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## Molecular Physiology of Ion Channels That Control Cardiac Repolarization

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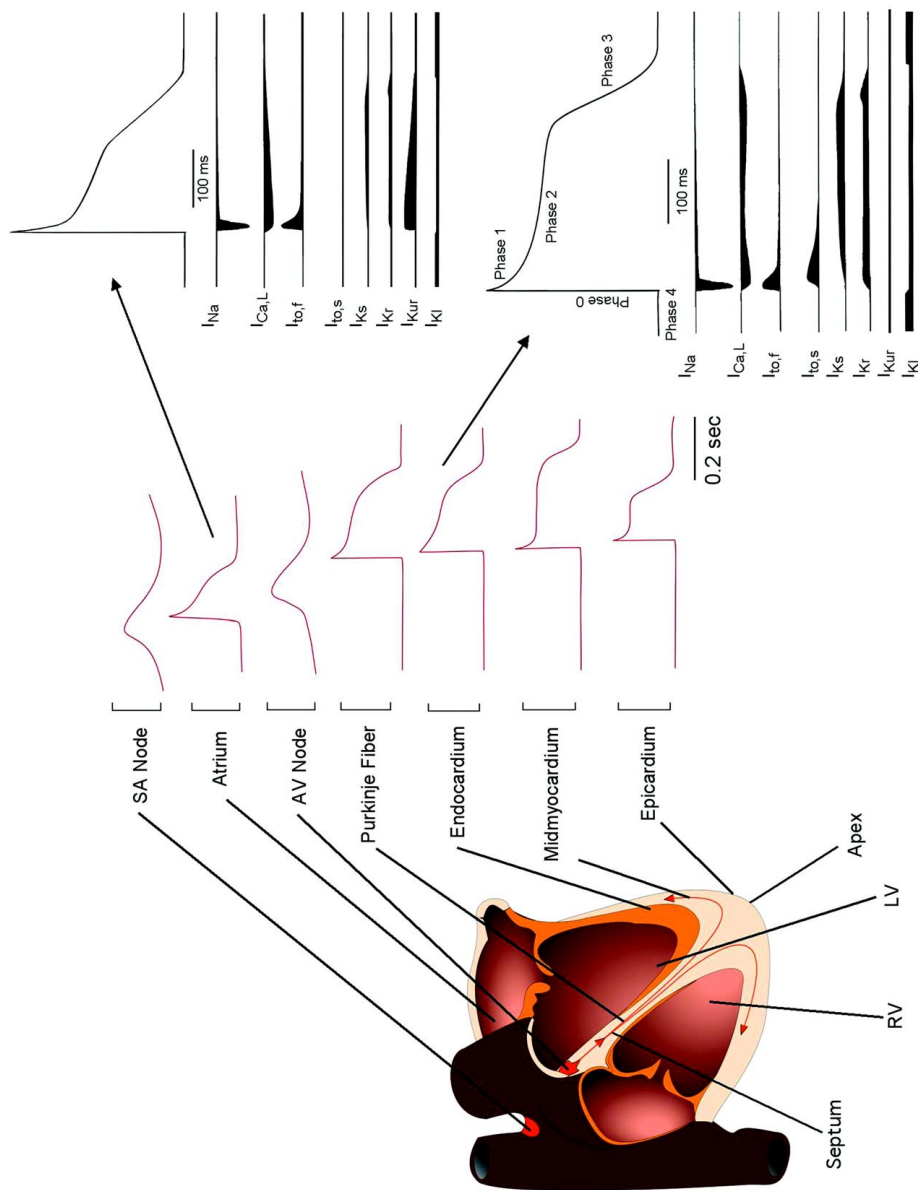
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### INTRODUCTION

The mammalian heart operates as an electromechanical pump, the proper functioning of which depends critically on the sequential activation of cells throughout the myocardium and the coordinated activation of the ventricles (Fig. 1). Electrical signaling in the heart is mediated through regenerative action potentials that reflect the synchronized activity of multiple ion channels that open, close, and inactivate in response to changes in membrane potential (Fig. 1). The rapid upstroke of the action potential (phase 0) in ventricular and atrial cells, for example, is attributed to inward currents through voltage-gated  $\text{Na}^+$  (Nav) channels. Phase 0 is followed by a rapid phase of repolarization (phase 2), reflecting Nav channel inactivation and the activation of voltage-gated outward  $\text{K}^+$  (Kv)

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**Fig. 1.** Regulation of cardiac membrane excitability. Left Panel: schematic of the human heart and the waveforms of the action potentials recorded in different regions of the heart. Right panel: action potentials and underlying ionic currents in adult human atrial (top) and ventricular (bottom) myocytes. The contributions of some  $K^+$  currents, such as  $I_{Kr}$  and  $I_{Kur}$ , are distinct in atrial and in ventricular cells.

currents (Fig. 1). In ventricular cells, this transient repolarization or *notch* influences the height and duration of the action potential plateau (phase 2), which depends on the balance of inward ( $\text{Ca}^{2+}$  and  $\text{Na}^+$ ) currents and outward ( $\text{K}^+$ ) currents. The main contributor of inward current during the plateau phase is  $\text{Ca}^{2+}$  influx through high threshold, L-type voltage-gated  $\text{Ca}^{2+}$  ( $\text{Cav}$ ) channels. The (L-type  $\text{Ca}^{2+}$ ) channels undergo  $\text{Ca}^{2+}$  and voltage-dependent inactivation and, as these channels inactivate, the outward  $\text{K}^+$  currents predominate resulting in a second, rapid phase (phase 3) of repolarization back to the resting potential (Fig. 1). The height and duration of the plateau, as well as the time- and voltage-dependent properties of the underlying  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$  channels determine action potential durations in individual cardiac cells. Changes in the properties or the densities of any of these channels, owing to underlying cardiac disease or as a result of the actions of cardiac and noncardiac drugs, therefore, is expected to have dramatic effects on action potential waveforms, refractory periods, and cardiac rhythms.

Electrophysiological studies have detailed the properties of the major voltage-gated inward ( $\text{Na}^+$  and  $\text{Ca}^{2+}$ ) and outward ( $\text{K}^+$ ) currents (Table 1) that determine the heights and the durations of cardiac action potentials. In contrast to the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  currents, there are multiple types of myocardial  $\text{K}^+$  currents, particularly  $\text{Kv}$  currents. At least two types of transient outward currents,  $\text{I}_{\text{to},f}$  and  $\text{I}_{\text{to},s}$ , and several components of delayed rectification, including  $\text{I}_{\text{Kr}}$  ( $\text{I}_{\text{K}(\text{rapid})}$ ) and  $\text{I}_{\text{Ks}}$  ( $\text{I}_{\text{K}(\text{slow})}$ ), for example, have been distinguished (Table 1). There are marked regional differences in the expression patterns of these currents, differences that contribute to regional variations in action potential waveforms (1–3). The time- and voltage-dependent properties of the  $\text{Kv}$  currents in myocytes isolated from different species and/or from different regions of the heart are similar, however, suggesting that the molecular correlates of the underlying channels are also the same. The pore forming ( $\alpha$ ) and accessory ( $\beta$ ,  $\delta$ , and  $\gamma$ ) subunits encoding myocardial  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$  channels have been identified, and considerable progress has been made in defining the relationships between these subunits and functional cardiac  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$  channels.

