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Cardiac ion channels

Birgit T Priest* and Jeff S McDermott

Lilly Research Laboratories; Indianapolis, IN USA

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Abbreviations: AF, Atrial Fibrillation; CiPA, Comprehensive *in vitro* Proarrhythmia Assay; VGCC, Voltage-gated calcium channel.

Ion channels are critical for all aspects of cardiac function, including rhythmicity and contractility. Consequently, ion channels are key targets for therapeutics aimed at cardiac pathophysiologies such as atrial fibrillation or angina. At the same time, off-target interactions of drugs with cardiac ion channels can be the cause of unwanted side effects. This manuscript aims to review the physiology and pharmacology of key cardiac ion channels. The intent is to highlight recent developments for therapeutic development, as well as elucidate potential mechanisms for drug-induced cardiac side effects, rather than present an in-depth review of each channel subtype.

Introduction

A number of ion channels are expressed in the heart and in the vasculature and are the target for important therapeutics as well as for off-target cardiac side effects of drugs in development. In the following chapters, we aim to briefly discuss the key players, their expression profiles, physiological roles and pharmacology.

Sodium Channels

Voltage-gated sodium channels typically exist as a protein complex consisting of a large α subunit (~260 kDa) with one or two β subunits. To date, 9 α subunits, Nav1.1–1.9, have been identified. The accessory β subunits (β1−β4) are not required to form functional channels but may affect trafficking and/or biophysical characteristics of the channel.[1] The main sodium channel subtypes found in the heart are the tetrodotoxin (TTX) insensitive subtypes Nav1.5 and Nav1.8. Expression of TTX-sensitive sodium channels has been described,[2] but the lack of cardiovascular effects of TTX in animals and human victims of accidental TTX poisoning suggests that these channels contribute little to normal cardiac function.[3] Expression of Nav1 channels in smooth muscle cells of the vasculature has been reported,[4] and the sodium channel activator veratridine induces contraction of several types of rodent blood vessels. The contractile effects of veratridine are partly mediated through actions on sympathetic nerves, but may also involve a direct effect on smooth muscle myocytes.[5] However, the lack of effect of sodium channel blockers suggests that they do not contribute to vascular tone under physiological conditions.

*Correspondence to: Birgit T Priest; Email: priest_birgit_t@lilly.com
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Nav1.5

Nav1.5, encoded by the SCN5A gene, is known as the cardiac sodium channel. However, it is expressed to some degree in other excitable and nonexcitable tissues.[6] With the exception of the sinoatrial (SA) node and atrioventricular (AV) node,[7] Nav1.5 activation is responsible for action potential upstroke throughout the myocardium. This initial influx of Na^+ provides the depolarization trigger for voltage-gated calcium channel activation, subsequent calcium dependent calcium release from the sarcoplasmic reticulum, and finally, contraction of the sarcomeres. In addition to their role in contraction, sodium channels are also the key driver of cardiac conduction. In the ventricles, the summation of the individual action potential upstrokes forms the ventricular depolarization wave responsible for the QRS complex in the electrocardiogram (**Fig. 1**). The atrial depolarization wave, reflected in the PR interval, while also sodium channel dependent, has a calcium channel dependent component as well. Conduction between adjacent cardiomyocytes involves gap junctions at the intercalated disc regions that express connexin proteins and high local concentrations of Nav1.5, and it is thought that local electrical fields may contribute to sodium channel activation in adjacent myocytes independent of gap junctional communication.[8]

Human mutations in SCN5A have been linked to multiple perturbations in cardiac function: loss-of-function mutations are the cause of approximately 20% of Brugada syndrome cases[9]; whereas gain-of-function mutations cause long QT Syndrome type 3.[10] SCN5A constitutive KO mice are embryonic lethal, with the SCN5A+/− heterozygotes displaying some of the conduction-related deficiencies seen in the human mutant population.[11]

