Molecular Physiology of Cardiac Repolarization

JEANNE M. NERBONNE AND ROBERT S. KASS

Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, Missouri; and Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York

I. Introduction
II. Myocardial Action Potentials and Voltage-Gated Inward Sodium and Calcium Currents
   A. Voltage-gated Na⁺ (Nav) currents
   B. Voltage-gated Ca²⁺ (Cav) currents
III. Myocardial Action Potentials and Repolarizing Voltage-Gated Potassium Currents
   A. Transient outward Kv currents
   B. Delayed rectifier Kv currents
   C. Regional differences in Kv current expression and properties
IV. Other Myocardial Potassium Currents Contributing to Repolarization
V. Molecular Components of Myocardial Nav and Cav Channels
   A. Nav channel pore-forming α-subunits
   B. Nav channel accessory subunits and other interacting proteins
   C. Cav channel pore-forming α-subunits
   D. Cav channel accessory subunits and other interacting proteins
VI. Molecular Components of Myocardial Kv Channels
   A. Kv channel pore-forming α-subunits
   B. Kv channel accessory subunits
   C. Molecular correlates of cardiac transient outward Kv channels
   D. Molecular correlates of cardiac delayed rectifier Kv channels
VII. Molecular Components of Other Cardiac Potassium Channels
   A. Inwardly rectifying cardiac K⁺ (Kir) channel pore-forming α-subunits
   B. Two-pore domain K⁺ (K2P) channel pore-forming α-subunits
VIII. Myocardial Potassium Channels and the Actin Cytoskeleton
IX. Summary and Conclusions

Nerbonne, Jeanne M., and Robert S. Kass. Molecular Physiology of Cardiac Repolarization. Physiol Rev 85: 1205–1253, 2005; doi:10.1152/physrev.00002.2005.—The heart is a rhythmic electromechanical pump, the functioning of which depends on action potential generation and propagation, followed by relaxation and a period of refractoriness until the next impulse is generated. Myocardial action potentials reflect the sequential activation and inactivation of inward (Na⁺ and Ca²⁺) and outward (K⁺) current carrying ion channels. In different regions of the heart, action potential waveforms are distinct, owing to differences in Na⁺, Ca²⁺, and K⁺ channel expression, and these differences contribute to the normal, unidirectional propagation of activity and to the generation of normal cardiac rhythms. Changes in channel functioning, resulting from inherited or acquired disease, affect action potential repolarization and can lead to the generation of life-threatening arrhythmias. There is, therefore, considerable interest in understanding the mechanisms that control cardiac repolarization and rhythm generation. Electrophysiological studies have detailed the properties of the Na⁺, Ca²⁺, and K⁺ currents that generate cardiac action potentials, and molecular cloning has revealed a large number of pore forming (α) and accessory (β, δ, and γ) subunits thought to contribute to the formation of these channels. Considerable progress has been made in defining the functional roles of the various channels and in identifying the α-subunits encoding these channels. Much less is known, however, about the functioning of channel accessory subunits and/or posttranslational processing of the channel proteins. It has also become clear that cardiac ion channels function as components of macromolecular complexes, comprising the α-subunits, one or more accessory subunit, and a variety of other regulatory proteins. In addition, these macromolecular channel protein complexes appear to interact with the actin cytoskeleton and/or the extracellular matrix, suggesting important functional links between channel complexes, as well as between cardiac structure and electrical functioning. Important areas of future research will be the identification of (all of) the molecular components of functional cardiac ion channels and delineation of the molecular mechanisms involved in regulating the expression and the functioning of these channels in the normal and the diseased myocardium.
I. INTRODUCTION

The normal mechanical (pump) functioning of the mammalian heart depends on proper electrical functioning (56, 173), reflected in the sequential activation of cells in specialized, “pacemaker” regions of the heart and the propagation of activity through the ventricles (Fig. 1). Myocardial electrical activity is attributed to the generation of action potentials in individual cardiac cells, and the normal coordinated electrical functioning of the whole heart is readily detected in surface electrocardiograms (Fig. 1). The propagation of activity and the coordination of the electromechanical functioning of the ventricles also depend on electrical coupling between cells, mediated by gap junctions (251, 435). The generation of myocardial action potentials reflects the sequential activation and inactivation of ion channels that conduct depolarizing, inward (Na\(^+\) and Ca\(^{2+}\)), and repolarizing, outward (K\(^+\)), currents (24, 375). The waveforms of action potentials in different regions of the heart are distinct (Fig. 1), owing to differences in the expression and/or the properties of the underlying ion channels (24, 374). These differences contribute to the normal unidirectional propagation of excitation through the myocardium and to the generation of normal cardiac rhythms (23, 24, 259, 374, 375). Changes in the properties or the functional expression of myocardial ion channels, resulting from inherited mutations in the genes encoding these channels (23, 36, 51, 102, 204, 243, 253) or from myocardial disease (34, 49, 67, 184, 365, 496, 501–503, 510), can lead to changes in action potential waveforms, synchronization, and/or propagation, thereby predisposing the heart to potentially life-threatening arrhythmias (13, 14, 16, 24, 127, 259, 436). There is, therefore, considerable interest in delineating the molecular, cellular, and systemic mechanisms contributing to the generation and maintenance of normal cardiac rhythms, as well as in understanding how these mechanisms are altered in the diseased myocardium.