The densities and the properties of voltage-gated cardiac  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$  currents change during development, reshaping action potential waveforms (4) and modifying the sensitivity to cardiac, as well as noncardiac, drugs. Alterations in the densities and properties of voltage-gated  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{K}^+$  currents also occur in a number of myocardial disease states (5–12). These changes can lead directly or indirectly to arrhythmia generation, as well as influence the sensitivity of individuals to the effects of cardiac and noncardiac drugs that influence the properties and/or the functional expression of these channels. There is, therefore, considerable interest in defining the properties of myocardial ion channels, as well as in delineating molecular mechanisms controlling the regulation, the modulation, and the functional expression of these channels.

## INWARD VOLTAGE-GATED $\text{Na}^+$ CURRENTS IN THE MYOCARDIUM

Voltage-gated  $\text{Nav}$  channels open rapidly on membrane depolarization and underlie the rising phases of the action potentials in ventricular and atrial myocytes (Fig. 1). The threshold for  $\text{Nav}$  channel activation is quite negative ( $-55 \text{ mV}$ ), and activation is steeply voltage-dependent (13). In addition,  $\text{Nav}$  channels inactivate rapidly and, during the plateau phase of ventricular action potentials, most of the  $\text{Nav}$  channels are in an inactivated and nonconducting state (14–16). There is, however, a finite probability (approx 1%) of channel reopening at voltages corresponding to the action potential plateau (14–17). Although the resulting plateau (or “window”)  $\text{Nav}$  current is small in magnitude (18),

Table 1  
Cardiac Currents Contributing to Action Potential Repolarization

Channel Type	Current Name	Activation	Inactivation	Recovery	Species	Tissue <sup>1</sup>
NaV	I <sub>Na</sub>	very fast	fast	fast	cat, dog, ferret, human, mouse, rat	A, P, V, SAN <sup>3</sup> , AVN <sup>3</sup>
CaV	I <sub>Ca(L)</sub>	fast	moderat	fast	cat, dog, ferret, human, mouse, rat	A, P, V, SAN, AVN
Kv(I <sub>To</sub> )	I <sub>Ca(T)</sub>	fast	fast	slow	cat, dog, guinea pig, rat	A, P, SAN, AVN
	I <sub>To,f</sub>	fast	fast	fast	cat, dog, ferret, human, mouse, rat	A, P, V
	I <sub>To,s</sub>	fast	moderate	slow	ferret, human, mouse, rat, rabbit	V (A, AVN, SA) <sup>4</sup>
Kv(I <sub>K</sub> )	I <sub>Kr</sub>	moderate	fast	slow	cat, dog, guinea pig, human, mouse, rabbit, rat	A, P, V, SAN, AVN
	I <sub>Ks</sub>	very slow	no	—	dog, guinea pig, human, rat	A, P, V, SAN
	I <sub>Kur</sub>	very fast	very slow	slow	dog, human	A
	I <sub>K,slow1</sub>	very fast	slow	slow	mouse	A, V
	I <sub>Kp</sub>	fast	no	—	guinea pig	V
Kir	I <sub>K,slow2</sub>	fast	very slow	slow	mouse	A, V
	I <sub>K</sub>	slow	slow	slow	rat	V
	I <sub>ss</sub>	slow	no	—	dog, human, mouse, rabbit, rat	A, V, AVN
	I <sub>K1</sub>	—	—	—	cat, dog, ferret, human, mouse, rabbit, rat	A, P, V

<sup>1</sup> A = atrial; P = Purkinje; V = ventricular; SAN = sinoatrial node; AVN = atrioventricular node.

<sup>2</sup> Inactivation is Ca<sup>2+</sup>-, as well as voltage dependent.

<sup>3</sup> Seen in some, but not all, AV and SAN cells.

<sup>4</sup> Seen in atrial and nodal cells only in rabbit.

particularly when compared with the Nav current during phase 0, it does contribute to maintaining the depolarized state, and plays a role in action potential repolarization, particularly in the ventricles.

Although the Nav channel “window” current has been recognized as a determinant of cardiac action potential waveforms for a great many years now (19,20), the identification of inherited mutations in the genes encoding myocardial Nav channels and the delineation of the molecular consequences of these mutations (14–16) have clearly demonstrated that plateau Nav currents play a very important role in action potential repolarization. Interestingly, there are regional differences in the expression of the persistent Nav current component (21), differences that may contribute to regional heterogeneities in action potential amplitudes and durations (1–3), as well as impact arrhythmia susceptibility.

### INWARD VOLTAGE-GATED MYOCARDIAL $\text{Ca}^{2+}$ CURRENTS

Two broad classes of voltage-gated  $\text{Ca}^{2+}$  (Cav) currents/channels, low-voltage-activated (LVA) and high-voltage-activated (HVA), Cav channels, have been distinguished based primarily on differences in the (voltage) threshold of channel activation (22). Similar to Nav channels, the LVA Cav channels activate at relatively hyperpolarized membrane potentials, and these channels activate and inactivate rapidly. HVA Cav channels, in contrast, open on depolarization to membrane potentials more positive than  $-20$  mV, and these channels inactivate in tens to hundreds of milliseconds. There is considerable variability in the detailed kinetic and pharmacological properties of HVA  $\text{Ca}^{2+}$  channels expressed in different cell types, and multiple HVA channel types, referred to as L, N, P, Q, or R, have been described (22,23). LVA channels are also often referred to as T (transient) type  $\text{Ca}^{2+}$  channels (23).

In mammalian cardiac myocytes, L-type HVA Cav currents predominate (24). In response to membrane depolarization, L-type cardiac Cav channels open with a delay relative to the Nav channels, and these channels contribute little to phase 0 (Fig. 1). The  $\text{Ca}^{2+}$  influx through the L-type Cav channels, however, triggers the release of  $\text{Ca}^{2+}$  from intracellular  $\text{Ca}^{2+}$  stores and excitation-contraction coupling (24). At positive potentials, L-type Cav channels undergo rapid voltage- and  $\text{Ca}^{2+}$ -dependent inactivation, contributing to the termination of action potential plateau and repolarization. It is clear, therefore, that cardiac and noncardiac drugs that modulate the influx of  $\text{Ca}^{2+}$  through these channels could have profound effects on action potential waveforms and the generation of normal cardiac rhythms.

### DIVERSITY OF VOLTAGE-GATED MYOCARDIAL $\text{K}^{+}$ CURRENTS

Voltage-gated  $\text{K}^{+}$  (Kv) channel currents influence the amplitudes and durations of cardiac action potentials and, in most cells, two classes of Kv currents have been distinguished: 1. transient outward  $\text{K}^{+}$  currents,  $\text{I}_{\text{to}}$ , and 2. delayed, outwardly rectifying  $\text{K}^{+}$  currents,  $\text{I}_{\text{K}}$  (Table 1).  $\text{I}_{\text{to}}$  channels activate and inactivate rapidly and underlie the early phase (phase 1) of repolarization, whereas  $\text{I}_{\text{K}}$  channels determine the latter phase (phase 3) of repolarization (Fig. 1). These are broad classifications, however, and there are multiple Kv currents (Table 1) expressed in cardiac cells. Differences in the expression patterns and the properties of these currents contribute to the observed variations in action potential waveforms recorded in different cardiac cell types (Fig. 1) and in different species (1–3).

