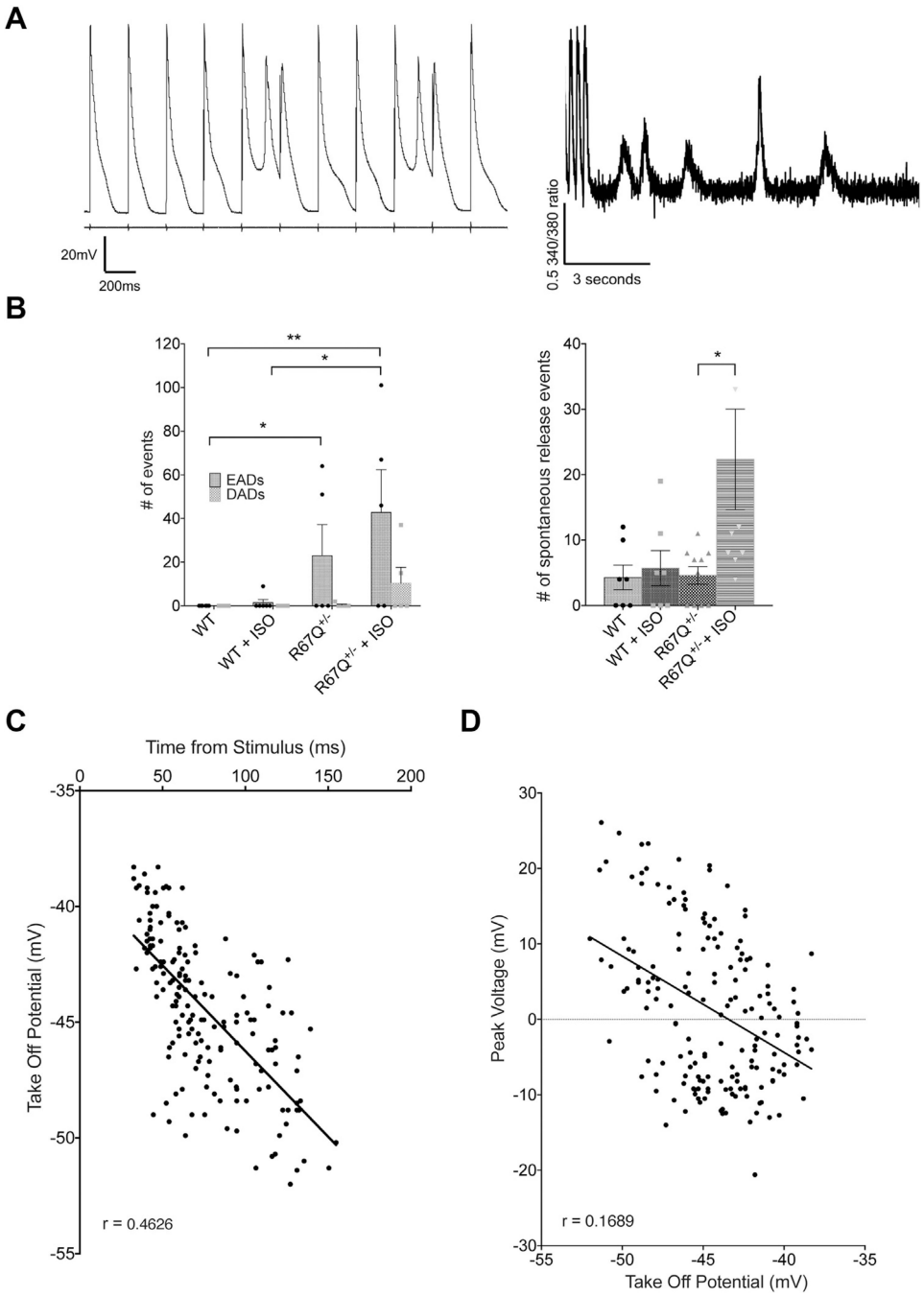

Figure 3.

Adrenergic-dependent phase 3 early afterdepolarizations (EADs) in R67Q$^{+/-}$ mice. **A:** Representative traces from action potential recordings **(left)** and calcium transients **(right).** **B:** R67Q$^{+/-}$ have more EADs and delayed afterdepolarizations (DADs) following adrenergic stimulation. **C:** Takeoff potential of EADs in R67Q$^{+/-}$ myocytes. **D:** Linear regression of takeoff potential vs peak voltage of phase 3 EADs. ISO = isoproterenol; WT = wild type.