Myocardial electrical activity is initiated in the pacemaker cells in the sinoatrial (SA) node and then propagated through the atria to the atrioventricular (AV) node (Fig. 1). Following a brief pause in the AV node, excitation spreads in the conducting Purkinje fibers to the apex of the heart and into the working, ventricular myocardium (Fig. 1). In cells in each of these specialized regions, excitation results in action potential generation, followed by relaxation and a period of refractoriness until the next impulse is generated and propagated. The observed heterogeneity in action potential waveforms in different cell types (Fig. 1) reflects differences in ion channel expression levels, and modeling studies suggest that small changes in the time- and/or voltage-dependent properties of cardiac sarcolemmal ion channels can have rather profound effects on action potential durations, as well as impact refractoriness and rhythmicity (105, 127, 311, 312). In ventricular and atrial myocytes and in Purkinje fibers (Fig. 1), the upstroke of the action potential (phase 0) is rapid, resulting from the activation of voltage-gated Na\(^+\).
(Nav) channels (172) (Fig. 2). In pacemaker cells in the SA node (SAN) and AV node (AVN), however, phase 0 is markedly slower than in atria/ventricles (Fig. 1), suggesting that Nav channels do not play a prominent role in depolarization. Nevertheless, Nav currents have been described in subsets of rabbit and guinea pig AVN cells (361, 579), as well as in rabbit SAN cells (173). The properties of the Nav currents expressed in cardiac cells from different species, as well as in different cell types in the same species (Table 1), are similar, an observation that might be interpreted as suggesting that the molecular correlates of the underlying channels are the same (see sect. vA).

Phase 0 of the action potential in Purkinje fibers and in atrial and ventricular myocytes is followed by a transient repolarization (phase 1), reflecting Nav channel inactivation and the activation of the fast transient voltage-gated outward K⁺ current (I_{to,f}) (Fig. 2). This transient repolarization or “notch,” which can be quite prominent in Purkinje and ventricular cells (Fig. 1), influences the height and duration of the action potential plateau (phase 2). Membrane depolarization also activates voltage-gated Ca²⁺ (Cav) currents, and the influx of Ca²⁺ through L-type Cav channels during the phase 2 plateau is the main trigger for excitation-contraction coupling in the working myocardium (57, 154). In SAN and AVN cells, activation of (L-type) Cav channels also contributes to action potential generation, particularly in cells expressing low levels of functional Nav channels (57). In some cardiac cells/species, another class of Cav channels, the T-type Cav channels (Table 1), has been distinguished and suggested to play a role in automaticity (57, 394). As with cardiac Nav channels, however, the properties of the L- and T-type cardiac Cav channels characterized electrophysiologically in different cell types and in different species (Table 1) are quite similar, suggesting that the molecular correlates of the underlying L- and T-type (Cav) channels are also similar throughout the myocardium (see sect. vC).

The driving force for K⁺ efflux is high during the plateau phase of the action potential in ventricular and atrial myocardium and, as the Cav channels inactivate, the outward K⁺ currents predominate, resulting in (phase 3) repolarization, bringing the membrane voltage back to the resting potential (Fig. 2). In contrast to Nav and Cav currents, however, there are multiple types of voltage-gated K⁺ (Kv) currents, as well as non-voltage-gated, inwardly rectifying K⁺ (Kir) currents (Table 1), that contribute to myocardial action potential repolarization. The greatest functional diversity is among Kv channels (Table 1). At least two types of transient outward currents, I_{to,f} and I_{to,s}, and several components of delayed rectification, including I_Kr [I_{K(rapid)}], I_Ks [I_{K(slow)}], and I_Kur [I_{K(ultrarapid)}], for example, have been distinguished (Table 1). The time- and voltage-dependent properties of the various Kv currents identified in myocytes isolated from different species and/or different regions of the heart in the same species, however, are remarkably similar, suggesting that the same (or very similar) molecular entities contribute to the generation of each of the various types of Kv channels (Table 1) in different cells/species. The relative Kv channel expression levels vary in cardiac cells in different regions (i.e., atria, ventricles) of the heart, and this hetero-