The early phase (phase 1) of repolarization is attributed to the activation of  $\text{Ca}^{++}$ -independent, 4-aminopyridine-sensitive transient outward  $\text{K}^+$  currents, variably referred to as  $I_{\text{to}}$ ,  $I_{\text{to}1}$ , or  $I_{\text{t}}$  (25,26). Electrophysiological and pharmacological studies, however, have now clearly demonstrated that there are actually two distinct cardiac transient outward  $\text{K}^+$  currents,  $I_{\text{to, fast}}$  ( $I_{\text{to,f}}$ ) and  $I_{\text{to, slow}}$  ( $I_{\text{to,s}}$ ) (27–30). Rapidly activating and inactivating transient outward  $\text{K}^+$  currents that are also characterized by rapid recovery from steady-state inactivation are referred to as  $I_{\text{to, fast}}$  ( $I_{\text{to,f}}$ ) (28). The rapidly activating transient outward  $\text{K}^+$  currents that recover slowly from inactivation are referred to as  $I_{\text{to, slow}}$  ( $I_{\text{to,s}}$ ) (28).  $I_{\text{to,f}}$  is a prominent repolarizing current in ventricular and atrial cells in most species (27–37), and is readily distinguished from other  $\text{Kv}$  currents, including  $I_{\text{to,s}}$ , using the spider  $\text{K}^+$  channel toxins, *Heteropoda* toxin-2 or -3 (38). The fact that the properties of  $I_{\text{to,f}}$  in different species and cell types are similar led to the suggestion that the molecular correlates of the underlying ( $I_{\text{to,f}}$ ) channels are the same (25), and considerable experimental evidence in support of this hypothesis has now been provided. Nevertheless, there are differences in the detailed biophysical properties of  $I_{\text{to,f}}$  channels (39), suggesting that there likely are subtle, albeit important, differences in the molecular compositions of these channels in different cells/species.

In rabbit myocardium, the prominent transient outward  $\text{K}^+$  current ( $I_{\text{t}}$ ) inactivates slowly and recovers from steady-state inactivation very slowly (40–42), and would be classified as  $I_{\text{to,s}}$ . In some species,  $I_{\text{to,f}}$  and  $I_{\text{to,s}}$  are co-expressed and differentially distributed (28–30). In all cells isolated from adult mouse right (RV) and left (LV) ventricles, for example,  $I_{\text{to,f}}$  is expressed, whereas  $I_{\text{to,s}}$  is undetectable (28–30). In the mouse inter-ventricular septum, in contrast,  $I_{\text{to,f}}$  and  $I_{\text{to,s}}$  are co-expressed in approx 80% of the cells, and in  $\approx 20\%$  of the cells, only  $I_{\text{to,s}}$  is evident.

Delayed rectifier  $\text{Kv}$  currents,  $I_{\text{K}}$ , have also been characterized extensively in cardiac myocytes and, in most cells, multiple components of  $I_{\text{K}}$  (Table 1) are co-expressed. Two prominent components of  $I_{\text{K}}$ ,  $I_{\text{Kr}}$  ( $I_{\text{K,rapid}}$ ) and  $I_{\text{Ks}}$  ( $I_{\text{K,slow}}$ ), for example, were first distinguished in guinea pig myocytes based on differences in time- and voltage-dependent properties (43–47).  $I_{\text{Kr}}$  activates rapidly, inactivates very rapidly, displays marked inward rectification and is selectively blocked by several class III antiarrhythmics (44,47). In contrast, no inward rectification is evident for  $I_{\text{Ks}}$ , and this current is not blocked by the compounds that affect  $I_{\text{Kr}}$  (44,47). In human (48,49), canine (50), and rabbit (51) ventricular cells, both  $I_{\text{Kr}}$  and  $I_{\text{Ks}}$  are expressed and contribute to repolarization. In adult rodent hearts, however, neither  $I_{\text{Kr}}$  nor  $I_{\text{Ks}}$  is a prominent repolarizing  $\text{Kv}$  current, and there are additional components of  $I_{\text{K}}$  (Table 1). In rat ventricular myocytes, for example, there are novel delayed rectifier  $\text{Kv}$  currents, referred to as  $I_{\text{K}}$  and  $I_{\text{ss}}$  (Table 1) (33,52). In mouse ventricular myocytes, three distinct  $\text{Kv}$  currents,  $I_{\text{K,slow1}}$ ,  $I_{\text{K,slow2}}$ , and  $I_{\text{ss}}$ , are co-expressed (28,53–59). It is clear, therefore, that in efforts focused on evaluating the possibility that there will be unwanted cardiac effects of drugs with clinical potential, it will be important to select the experimental species used in the assays carefully.

In rat (60), canine (61), and human (62,63) atrial myocytes, a novel, rapidly activating and slowly inactivating outward  $\text{K}^+$  current, referred to as  $I_{\text{Kur}}$  ( $I_{\text{K,ultra rapid}}$ ), is expressed (Table 1). It has been suggested that the expression of  $I_{\text{Kur}}$ , together with  $I_{\text{to,f}}$  in atrial myocytes, contributes to the more rapid repolarization evident in atrial, compared with ventricular, myocytes (Fig. 1). However, in guinea pig (64) and mouse (53,57–59) ventricular myocytes there are voltage-gated outward  $\text{K}^+$  currents with biophysical properties similar to atrial  $I_{\text{Kur}}$ . The rapidly activating  $\mu\text{M}$  4-AP-sensitive component of mouse ventricular  $I_{\text{K,slow}}$ ,  $I_{\text{K,slow1}}$  (57,59) should probably be renamed  $I_{\text{Kur}}$  (Table 1). Import-

tantly,  $I_{K_{ur}}$  is not expressed in human ventricular myocytes or in Purkinje fibers, suggesting that  $I_{K_{ur}}$  channels might represent a therapeutic target for the treatment of atrial arrhythmias without complicating effects on ventricular function or performance. The potential of this pharmacological strategy, however, will have to be determined by the atrial specificity/selectivity of the reagents to be developed.

## REGIONAL AND DEVELOPMENTAL DIFFERENCES IN ACTION POTENTIAL WAVEFORMS AND IONIC CURRENTS

There are marked regional differences in action potential waveforms in the myocardium (Fig. 1), and these contribute to the normal propagation of activity through the heart and the generation of normal cardiac rhythms. An important determinant of the observed regional differences in action potential waveforms is heterogeneity in Kv current expression (1–3). There are, for example, large variations in ventricular  $I_{to,f}$  densities (27–29,31,32,35,36,65–67). In (canine) LV,  $I_{to,f}$  density is five- to sixfold higher in epicardial and midmyocardial, than in endocardial, cells (65). The density of  $I_{to,s}$  is quite variable (27–30), being detected only in endocardial (27) and septum (28,29) cells. There are also regional differences in  $I_{K_s}$  and  $I_{K_r}$  densities. In (canine) LV, for example  $I_{K_s}$  density is higher in epicardial and endocardial cells than in M cells (49). In cells isolated from the (guinea pig) LV free wall,  $I_{K_r}$  density is higher in subepicardial, than in midmyocardial or subendocardial, myocytes (68). At the base of the LV, in contrast,  $I_{K_r}$  and  $I_{K_s}$  densities are significantly lower in endocardial than in midmyocardial or epicardial cells (69). These differences contribute to the variations in action potential waveforms recorded in different regions (right vs left; apex vs base) and layers (epicardial, midmyocardial, and endocardial) of the ventricles. In addition, these electrophysiological differences clearly suggest that there will be regional differences in the physiological effects of drugs that affect the properties and/or the functional expression of cardiac Kv channels, differences that could increase the propensity to develop life-threatening arrhythmias.