Nav1.5 is the target of many common antiarrhythmic therapies. Based on the Vaughan-Williams schema, sodium channel blockers are grouped into Class I based on their propensity to decrease the upstroke velocity (Vmax or dV/dt) of ventricular cardiac action potentials. Sodium channel blockers are further subdivided based on their effects on the QRS interval and the effective refractory period (ERP). While all Class I antiarrhythmics have higher channel affinity at depolarized membrane

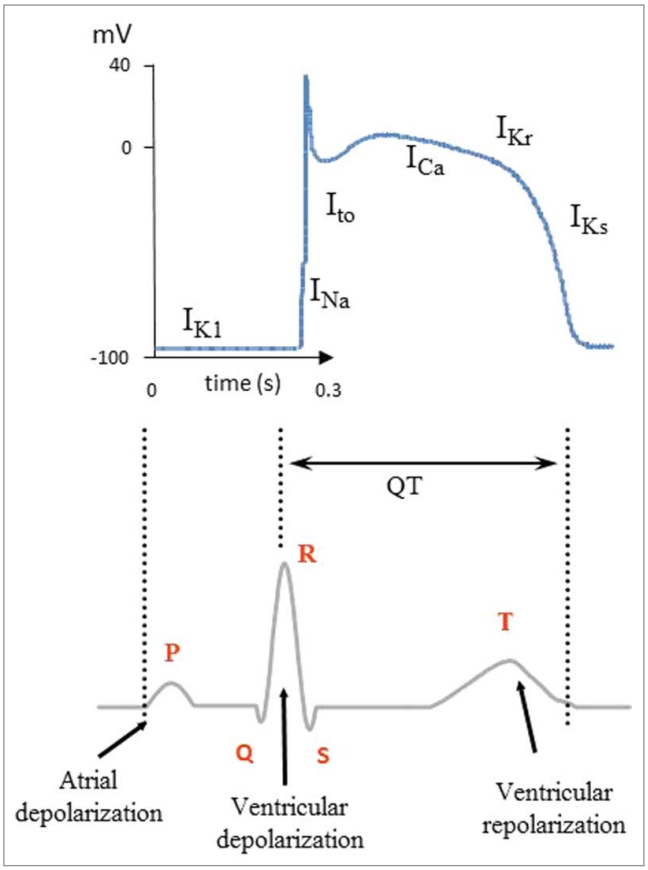

Figure 1. The top panel shows the ventricular action potential and the currents contributing to each phase. The bottom panel depicts the features and intervals seen in a typical electrocardiogram (e.g., the PR interval is the time between the P wave and the R wave).

potentials, their on- and off rates vary significantly. Compounds that prolong ERP with little effect on QRS (Class Ib) show fast on- and off-rates, whereas Class Ic compounds, which prolong QRS without major effects on ERP, were found to have slow kinetics.[12] Class Ic drugs carry an increased risk of cardiac arrest, and consequently Nav1.5 inhibition and QRS prolongation are frequently studied endpoints in preclinical cardiac toxicology assays. The degree of Nav1.5 inhibition necessary to result in significant QRS prolongation is the subject of significant debate.[13]

Nav1.8

Like Nav1.5, Nav1.8 is a member of the TTX-resistant sodium channel family. As recently as 2010, Nav1.8 was thought to function exclusively in the peripheral nervous system. However, recently several genome-wide association studies have linked polymorphisms in SCN10A, the gene encoding Nav1.8, to prolongation of PR and QRS intervals.[14,15] Subsequently, Nav1.8 expression was demonstrated in mouse and human cardiac myocytes and intracardiac neurons.[16] In cardiac myocytes, block of Nav1.8 reduces late Na^+ current and shortens action potential duration,[17] while block of Nav1.8 in intracardiac neurons reduces action potential frequency.[18] How these observations relate to cardiac conduction is not entirely clear. The picture is further complicated by the findings that SCN10A knock-out mice display a decreased PR interval, while pharmacological inhibition of Nav1.8 causes PR prolongation.[19] The human polymorphisms occur in non-coding regions of SCN10A and their functional consequences are not known. Recent data suggest that at least one of these polymorphisms may affect transcriptional regulation of SCN5A and/or SCN10A.[20]