![FIG. 2. Action potential waveforms and underlying ionic currents in adult human and ventricular (left) and atrial (right) myocytes. The time- and voltage-dependent properties of the voltage-gated inward Na⁺ (Nav) and Ca²⁺ (Cav) currents expressed in human atrial and ventricular myocytes are similar. In contrast, there are multiple types of K⁺ currents, particularly Kv currents, contributing to atrial and ventricular action potential repolarization. The properties of the various Kv currents are distinct, and in contrast to the inward currents, there are multiple Kv currents expressed in individual myocytes throughout the myocardium.](image-url)
geneity contributes importantly to the observed regional differences in action potential waveforms (24, 127, 373, 374). Changes in the properties or the functional expression of Kv channels, as occurs in a variety of myocardial diseases (34, 49, 67, 365, 496, 503, 510), can, therefore, have dramatic effects on action potential waveforms, propagation, and rhythmicity. A large number of pore-forming (α) subunits, encoding Nav, Cav, Kv, and Kir channels, and a variety of channel accessory (β, δ, and γ) subunits have been identified (Tables 2–6), and considerable progress has been made in defining the expression patterns of these subunits in the heart and the roles of the individual subunits in the generation of functional cardiac (Nav, Cav, Kv, and Kir) channels (Tables 2–6). These studies have demonstrated that distinct molecular entities underlie the various cardiac ion channels/currents that have been distinguished electrophysiologically and shown to contribute to myocardial action potential repolarization. It also has now been shown that mutations in the genes encoding the subunits involved in the generation of functional cardiac Nav, Cav, Kv, and Kir channels underlie several inherited cardiac arrhythmias (23, 36, 51, 102, 204, 253, 479, 481). Although inherited rhythm disorders are rare, these mutations belong to an ever-increasing number of “channelopathies,” i.e., diseases linked to genes encoding ion channels (35, 123, 204, 220, 234, 243, 267, 309, 359, 403, 442, 459). Based on the rapid progression of this field (249) and the growing molecular complexity of ion channels, it seems certain that the number of genes encoding ion channels or ion channel regulatory molecules linked to inherited and acquired disorders of the cardiovascular (and other) system will continue to increase, perhaps dramatically, in the future. The densities and the functional properties of myocardial Nav, Cav, Kv, and Kir currents also change in a number of acquired myocardial disease states (34, 49, 67, 365, 496, 503, 510), and these changes can lead to the generation of potentially life-threatening cardiac arrhythmias. At present, therefore, there is considerable interest in understanding the detailed molecular mechanisms controlling the properties and the functional cell surface expression of the various ion channels controlling myocardial action potential repolarization, as well as the impact of genetic and epigenetic factors, including cardiac and noncardiac disease, on the functioning of these channels.

II. MYOCARDIAL ACTION POTENTIALS AND VOLTAGE-GATED INWARD SODIUM AND CALCIUM CURRENTS

A. Voltage-Gated Na⁺(Nav) Currents

Voltage-gated cardiac Na⁺ (Nav) channels open rapidly on membrane depolarization (Fig. 2) and underlie the rapidly rising phases of the action potentials recorded in mammalian ventricular and atrial myocytes and in cardiac Purkinje fibers (93, 375). Although not evident in all cells, Nav channels with similar properties are also expressed in subsets of mammalian SAN and AVN cells, and differences in functional Nav channel expression likely contrib-
ute to action potential heterogeneity in pacemaker cells (262, 361, 579, 582). Although the properties of the Nav channels expressed in different cardiac cells are similar, the biophysical and pharmacological properties of these channels are distinct from Nav channels expressed in other excitable cells, such as neurons and skeletal muscle (93, 573). Cardiac Nav channels, for example, are remarkably insensitive to the Nav channel toxin tetrodotoxin (TTX), which binds with high (nM) affinity to neuronal and skeletal muscle Nav channels and blocks Na⁺ influx (93, 573). This observation was probably the first indication that the molecular identities of the Nav channels in cardiac myocytes, neurons, and skeletal muscle were distinct, and as detailed in section V, this has now been demonstrated.

On membrane depolarization, cardiac Nav channels activate and inactivate rapidly (172, 174). The threshold for Nav channel activation is quite negative (approximately −55 mV), and the activation of these channels is steeply voltage dependent. Importantly, inactivation is also voltage dependent, and cardiac Nav channels can undergo voltage-dependent inactivation without ever opening (174). Nevertheless, persistent openings of cardiac Nav channels are occasionally observed, even at depolarized membrane potentials (437, 591). At potentials corresponding to the action potential plateau in ventricular myocytes, present estimates are that ~99% of the Nav channels are in an inactivated, nonconducting state (423, 525). There is, therefore, a finite, albeit small (~1%), probability of Nav channels being open at potentials corresponding to the action potential plateau (525). A slow component of Nav channel inactivation has indeed been described in normal human ventricular myocytes (323). This current is modulated by lysolipids (501) and appears to be upregulated in failing myocardium (501–503). This observation was probably the first indication that the molecular identities of the Nav channels in cardiac myocytes, neurons, and skeletal muscle were distinct, and as detailed in section V, this has now been demonstrated.