During postnatal development, myocardial action potentials shorten markedly (4). In ventricular myocardium, for example, phase 1 repolarization becomes more pronounced with age, and functional  $I_{to,f}$  density is increased (52,70–77). In addition, action potentials in neonatal cells are insensitive to 4-AP, and voltage-clamp recordings reveal that  $I_{to,f}$  is undetectable, whereas, in cells from 60 d postnatal animals,  $I_{to,f}$  is present and phase 1 repolarization is clearly evident (71).  $I_{to,f}$  density is also low in neonatal mouse (75) and rat (52,70,72,74,76) ventricular myocytes, and increases several fold during early postnatal development. In rat, the properties of the currents in 1–2 d ventricular myocytes (76) are also distinct from those of  $I_{to,f}$  in postnatal d 5 to adult cells (52) in that inactivation and recovery from inactivation are slower. Indeed, the properties of the transient outward currents in postnatal d 1–2 rat ventricular cells (76) more closely resemble  $I_{to,s}$  than  $I_{to,f}$ . In rabbit ventricular myocytes, transient outward  $K^+$  current density increases and the kinetic properties of the currents also change during postnatal development (73). In this case, however, the rate of recovery of the currents is ten times faster in neonatal (mean recovery time ~ 100 ms) than in adult (mean recovery time ~ 1300 ms) cells (73). The slow recovery of the transient outward currents underlies the marked broadening of action potentials at high stimulation frequencies in adult (but not in neonatal) rabbit ventricular myocytes (73). These observations suggest that  $I_{to,f}$  is prominent in neonatal rabbit cells and that  $I_{to,s}$  dominates repolarization in adult cells. In addition, these observations again

reveal species differences in the ionic currents shaping action potential waveforms, again demonstrating the importance of the selection of species in efforts focused on determining drug effects in the myocardium.

Delayed rectifier  $K^+$  current expression also changes during postnatal development. For example, both  $I_{Kr}$  and  $I_{Ks}$  are readily detected in neonatal mouse ventricular myocytes (77), whereas these currents are not detected in adult cells (28,29). Because  $I_{Kr}$  and  $I_{Ks}$  are prominent repolarizing  $K^+$  currents in adult human cardiac cells, developmental changes in the expression and/or the properties of these currents will lead to marked changes in action potential waveforms and altered sensitivity to drugs that affect the properties and the functioning of these channels.

### INWARDLY RECTIFYING $K^+$ CHANNELS CONTRIBUTE TO ACTION POTENTIAL REPOLARIZATION

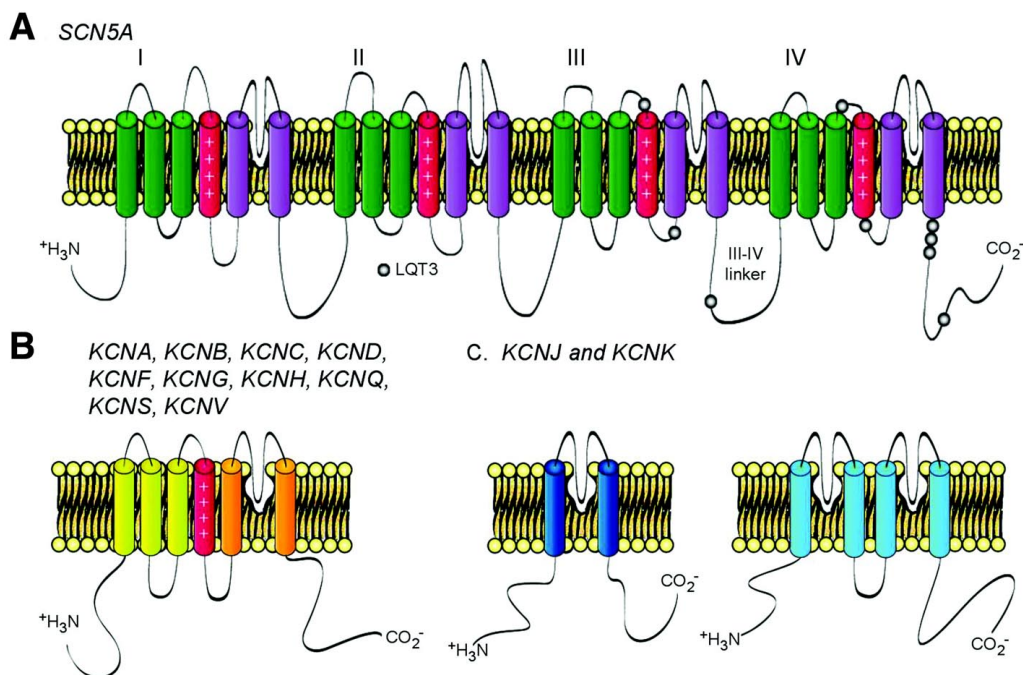
In addition to  $K_v$  currents, the inwardly rectifying  $K^+$  ( $K_{ir}$ ) current ( $I_{K1}$ ) plays a role in myocardial action potential repolarization (Table 1), and there are marked regional differences in  $I_{K1}$  expression in atria, ventricles and conducting tissues (78,79). In atrial and ventricular myocytes and in cardiac Purkinje cells,  $I_{K1}$  plays a role in establishing the resting membrane potential, the plateau potential and contributes to phase 3 repolarization (Fig. 1). The strong inward rectification evident in these channels is attributed to block by intracellular  $Mg^{2+}$  (80) and by polyamines (81,82). The fact that channel conductance is high at negative membrane potentials underlies the contribution of  $I_{K1}$  to resting membrane potentials (79). The voltage dependent properties of  $I_{K1}$  channels, however, are such that the conductance is very low at potentials positive to approx  $-40$  mV (78). Nevertheless, because the driving force on  $K^+$  is high at depolarized potentials,  $I_{K1}$  channels do contribute outward  $K^+$  current during the plateau phase of the action potential, as well as during phase 3 repolarization (Fig. 1), particularly in ventricular cells. Cardiac and noncardiac drugs that affect the properties or the functioning of  $I_{K1}$  channels, therefore, could have rather profound effects on myocardial action potential waveforms, propagation, and rhythmicity and these effects are expected to be region specific, owing to the differential expression of these channels.

### MOLECULAR CORRELATES OF VOLTAGE-GATED CARDIAC $Na^+$ (NAV) CHANNELS

Functional cardiac Nav channels reflect the coassembly of Nav pore-forming ( $\alpha$ ) subunits and accessory ( $\beta$ ) subunits. The Nav channel  $\alpha$  subunits (Fig. 2A) belong to the "S4" superfamily of voltage-gated ion channel genes. Although a number of Nav  $\alpha$  subunits have been identified, Nav1.5 (*SCN5A*) is the one predominantly expressed in the myocardium, and Nav1.5 is the locus of mutations linked to one form of inherited long QT syndrome, LQT3 (Fig. 2A), as well as Brugada syndrome and conduction defects (14–17). Each Nav  $\alpha$  subunit has four homologous domains (I to IV), and each domain contains six  $\alpha$ -helical transmembrane repeats (S1–S6) (Fig. 2A). The cytoplasmic linker between domains III and IV is a pivotal component of Nav channel inactivation, and a critical isoleucine, phenylalanine, and methionine (IFM) motif in this linker has been identified as the inactivation gate (84–86).