Calcium Channels

Voltage-gated calcium channels (VGCCs) are critical for all aspects of cardiovascular physiology. In mammalian species, 10 genes have been cloned that encode pore forming subunits of VGCCs.[21] In native tissues, VGCCs exist as a complex of the pore-forming α-subunit, one of 4 distinct β-subunits, one of 4 α2δ-subunits and potentially one of 8 γ-subunits. The β-subunits and α2δ-subunits have profound effects on surface membrane expression and voltage dependence of gating, whereas much less is known about the role of the γ-subunits. Depending on cell type and subcellular location, VGCCs further co-assemble with a number of proteins, including proteins such as calmodulin and calcium-binding protein1 that regulate their activity, proteins that are involved in vesicle docking and neurotransmitter release and intracellular signaling molecules.[22,23]

L-type (Cav1.x) channels

Physiology

In adult cardiac myocytes, calcium influx through Cav1.2 is responsible for the majority of the inward current during the plateau phase of the cardiac action potential, and Cav1.2 is the dominant channel involved in excitation-contraction coupling. Calcium currents also contribute to the electrical properties of cardiomyocytes, and channel mutations are associated with different cardiac arrhythmias. In ventricular myocytes, Cav1.2 is the only VGCC, whereas both Cav1.2 and Cav1.3 are expressed in atrial myocytes, in the autonomously beating cells of the SA and AV node and in vascular smooth muscle cells. Currently, alternative splicing of VGCCs has attracted attention as a means of tissue specificity, and the dominant Cav1.2 variant was found to differ between smooth muscle and cardiac cells.[24]

Gain-of-function mutations in CACNA1C, the gene encoding Cav1.2, are associated with long-QT syndrome,[25] as well as with the multisystem disorder known as Timothy syndrome. Timothy syndrome mutations are located in 2 mutually exclusive and tissue-specific exons. Mutations in both exons are associated with increased risk for ventricular arrhythmias and sudden cardiac death, a phenotype that is more pronounced if the mutation is found in the cardiac preferred exon.[26]. Loss-of-function mutations in CACNA1C, as well as mutations in the genes encoding the associated subunits β2 and α2δ, account for 12–13% of patients with Brugada syndrome, characterized by an elevated ST segment and increased risk for ventricular fibrillation and sudden cardiac death.[27] In mice, global and cardiac-specific deletion of

Cav1.2 is lethal, whereas smooth muscle specific knockout lowered blood pressure and reduced myogenic tone and contractility in isolated small-diameter arteries.[28]

A role for Cav1.3 in modulating heart rate has only recently been appreciated. Relative to other L-type calcium channels, Cav1.3 activates rapidly and at more hyperpolarized voltages; key attributes that support the role of Cav1.3 in pace making.

The first human channelopathy involving CACNA1D, the gene encoding Cav1.3, was identified in 2011.[29] The loss-of-function mutation in an alternatively spliced exon is associated with congenital deafness. On further examination, affected individuals presented with bradycardia and impaired SA node function but normal QRS and QT intervals. In mice, global knockout of Cav1.3 results in viable, fertile offspring presenting with deafness, bradycardia and SA and AV node dysfunction, much like their human counterparts. Recent evidence suggests that the C-terminus of Cav1.3 may function as a transcriptional regulator in atrial myocytes and modulate the expression of myosin light chain II and small conductance calcium-activated K^+ channel.[30]

In addition to the classical role in conducting calcium currents, recent work highlights a metabotropic role of L-type calcium channels as signaling receptors activated by extracellular calcium binding.[31] These findings can account for the rapid kinetics of excitation-contraction and excitation-secretion coupling; however sustained or repetitive contraction and neurotransmitter release requires calcium influx through VGCCs.

Pharmacology

L-type calcium channel blockers are used clinically to treat hypertension, angina, and/or atrial dysrhythmias. They are generally well tolerated, despite the wide-spread expression of Cav1 channels. Factors that affect the tolerability of L-type calcium channel blockers include the degree of voltage dependence and kinetics of channel block.