During the plateau phase of ventricular action potentials, approx 99% of the Nav channels are in an inactivated, nonconducting state in which the inactivation gate is





**Fig. 2.** Pore-forming ( $\alpha$ ) subunits of cardiac ion channels. Membrane topologies of the  $\alpha$  subunits encoding Nav (A), Kv (B), and Kir channels (C) are illustrated. A four transmembrane, two-pore domain  $K^+$  (K2P) channel  $\alpha$  subunit is also illustrated in C.

thought to occlude the inner mouth of the pore through specific interactions with sites on S6 (87) or the S4-S5 loop (88) in domain IV. Inherited LQT3 mutations (i.e.,  $\Delta$ KPQ) in the domain III–IV linker in Nav1.5 disrupt inactivation (89). This ( $\Delta$ KPQ) and other LQT3 mutations result in sustained (bursting) Nav current activity (89), resulting in action potential prolongation in theoretical models (90) and in mice genetically engineered with LQT3 mutant Nav channels (91). Analysis of other *SCN5A* mutations, linked both to LQT3 and the Brugada syndrome, however, has revealed that this is not the only mechanism by which altered Nav channel function can prolong cardiac action potentials. A critical role for the carboxy (C)-terminal tail of Nav1.5 channel in the control of channel inactivation, for example, has now been defined (92–94). Point mutations in the C-terminus shift the voltage-dependence of inactivation, promote sustained  $Na^+$  channel activity, change the kinetics of both the onset of and recovery from inactivation, and alter drug-channel interactions (95–98). Single channel studies reveal that the C-terminus has pronounced effects on repetitive channel openings (99). Modeling studies suggest that this (C-terminal) domain can adopt a predominantly  $\alpha$ -helical structure and that only the proximal region of the C-terminus, which contains this helical domain, appears to measurably affect channel inactivation. Interactions likely occur, therefore, between the structured region of the C-terminus and other components of the channel protein, and these interactions appear to function to stabilize the channel in an inactivated state at depolarized membrane potentials. Drugs that affect these interactions, therefore, will alter Nav channel inactivation, influence action potential waveforms, and affect rhythmicity.

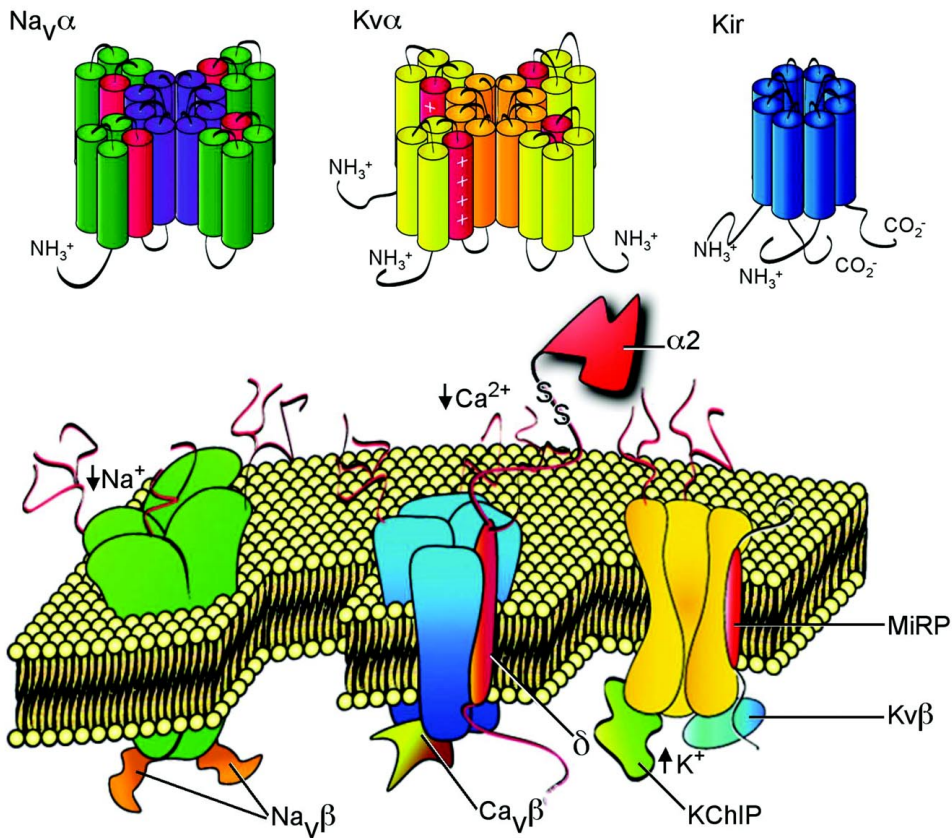
Modeling studies (100,101) have also provided insights into the mechanistic basis of the pathophysiology of other LQT3 mutations. The I1768V mutation, for example, does not cause channel bursting, but rather speeds recovery (from inactivation) at hyperpolarized potentials. Computational analysis predicts that this mutation will have a significant effect during action potential repolarization, a prediction that was verified experimentally (102). Similarly, subtle changes in Nav channel gating are caused by a commonly occurring *SCN5A* variant (S1102Y) which is associated with an elevated arrhythmia risk in African Americans (103). This variant causes subtle changes in channel activation and inactivation that are not likely to alter myocyte functioning in mutation carriers, unless these carriers are treated with drugs that block cardiac  $K^+$  channels (103). In this case, computational analysis, in combination with the experimental data, suggests a novel mechanism underlying susceptibility to drug-induced QT prolongation (103).

Functional Nav channels (Fig. 3) are thought to reflect the coassembly of Nav  $\alpha$  subunits with accessory Nav  $\beta$  subunits (104), and three different Nav  $\beta$  subunit genes, *SCN1b* (105,106), *SCN2b* (107,108), and *SCN3b* (109) have been identified. Co-expression of either *SCN1b* or *SCN3b* with *SCN5A* affects Nav channel kinetics and current densities (110), and *SCN2b* (111) co-expression affects the  $Ca^{2+}$  permeability of functional Nav channels (112). The fact that Nav $\beta$  subunits interact with ankyrin B (113), a cytoskeletal adaptor protein (114), suggests that an important function of these subunits may be to regulate Nav channel function through the cytoskeleton. Consistent with this hypothesis, electrophysiological recordings from myocytes isolated from ankyrin B $^{+/-}$  hearts reveal increased Nav channel bursting (115). Interestingly, molecular genetic studies have revealed that a loss-of-function mutation in ankyrin B (E1425G) underlies LQT4 (116).

## MOLECULAR CORRELATES OF VOLTAGE-GATED CARDIAC $Ca^{2+}$ (CAV) CHANNELS

Similar to Nav channels, Cav channel pore-forming ( $\alpha$ ) subunits belong to the “S4” superfamily of voltage-gated ion channel genes, and these subunits combine with auxiliary  $\beta$  and  $\alpha_2\delta$  subunits to form functional Cav channels (Fig. 3). Four distinct subfamilies of Cav  $\alpha_1$  subunits, Cav1, Cav2, Cav3, and Cav4 (117), have been identified, each with many subfamily members. Expression studies reveal that these genes encode Cav channels with distinct time- and voltage-dependent properties and pharmacological sensitivities. Functional expression of any of the Cav1  $\alpha$  subunits, Cav1.1, Cav1.2, Cav1.3, or Cav1.4, for example, reveals L-type HVA  $Ca^{2+}$  channel currents, which activate at approx  $-20$  mV and are selectively blocked by dihydropyridine  $Ca^{2+}$  channel antagonists. One member of this Cav1 subfamily, Cav1.2, is composed of 44 invariant and six alternative exons (118). Cav1.2 encodes the  $\alpha_{1C}$  ( $\alpha_1$ 1.2) protein, and three different isoforms of the  $\alpha_{1C}$  protein,  $\alpha_1$ 1.2a,  $\alpha_1$ 1.2b, and  $\alpha_1$ 1.2c (119,120), have been identified. Although nearly identical (>95 %) in amino acid sequences, these isoforms are differentially expressed, and the cardiac specific isoform is  $\alpha_1$ 1.2a (119).

There are two distinct types of Cav accessory subunits, Cav $\beta$  and Cav $\alpha_2\delta$  subunits. The  $\beta$  subunits are cytosolic proteins that are believed to form part of each functional L-type Cav channel protein complex (Fig. 3). Four different Cav $\beta$  subunits, Cav $\beta_1$  (121,122), Cav $\beta_2$  (123,124), Cav $\beta_3$  (123–125), and Cav $\beta_4$  (125,126) have been identified. Each Cav $\beta$  subunit has three variable regions (the carboxyl terminus, the amino terminus, and small region in the center of the linear protein sequence) flanking two highly conserved domains. The conserved domains mediate the interaction(s) with Cav $\alpha_1$  subunits, and the variable domains determine the functional effects of Cav $\beta$  subunit



**Fig. 3.** Molecular compositions of functional cardiac Nav, Cav, and Kv channels. Upper panel: the four domains of Nav (and Cav)  $\alpha$  subunit form monomeric Nav (and Cav) channels, whereas four Kv or Kir  $\alpha$  subunits combine to form tetrameric Kv and Kir channels. Lower panel: schematic illustrating functional cardiac Nav, Cav, and Kv channels, composed of the pore-forming  $\alpha$  subunits and a variety of accessory subunits.

co-expression (127). In co-expression studies, all four Cav $\beta$  subunits associate with Cav $\alpha_1$  subunits and modify the amplitudes, as well as the time- and voltage-dependent properties, of the currents (128–133).

In addition to Cav $\beta$  subunits, a disulfide-linked, transmembrane accessory subunit, Cav $\alpha_2\delta$ , is also found in the complex of functional Cav channels (Fig. 3). The first Cav $\alpha_2\delta$  subunit was cloned from skeletal muscle (134), and there are several members of the Cav $\alpha_2\delta$ –1 subfamily, as well as two homologous, Cav $\alpha_2\delta$ –2 and Cav $\alpha_2\delta$ –3, subfamilies (135). The Cav $\alpha_2\delta$  subunits are heavily glycosylated proteins that are cleaved posttranslationally to yield disulfide-linked  $\alpha_2$  and  $\delta$  proteins. The Cav $\alpha_2$  domain is extracellular and the Cav $\delta$  domain has a large hydrophobic region, which inserts into the membrane (Fig. 3) and anchors the Cav $\alpha_2\delta$  complex (136–138). The functional roles of Cav $\alpha_2\delta$  are somewhat variable and seem to depend on the identities of the co-expressed Cav $\alpha_1$  and Cav $\beta$  subunits and the expression environment. In general, co-expression of Cav $\alpha_2\delta$ –1 alters channel gating and increases current amplitudes, compared with the currents produced on expression of Cav $\alpha_1$  and Cav $\beta$  subunits alone (135,136,138–140). The increase in current density reflects improved targeting of Cav $\alpha_1$  subunits to the membrane, an effect attributed to the  $\alpha_2$  subunit domain (141).

## MOLECULAR CORRELATES OF VOLTAGE-GATED CARDIAC K<sup>+</sup> (Kv) CHANNELS

Kv channel pore-forming ( $\alpha$ ) subunits are six transmembrane spanning domain proteins with an "S4" domain and a K<sup>+</sup>-selective pore (Fig. 2), and functional Kv channels reflect the tetrameric assembly of four  $\alpha$  subunits (Fig. 3). Ten homologous Kv  $\alpha$  subunit subfamilies, *KCNA*, *KCNB*, *KCNC*, *KCND*, *KCNF*, *KCNG*, *KCNH*, *KCNQ*, *KCNS*, and *KCNV*, have been identified (Fig. 2) and in most subfamilies, there are several members (2). In addition to the multiplicity of Kv  $\alpha$  subunits, further functional Kv channel diversity can arise through alternative splicing of transcripts and through the formation of heteromultimeric channels (2).

The *KCNH2* subunit, which encodes the ether-a-go-go-related (143) or ERG1 protein (144), is the locus of mutations (Fig. 2B) underlie familial long QT syndrome, LQT2 (145). Expression of ERG1 in *Xenopus* oocytes reveals inwardly rectifying voltage-gated, K<sup>+</sup>-selective currents (146,147) with properties similar to cardiac I<sub>Kr</sub>. There are additional members of the *KCNH* subfamily (2), although none of these appear to be expressed in the heart. Another Kv $\alpha$  subunit, KvLQT1 (*KCNQ1*), has also been linked to inherited arrhythmias, and mutations in *KCNQ1* (Fig. 2C) underlie LQT1 (147). Although heterologous expression of *KCNQ1* alone reveals rapidly activating and noninactivating K<sup>+</sup> currents, co-expression with minK produces slowly activating K<sup>+</sup> currents that resemble the slow component of cardiac delayed rectification, I<sub>Ks</sub> (149,150). There are additional *KCNQ* subfamily members, *KCNQ2* and *KCNQ3*, although these are not expressed in the heart (151–153). Interestingly, however, *KCNQ2* and *KCNQ3* have been identified as loci of mutations leading to benign familial neonatal convulsions (151,153).

Similar to cardiac Nav and Cav channels, accessory subunits also contribute to the generation of functional cardiac Kv channels. The first identified Kv accessory subunit, minK (*KCNE1*), is a small (130 amino acids) protein with a single membrane spanning domain (154–156) that appears to co-assemble with KvLQT1 to form functional I<sub>Ks</sub> channels (149,150). Additional minK homologues, MiRP1 (*KCNE2*), MiRP2 (*KCNE3*), and MiRP3 (*KCNE4*) have also been identified, and it has been suggested that MiRP1 functions as an accessory subunit of ERG1 in the generation of I<sub>Kr</sub> (157,158). Although it is unclear whether minK, MiRP1 or other *KCNE* subfamily members contribute to the formation of cardiac Kv channels in addition to I<sub>Ks</sub> and I<sub>Kr</sub>, it has been reported that MiRP2 assembles with Kv3.4 in mammalian skeletal muscle (159) and with Kv4.x  $\alpha$  subunits in heterologous expression systems (160). These observations suggest the interesting possibility that *KCNE* subunits can assemble with different Kv  $\alpha$  subunits and contribute to the formation of multiple types of myocardial Kv channels.