Based on their structures, calcium channel blockers can be classified as dihydropyridines (e.g., nifedipine, amlodipine), benzothiazepines (e.g., diltiazem) and phenylalkylamines (e.g., verapamil). The three classes of ligands bind to distinct sites located near the outer pore of the α-subunit. All L-type calcium channel blockers lower systolic and diastolic blood pressure by reducing peripheral vascular tone and improve blood supply to the heart by dilating coronary arteries.[32] However, dihydropyridine (DHP) and non-dihydropyridine (non-DHP) calcium channel blockers differ in their effect on cardiac contractility and heart rate (**Table 1**). Non-DHP calcium channel blockers decrease contractility and heart rate; factors that decrease cardiac workload and are beneficial in patients suffering from angina, but are contra-indicated in patients with heart failure. In contrast, DHPs show functional selectivity for L-type channels in the vasculature, based in part on their more pronounced voltage dependence combined with the more depolarized resting membrane potential in the vasculature.[33] DHPs typically do not affect contractility and differ in their effect on heart rate. Especially short-acting DHPs, associated with rapid fluctuation in blood pressure, can increase sympathetic activity, resulting in reflex tachycardia. This effect is largely mitigated in long-acting drugs that reach their steady-state plasma levels more slowly. Differing selectivity for Cav1.2 over Cav1.3, alternative splicing and different β-subunits may also contribute to the selectivity for cardiac versus vascular targets in vivo.[33] Increased activity on Cav1.3 may be associated with less tachycardia, considering the role of Cav1.3 in heart rate modulation. DHP structure-activity relationships have been reviewed recently.[34]

Weak inhibition of calcium channels, with IC_{50}s in the range of 5–50 μM, is found in many Medicinal Chemistry compounds. Most of these small molecules act in a voltage dependent manner and rarely result in adverse events. A decrease in systolic and diastolic blood pressure without effects in the electrocardiogram is suggestive of L-type calcium channel block. In the clinic, calcium channel blockers afford blood pressure lowering at free plasma concentrations close to the IC_{50}.

N-type (Cav2.2) and T-type (Cav3.x) channels

The autonomic nervous system plays a key role in the control of heart rate. A number of voltage gated calcium channels are found in neurons of the autonomic nervous system. Of particular importance is the N-type calcium channel Cav2.2, because of its role in the release of norepinephrine from synaptic terminals in the sympathetic nervous system.[35] Cav2.2 also plays a dominant role controlling the exercise pressor reflex through its expression in muscle afferents.[36]

T-type channels differ from other VGCCs by their lack of accessory subunits and by their activation at more hyperpolarized voltages. T-type channels are expressed widely and play a key role in modulating excitability and burst firing in many cell types. In the cardiovascular system, expression of Cav3.1 and Cav3.2 has been found in the SA and AV nodes and in arterial smooth muscle cells, where they play a role in pace-making and control of vascular tone, respectively.[37,38] Interestingly, the endogenous vasodilator nitric oxide suppresses T-type calcium currents in aterial smooth mucle cells.[39]

No selective N-type or T-type small molecule blockers have been approved for clinical use. Recent developments suggest that mixed L-/N-type blockers (e.g., cilnidipine) and mixed L-/T-type

Table 1. Pharmacology of L-type Calcium Channels

	DHPs short-acting	DHPs long-acting	Non-DHP L-type blocker	L-/N-type blocker	L-/T-type blocker
Blood pressure	⇓	⇓	⇓	⇓	⇓
Vasodilation	⇑	⇑	⇑	⇑	⇑
Heart Rate	⇑	⇔	⇓	⇔	⇓
Myocardial Contractility	⇔	⇔	⇓	⇔	⇔
Sympathetic activity	⇑	⇔	⇑	⇓	⇓

(e.g. efonidipine, benidipine) may offer benefits over selective L-type blockers as anti-hypertensives.[40] Mixed blockers are expected to lower blood pressure, while avoiding the reflex tachycardia caused by sympathetic activation. Indeed, cilnidipine has demonstrated efficacy in patients with morning hypertension, caused by increased sympathetic tone in the morning and associated with an elevated risk for heart attack and stroke in the early morning hours.[41] Mixed L-/N-type should also avoid the exercise pressor reflex that can lead to an increased risk for heart attacks.