The accessory Kv $\beta$  subunits are low molecular weight (~ 45 kD) cytosolic proteins, first identified in the brain (161,162). There are four homologous Kv  $\beta$  subunits, Kv  $\beta$ 1, Kv  $\beta$ 2, Kv  $\beta$ 3, and Kv  $\beta$ 4, and both Kv  $\beta$ 1 and Kv  $\beta$ 2 are expressed in the heart (163–168). The presence of Kv $\beta$  subunits affects the properties and the cell surface expression of Kv  $\alpha$  subunit-encoded K<sup>+</sup> channels (163–167). Heterologous expression studies suggest that the effects of the Kv  $\beta$  subunits are subfamily specific, i.e., Kv  $\beta$ 1, Kv  $\beta$ 2, and Kv  $\beta$ 3 interact with the Kv 1 subfamily of  $\alpha$  subunits (169,170), whereas Kv  $\beta$ 4 is specific for the Kv2 subfamily (171). It is not known, however, which Kv  $\alpha$  subunit(s) the Kv  $\beta$ 1 and Kv  $\beta$ 2 subunits associate with in the myocardium.

A distinct Kv channel accessory protein, referred to as KChAP (K<sup>+</sup> channel accessory protein), was identified in a yeast two hybrid screen (172). Co-expression of KChAP with

Kv2.1 (or Kv2.2) increases functional Kv2.x-induced current densities without measurably affecting the time-dependent and/or the voltage-dependent properties of the currents, suggesting that KChAP functions as a chaperon protein (172). Interestingly, KChAP can also interact with the N-termini of Kv1.x  $\alpha$  subunits and with the C-termini of Kv  $\beta$ 1.x subunits (172), suggesting that KChAP may be a multifunctional protein contributing to the generation of several cardiac Kv channels.

A yeast two hybrid screen also lead to the identification of the KChIPs (Kv channel interacting proteins) (173). Of these, only KChIP2 appears to be expressed in the heart (173,174), although there are several splice variants of KChIP2 (174–176). The KChIPs contain multiple EF-hand domains and belong to the recovering family of neuronal  $\text{Ca}^{2+}$ -sensing (NCS) proteins (177). When co-expressed with Kv4  $\alpha$  subunits, the KChIPs increase current densities, slow current inactivation, speed recovery from inactivation and shift the voltage-dependence of activation (173). However, KChIP co-expression reportedly does not affect the properties or the densities of Kv1.4- or Kv2.1-encoded  $\text{K}^+$  currents, suggesting that the modulatory effects of the KChIP proteins are specific for  $\alpha$  subunits of the Kv4 subfamily (173). In addition, although the binding of the KChIP proteins to Kv4  $\alpha$  subunits is not  $\text{Ca}^{2+}$ -dependent, mutations in EF hand domains 2, 3, and 4 eliminate the modulatory effects of KChIP1 on heterologously expressed Kv4.2-encoded  $\text{K}^+$  channels (173). It has also been shown that KChIP2 co-immunoprecipitates with Kv4.2 and Kv4.3  $\alpha$  subunits from adult mouse ventricles, consistent with a role for this subunit in the generation of Kv4-encoded cardiac  $\text{I}_{\text{to,f}}$  channels (178). Interestingly, a gradient in KChIP2 message expression is observed through the thickness of the ventricular wall in canine and human heart, suggesting that KChIP2 underlies the observed differences in  $\text{I}_{\text{to,f}}$  densities in the epicardium and endocardium in human and canine ventricles (174,179). However, in rat and mouse there is no gradient in KChIP2 expression, and it appears that differences in Kv4.2 underlie the regional variations in  $\text{I}_{\text{to,f}}$  densities in rodents (178,180).

Although the link is less clear than for Nav channels (113–115), there is evidence to suggest that myocardial Kv channels are also regulated through interactions with the actin cytoskeleton. Using a yeast two-hybrid screen, for example, it has been shown that Kv1 $\alpha$  subunits, Kv1.5 and Kv1.4, bind to  $\alpha$ -actinin-2 (181,182). In addition, when heterologously expressed, Kv1.5 and  $\alpha$ -actinin-2 co-immunoprecipitate, and treatment of cells with cytochalasin B or D reduces the functional cell surface expression of Kv1.5-encoded  $\text{K}^+$  channels (181,182). It has also been reported that  $\alpha$  subunits of the Kv4 subfamily interact directly with another actin-binding protein, filamin (183), and that disruption of the cytoskeleton increases the density of heterologously expressed Kv4-encoded  $\text{K}^+$  currents (184). Although the role of cytoskeletal-channel interactions in the myocardium and the molecular mechanisms involved in mediating these interactions remain to be defined, it seems reasonable to suggest that cardiac Kv (and other) channels function as components of macromolecular complexes. Clearly, drugs that interact with any of the complex components or that affect interactions between complex components could, in principle, affect channel properties and myocardial function.

## RELATIONS BETWEEN KV SUBUNITS AND FUNCTIONAL CARDIAC KV CURRENTS

Considerable experimental evidence has accumulated documenting a role for Kv  $\alpha$  subunits of the Kv4 subfamily in the generation of cardiac  $\text{I}_{\text{to,f}}$  channels (185–188). In

ventricular myocytes isolated from transgenic mice expressing a dominant negative pore mutant of Kv4.2 (Kv4.2W362F), Kv4.2DN in the myocardium, for example,  $I_{to,f}$  is eliminated (187). In addition, biochemical and electrophysiological studies suggest that Kv4.2 and Kv4.3 are associated in adult mouse ventricles and that functional mouse ventricular  $I_{to,f}$  are heteromeric (178). However, in large mammals, including humans, it appears that  $I_{to,f}$  channels are Kv4.3 homomultimers because Kv4.2 is not expressed (189). The fact that the properties of  $I_{to,s}$  are different from  $I_{to,f}$  (Table 1), suggested that the molecular correlates of  $I_{to,s}$  and  $I_{to,f}$  channels are also distinct. Direct experimental support for this hypothesis was provided with the demonstration that  $I_{to,s}$  is eliminated (29) in ventricular myocytes isolated from mice with a targeted deletion in the Kv1.4 gene (190). Given the similarities in current properties, it seems reasonable to suggest that Kv1.4 also encodes  $I_{to,s}$  in other species, including humans.

As noted earlier, *KCNH2* is the locus of mutations in LQT2 and has been shown to encode  $I_{Kr}$  (145–147), and *KCNQ1*, the locus of mutations leading in LQT1 (148), encodes cardiac  $I_{Ks}$  (149,140). The fact that mutations in the transmembrane domain of minK alter the properties of heterologously expressed *KCNQ1* and minK Kv channels suggests that the transmembrane segment of minK also contributes to the  $I_{Ks}$  channel pore (191–194). Alternative experimental strategies, primarily in mice, have been exploited to define the molecular correlates of several other myocardial Kv currents. A role for Kv2  $\alpha$  subunits in the generation of mouse ventricular  $I_{K,slow2}$ , for example, was revealed with the demonstration that  $I_{K,slow2}$  is selectively attenuated in ventricular myocytes isolated from transgenic mice expressing a truncated Kv2.1  $\alpha$  subunit that functions as a dominant negative (55). Subsequently, it was also shown that  $I_{K,slow1}$  is eliminated in ventricular myocytes isolated from mice with targeted deletion of Kv1.5 (57), revealing that Kv1.5 encodes mouse ventricular  $I_{K,slow1}$  (57,59). These findings, together with the previous results obtained on cells isolated from Kv1.4 null animals, in which  $I_{to,s}$  is eliminated (29), suggest that, in contrast to the Kv 4  $\alpha$  subunits (178), myocardial Kv 1  $\alpha$  subunits, Kv1.4 and Kv1.5, do not associate in situ. Rather, functional cardiac Kv1  $\alpha$  subunit-encoded  $K^+$  channels are homomeric, composed of Kv1.4  $\alpha$  subunits ( $I_{to,s}$ ) or Kv1.5  $\alpha$  subunits ( $I_{K,slow1}$ ,  $I_{Kur}$ ).