Future efforts in the development of calcium channel blockers are expected to focus on highly selective compounds, targeting a particular subtype or variant, and on mixed blockers demonstrating appropriate phenotypic responses and improved tolerability.

Voltage-Gated Potassium Channels

Several families of voltage-gated potassium channels are expressed in cardiac myocytes and together provide the majority of the outward current responsible for action potential repolarization. The activation and inactivation kinetics of each channel subtype determine their contribution to different phases of repolarization. In order of the repolarization phase they contribute to, the dominant Kv channel pore-forming subunits and corresponding currents are: Kv4.3 and Kv1.4/fast and slow components of the transient outward current (I_{to}), Kv1.5/ultra-rapid delayed-rectifier current (I_{Kur}), Kv11.1 aka hERG/rapid delayed-rectifier current (I_{Kr}), and Kv7.1 aka KvLQT1/slow delayed-rectifier current (I_{Ks}). In addition to the pore-forming subunit, cardiac myocytes express a variety of accessory proteins, including β-subunits, MinK, MinK-related proteins, potassium channel interacting proteins, and potassium channel accessory proteins.[42,43] Inhibition of Kv channels generally leads to a depolarized action potential plateau and/or prolonged action potential duration.

The use of potassium channel blockers to treat arrhythmias has generally been disappointing because of their pro-arrhythmic potential. Especially, the association of reduced Kv11.1/hERG activity, either drug-related or caused by channel mutations, with an increased risk for the arrhythmia known as Torsades de pointes is well established.[44] However two strategies have evolved to pharmacologically treat atrial fibrillation (AF), the most common form of arrhythmia. Both approaches involve selectively blocking atrial Kv channels, either by targeting Kv1.5 channels which are expressed in atrial but not ventricular myocytes, or through multichannel inhibitors with functional selectivity for the atrium.

Kv1.5

Kv1.5 is the gene product of KCNA5 and is responsible for the ultra-rapid delayed-rectifier current (I_{Kur}). I_{Kur} is expressed almost exclusively in atrial myocytes, making it a promising target in AF drug discovery.[45] Block of Kv1.5 contributes to the mechanism of action of several agents [46]; however no selective Kv1.5 blockers have been tested in patients suffering from AF. One compound, MK-0448, that is selective for Kv1.5 over other cardiac ion channels, showed efficacy in multiple preclinical models of AF and was examined in an invasive electrophysiological study in healthy volunteers.[47] In this study, MK-0448 did not prolong the atrial refractory period. Follow-up studies in anesthetized dogs attributed the lack of effect to the high vagal tone in young, healthy subjects such as those participating in the study.[47] Although vagal tone may be reduced in the patient population, the greater risk for episodes of AF associated with vagal stimulation does not bode well for this mechanism, and clinical development of MK-0448 was discontinued. However, a recent study with MK-0448 in human atrial tissue from sinus rhythm controls and from permanent AF patients showed that MK-0448 delayed the effective refractory period in atrial tissue from AF patients and not from control subjects. Therefore, the disappointing trial results with MK-0448 in healthy volunteers may not be predictive of eventual efficacy in an AF population.[48]

Multichannel inhibitors

Several compounds, that are relatively weak blockers of a number of cardiac ion channels, have entered development. Of these, intravenous formulations of vernakalant, AVE0118, and AZD7009 entered clinical development for acute conversion of AF to sinus rhythm (rhythm control). All three compounds inhibit Kv1.5, Kv4.3, Kir3.1/4 and block Nav1.5 at depolarized potentials. In addition, vernakalant blocks hERG, and AZD7009 blocks hERG and KvLQT1. All three compounds are effective in preclinical models of AF and relatively free of pro-arrhythmic risk.[49] While development of AVE0118 and AZD7009 has been discontinued, vernakalant (Brinavess™) is approved in many countries for hospital use. Vernakalant is relatively ineffective in patients with long-standing AF, questioning its potential for preventative use.[50]

Voltage-dependent block of Nav1.5 is more effective in the atria because of their more depolarized membrane potential, especially during periods of tachycardia. It increases the atrial refractory period and protects against the pro-arrhythmic action associated with the block of ventricular Kv channels. Consequently, the combination of blocking potassium channels, especially those expressed in atrial myocyte and absent (Kv1.5, Kir3.1/4) or less prominent (Kv4.3) in ventricular myoctes, and blocking Nav1.5 confers functional atrial selectivity.[51]

Nature may have evolved its own multichannel modulator in that the sodium channel β1 subunit also associates with Kv4.3. Recently, mutations in β1 were linked to Brugada syndrome and were shown, in recombinant expression systems, to reduce Na^+ current and increase Kv4.3 current.[52]

Inward Rectifying Potassium Channels

In contrast to voltage-gated potassium channels, inward rectifying potassium channels conduct current at hyperpolarized membrane potentials. The so-called weak inward rectifiers allow potassium flow across the entire voltage range; whereas in the strong inward rectifiers, outward potassium currents are largely blocked by voltage-dependent binding of intracellular Mg^{2+} and

polyamines.[53,54,55,56] Despite the greater capacity for passing inward currents, under physiological conditions inward rectifying potassium channels conduct outward potassium currents, hyperpolarizing the cell membrane. Seven families of inward rectifying potassium channels have been identified, and subunits of the Kir2, Kir3 and Kir6 families are expressed in the heart.[57] The pharmacology of inward rectifying potassium channels is the subject of a recent review.[58]

Kir2.1

The mammalian Kir2 family has 5 strongly rectifying members (Kir2.1–2.4, Kir2.6). Kir2.1 is the key component of the current I_{K1} in ventricular myocytes and is a critical regulator of resting membrane potential [59-61]; whereas Kir2.3 is the dominant Kir2 subunit in atrial myocytes.[62] In the vasculature, Kir2.1 channels are found in smooth muscle cells of small diameter arteries, where they control vascular tone (**Fig. 2**[63]). Block of Kir2 channels constricts these vessels, and indirect activation of Kir by adenosine causes vasodilation.[64]

Loss-of-function mutations in KCNJ2, the gene encoding Kir2.1, cause the long QT Syndrome known as Andersen-Tawil syndrome,[65] associated with cardiac arrhythmias, periodic paralysis and physical abnormalities. The phospholipid PIP_2 activates Kir2.1, and many mutations associated with Andersen-Tawil syndrome show reduced affinity of the channel for PIP_2 rather than complete channel absence.[66] Gain-of-function mutations have been identified in individuals presenting with short QT syndrome and families suffering from AF.[67] Kir2.1 deficient mice die shortly after birth; whereas Kir2.2−/− mice are viable and display no gross abnormalities.[68,69]

Potent blockers of Kir2 channels are rare. Weak, off-target Kir2 activity has been found in a few drugs, including the breast cancer drug tamoxifen, the anti-histamine diphenhydramine and the anti-malarial agent chloroquine. Chloroquine appears to bind within the pore[70]; whereas tamoxifen is thought to inhibit Kir2 channels by interfering with PIP_2 activation of the channel.[71] MicroRNA-26 has been identified as an endogenous modulator of Kir2.1 expression and reduced expression of MicroRNA-26 may promote AF.[72]