## MOLECULAR CORRELATES OF OTHER CARDIAC $K^+$ CURRENTS

In cardiac and other cells, the inwardly rectifying  $K^+$  (Kir) channels are encoded by a large and diverse subfamily of Kir channel  $\alpha$  subunit genes, each of which encodes a protein with two transmembrane domains (Fig. 2C). Similar to Kv channels, Kir subunits assemble as tetramers to form  $K^+$  selective pores (Fig. 3). Based on the properties of the currents produced in heterologous expression systems, it has been suggested that Kir2  $\alpha$  subunits encode cardiac  $I_{K1}$  channels (195), and several members of the Kir 2 subfamily are expressed in the myocardium (196). Direct support for a role for Kir 2  $\alpha$  subunits in the generation of  $I_{K1}$  channels was provided in studies completed on myocytes isolated from mice with a targeted deletion of Kir2.1 (Kir2.1 $^{-/-}$ ) or Kir 2.2 (Kir2.2 $^{-/-}$ ) (197,198). Although the Kir2.1 $^{-/-}$  mice have cleft palate and die shortly after birth (197), voltage-clamp recordings from newborn Kir2.1 $^{-/-}$  ventricular myocytes revealed that  $I_{K1}$  is absent (198). A small, slowly activating inward rectifier current, distinct from  $I_{K1}$ , however, is evident in Kir2.1 $^{-/-}$  myocytes. Voltage-clamp recordings from adult Kir2.2 $^{-/-}$  ventricular myocytes reveal that  $I_{K1}$  is reduced (198). Taken together, these results suggest that both Kir2.1 and Kir2.2 contribute to functional cardiac  $I_{K1}$  channels. The obser-

vation that Kir2.2 does not generate  $I_{K1}$  channels in the absence of Kir2.1, however, further suggests that functional cardiac  $I_{K1}$  channels are heteromeric.

A novel type of  $K^+$  channel  $\alpha$  subunit with four transmembrane spanning regions and two pore domains (Fig. 2C) was identified with the cloning of TWIK-1 (199). Both pore domains are functional and TWIK subunits assemble as dimers, rather than tetramers (200). There are a great many four transmembrane and two pore domain  $K^+$  (K2P) channel  $\alpha$  subunit (*KCNK*) genes, and expression studies suggest that the members of various K2P subunit subfamilies give rise to currents that display distinct current-voltage-relations and differential sensitivities to a variety of modulators, including pH and fatty acids (200). Nevertheless, the physiological roles of these subunits/channels in the myocardium, as well as in other cell types, remain to be determined. The K2P subunits TREK-1 and TASK-1, for example, are both expressed in the heart and heterologous expression of either of these subunits gives rise to instantaneous, noninactivating  $K^+$  currents that display little or no voltage-dependence (200). These properties have led to suggestions that these subunits contribute to “background” or “leak” currents (201), and interestingly, expressed TREK-1 or TASK-1 currents are similar to the current referred to as  $I_{Kp}$  in guinea pig ventricular myocytes (64,202).

## SUMMARY AND CONCLUSIONS

Electrophysiological studies have clearly identified multiple types of voltage-gated inward and outward currents that contribute to action potential repolarization in the mammalian myocardium (Table 1). Interestingly, the outward currents are more numerous and more diverse than the inward currents, and cardiac myocytes express a repertoire of  $K_v$  channels/currents that contribute importantly to shaping the waveforms of action potentials, as well as influencing automaticity and refractoriness. Changes in the properties or the functioning of cardiac  $K_v$  channels during development, owing to underlying cardiac disease or resulting from the actions of cardiac or non-cardiac drugs, can, therefore, have rather dramatic effects on myocardial action potential waveforms and the generation of normal cardiac rhythms.

In addition to the demonstrated importance of repolarizing  $K_v$  currents in the myocardium, however, it is also quite clear that Cav channel currents and the Nav channel “window” current also contribute importantly to action potential repolarization. This has been very elegantly demonstrated for Nav channels with the characterization of inherited mutations in the cardiac Nav *SCN5A* gene, mutations that underlie Long QT3, Brugada syndrome, and conduction defects. Functional characterization of these mutants and computer simulations of cellular electrical activity together have provided new insights into the effects of altered channel functioning on action potential waveforms and rhythmicity (100,101,117,138,139,203). These studies demonstrate that small changes in Nav channel currents can have profound effects on repolarization because the plateau phase of the action potential is maintained by the delicate balance of small (inward and outward) currents. It is very clear, therefore, that cardiac or noncardiac drugs that affect Nav channel currents will influence action potential durations. Because cardiac Cav channels also control the plateau phase of cardiac action potentials and action potential repolarization, changes in Cav channel currents will also have functional consequences. Indeed, it seems reasonable to suggest that drugs that affect the functional expression and/or the properties of any of the (inward or outward current) channels that contribute to shaping action potentials would have been expected to impact the propagation of activity and the

generation of cardiac rhythms. When considering screening of noncardiac (as well as cardiac) drugs, therefore, effects on all of the various cardiac ion channels that contribute to repolarization must be considered.

In addition to the diversity of voltage-gated ion channel pore-forming  $\alpha$  subunits, molecular and biochemical studies have now demonstrated that there are multiple accessory subunits that contribute to the formation of the various cardiac inward and outward current channels. Recent studies also suggest that channel subunit interactions with the actin cytoskeleton are important in determining functional cardiac (Nav and Kv) channel expression. Although it seems quite clear that the relationships between channel subunits and regulatory molecules are important in determining channel expression and/or properties, very little is presently known about the molecular interactions involved and/or the role(s) of these interactions in determining the properties and/or the functioning of the various cardiac ion channels involved in mediating repolarization. Nevertheless, these channel subunit-subunit and channel subunit-regulatory protein interactions are also potential sites of action of cardiac and noncardiac drugs. It seems reasonable to suggest, therefore, that defining the molecular correlates/compositions of the channels controlling cardiac action potential waveforms in detail will facilitate future efforts focused on delineating the mechanisms controlling the properties and the functional expression of these channels in the developing, aging, damaged, or diseased myocardium. In addition, however, this information will provide fresh new insights into the repertoire of proteins that play roles in regulating the properties and the expression of myocardial ion channels and into the detailed molecular mechanisms involved in mediating these effects. Probing these molecular mechanisms in detail is requisite to understanding the factors controlling channel expression and properties during normal cardiac development, as well as in the aged, damaged, and/or diseased myocardium. Also, because any step in the regulatory pathway could potentially be affected by cardiac and/or noncardiac drugs, it will become increasingly important to understand these pathways in detail, as well as how the various drugs might affect one or more steps in channel regulatory pathways, to minimize potentially dangerous, life-threatening drug effects. Clearly, a major focus of future research will be on defining the molecular mechanisms controlling the properties and the functioning of myocardial ion channels in great detail.

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