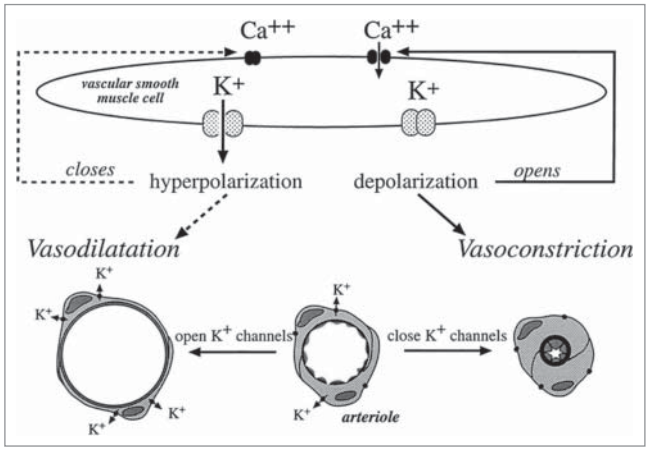

Figure 2. The interplay between voltage-dependent calcium and potassium channels in vascular smooth muscle cells controls blood vessel diameter. Reproduced from.[63] © Blackwell. Reproduced by permission of John Wiley and Sons. Permission to reuse must be obtained from the rightsholder.

Kir3.1/4 (I_{KACh})

Four mammalian Kir3 channels (Kir3.1–3.4) have been identified.[57] All conduct strongly inward rectifying currents and are distinguished from other Kir families by being activated by $G_{i/o}$-coupled GPCRs via Gβγ. Kir3 channels can form homo- and hetero-tetrameric channels. The predominant subtype combinations are Kir3.1/3.2 in the CNS and Kir3.1/3.4 in cardiac tissue.[57] The Kir3.1/3.4 heteromultimer gives rise to the current I_{KACh}, named for its activation following acetylcholine binding to muscarinic receptors, and is predominantly expressed in the atria and SA and AV nodes, with little expression in the ventricles.[73] In the SA node, I_{KACh} is responsible for vagal control of heart rate.[74] In atrial myocytes, I_{KACh} contributes to repolarization and channel activation decreases action potential duration. I_{KACh} inhibition prolongs the atrial refractory period without affecting the ventricles, making it an attractive target for drugs designed to treat AF.[75]

A loss-of-function mutation in KCNJ5, the gene encoding Kir3.4, has been reported as the cause of the long QT syndrome LQT13 in a single Chinese family. Given the lack of expression in ventricles, this finding is surprising and may in fact result from previously underestimated genetic 'noise'.[76] An association between early-onset AF and 2 common polymorphisms in KCNJ5 has been reported.[77] However, since neither polymorphism results in an amino acid change, the implications of this finding are unclear. Mice with genetic ablation of Kir3.1 or Kir3.4 are viable and lack any I_{KACh} current.[78,74] Mice from both strains have normal resting heart rates, but significantly diminished vagal control of heart rate. Mice deficient in Kir3.4 were shown to be resistant to experimentally-induced AF.[79]

Potent inhibitors of Kir3 channels are rare, and the therapeutic potential of selective Kir3 inhibitors remains to be determined. A number of non-selective agents in development for AF, such as vernakalant, NIP-142 and AVE0118, include inhibition of I_{KACh} in their mechanism of action.

Kir6

Only two Kir6 channels have been identified. They are unique in that they exist only in heteromeric complexes with accessory sulfonyl urea receptor (SUR) subunits. The heteromeric complex, formed by 4 Kir6 and 4 SUR subunits, gives rise to a current that is modulated by the intracellular concentrations of ATP and ADP and has been termed K_{ATP}.[80] K_{ATP} channels are weak inwardly rectifying and closed by high intracellular ATP concentrations, found in cardiac myocytes under physiological conditions.[81] Thus, channel open probability is typically low and can be regulated by other factors such as the intracellular concentration of MgADP and adenosine receptor activation.[82]

The sarcolemmal K_{ATP} channel is mainly formed from Kir6.2 and SUR2A in ventricular myocytes and from Kir6.2 and SUR1 in atrial myocytes; whereas the predominant vascular

smooth muscle K_{ATP} channel consists of Kir6.1 and SUR2B subunits.[83,80] A K_{ATP} current has also been reported in mitochondrial membranes. However, mitochondrial K_{ATP} is intact in mice lacking Kir6.1 or Kir6.2 and may represent a macro-molecular complex with Kir1.1 as the pore-forming subunit.[84]

K_{ATP} channels are important players in the cardioprotective mechanism known as ischemic preconditioning.[82] The drop in intracellular ATP concentration associated with ischemia, activates K_{ATP} currents. Activation of K_{ATP} shortens action potential duration and reduces calcium influx. This may prevent cellular toxicity from calcium overload. In addition, activation of K_{ATP} causes vasodilation, increasing tissue perfusion and counteracting the ischemic event.[85] This mechanism may also be important for the ability to adapt to increased oxygen demand during vigorous exercise.[86] The effect of K_{ATP} on vascular tone is highlighted by the activation and inhibition of K_{ATP} by vasodilators and vasoconstrictors, respectively.[64]

Kir6.2 is encoded by KCNJ11. Loss-of-function and gain-of-function mutations in KCNJ11 have been identified. Afflicted individuals suffer from abnormalities in glucose handling, due to the expression of KCNJ11 in the pancreas, but no signs of cardiovascular pathophysiology have been reported.[87] A gain-of-function variant of Kir6.1, encoded by KCNJ8, has been found in a small number of individuals showing symptoms of either Early Repolarization syndrome or Brugada syndrome; whereas loss-of-function mutations were found in 2 victims of sudden infant death syndrome.[86] Recently, mutations in SUR2 have been linked to Cantu syndrome, characterized by multi-organ developmental abnormalities.[88] These mutations are considered gain-of-function with regard to K_{ATP}, since they result in channels with reduced sensitivity to ATP inhibition. In mice, ablation and gain-of-function of Kir6.2 results mainly in metabolic deficiencies.[89] Kir6.1 KO mice die within a few weeks from birth due to coronary artery contraction,[89] whereas Kir6.1 gain-of-function animals are viable and have reduced blood pressure.

Unlike other Kir channel families, a rich pharmacology exists for K_{ATP} and has been reviewed extensively.[90] K_{ATP} inhibitors have long been used in the treatment of type 2 diabetes; whereas K_{ATP} activators are used clinically to treat refractory hypertension and angina. All known K_{ATP} modulators bind to the SUR subunits.

Integrated Cardiac Safety Assessment

A few Drug Discovery efforts target specific ion channel subtypes to treat cardiovascular pathophysiologies; however, off-target interactions with cardiovascular ion channels affect many, if not most, drug development programs. Following the withdrawal of several drugs from the market, based on their risk for inducing the life-threatening ventricular arrhythmia Torsades de pointes, guidelines S7B and E14 were issued that govern the strategy to evaluate the risk for Torsades associated with a new chemical entity.[91] These guidelines, issued in 2005, focus on the repolarizing potassium current I_{Kr}, the underlying channel hERG, and the heart rate-corrected QT interval. However, not all drugs that block hERG cause QT prolongation, and not all drugs that prolong the QT interval are proarrhythmic, presumably because of the complex interplay between multiple ion channels.[92] While current guidelines have been successful at preventing new torsadogenic drugs from reaching the market,[93] they may have inadvertently limited patient access to beneficial treatments.[91] Efforts are underway to define a new cardiac safety paradigm referred to as CiPA (Comprehensive *in vitro* Proarrhythmia Assay) that will categorize drug candidates into low-, medium- and high-risk with regard to proarrhythmic potential. This initiative represents a collaborative effort involving multiple pharmaceutical, regulatory and contract research organizations and is a first of its kind. CiPA consists of 3 components: *in vitro* characterization of electrophysiological effects on 7 cardiac ion channels expressed recombinantly, *in silico* modeling of the impact on the human ventricular action potential, and characterization of electrophysiological effects on human stem cell-derived cardiomyocytes using multi-electrode arrays and voltage-sensitive dye.[94] Expert working groups are tasked with developing and testing detailed protocols for each of the 3 components, which will be validated by testing 29 established drugs spanning the range from low to high proarrhythmic liability.[95,96] Once established, CiPA may remove the need for expensive Thorough QT studies for most drug candidates.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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