B. Voltage-Gated Ca²⁺ (Cav) Currents

In contrast to skeletal muscle, it has long been recognized that Ca²⁺ entry from the extracellular space is required for excitation-contraction coupling in the mammalian myocardium (56, 57, 154, 173). The pathway for plasmalemmal Ca²⁺ entry was first revealed in voltage-clamp recordings from multicellular (frog) atrial preparations and was termed the “slow inward” current pathway (416–418, 429). Subsequent studies revealed that this “slow inward” current is carried by Ca²⁺ through a membrane conductance distinct from the voltage-dependent (Nav channel) pathway for Na⁺ movement (45, 332, 386, 416–418, 429). Further studies detailed the time- and voltage-dependent properties of voltage-gated cardiac Ca²⁺ (Cav) currents, first, in multicellular preparations and later, in isolated single cardiac cells (45, 57, 172, 416–418).

Although the presence of two functionally distinct types of Cav currents in single (starfish egg) cells was first reported in 1975 (201), it was not until the late 1980s that the import and the generality of these observations became clear. Two types of Cav currents/channels, for example, were clearly distinguished in (chick and rat) sensory neurons, based primarily on differences in the thresholds for channel activation (85, 86). These channels were termed high voltage-activated (HVA) and low voltage-dependent (LVA) Cav currents.
age-activated (LVA) Cav channels. Cardiac HVA and LVA Cav channels were first described in isolated canine atrial cells (44). LVA Cav channels, also referred to as T-type Ca$^{2+}$/H11001 channels (394), activate at relatively hyperpolarized membrane potentials, i.e., approximately −50 mV, and these channels activate and inactivate rapidly (85, 86, 382). HVA Cav channels, in contrast, open on depolarization to membrane potentials positive to approximately −20 mV, and these channels inactivate over a time course of several tens of milliseconds to seconds, depending on the preparation and the charge carrying ion (85, 326). Under physiological conditions, with Ca$^{2+}$ as the charge carrier, HVA channels in most cells inactivate in <100 ms at depolarized voltages (44, 326).

The detailed kinetic, pharmacological, and voltage-dependent properties of HVA Cav channels in different cell types are distinct, suggesting that HVA Cav channels are heterogeneous, particularly compared with LVA Cav channels. Consistent with this view, multiple types of HVA channels have now been identified in different cell types, and these are referred to as L, N, P, Q, or R channels (276, 382, 394). Although all HVA Cav channels exhibit relatively large single-channel conductances (13–25 pS) and have similar permeation properties, the detailed biophysical properties and the pharmacological sensitivities of the various types of HVA Cav channels are distinct. In the mammalian heart, L-type HVA Cav currents appear to be ubiquitously expressed (44, 49, 326). In addition, the properties and the densities of L-type Cav channel currents in cells isolated from different regions of the myocardium, as well as in cardiac cells from different species, are quite similar, suggesting that the molecular compositions of the underlying channels and the molecular mechanisms controlling the functional expression of these channels are the same. Importantly, however, the time- and voltage-dependent properties of cardiac L-type HVA currents are distinct from the L-type HVA Cav currents expressed in skeletal muscle and in neurons (44, 326, 340), suggesting that, similar to the Nav channels, distinct molecular entities underlie the L-type HVA Cav channels in different tissues (see sect. V).

The opening of cardiac L-type Cav channels in response to membrane depolarization is delayed relative to the Nav channels (Fig. 2), and these channels, therefore, contribute little to phase 0 depolarization in Purkinje, atrial and ventricular cells. Rather, the opening of HVA L-type Cav channels and the Ca$^{2+}$ entry through these channels underlies the action potential plateau (phase 2), which is particularly prominent in ventricular and Purkinje cells (Fig. 2). In addition, Ca$^{2+}$ influx through the L-type HVA Cav channels triggers Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores and underlies excitation-contraction coupling in the working (ventricular) myocardium (56, 57, 154, 173). L-type HVA Cav channels are also expressed in SAN and AVN cells, where they play a role in action potential generation, as well as in regulating automaticity (49, 72, 262, 340, 361, 579). Cardiac L-type HVA Cav channels undergo rapid voltage- and Ca$^{2+}$-dependent inactivation (166, 281, 326), processes that will also influence action potential waveforms (Fig. 2) by affecting the duration of the plateau (phase 2) and the time course of action potential repolarization.
In addition to the ubiquitously expressed HVA L-type cardiac Cav currents, LVA or T-type Cav channel currents have also been identified in voltage-clamp recordings from adult atrial myocytes and conducting tissues in several different species (44, 200, 340, 394). Although not evident in normal adult ventricular myocytes (394), T-type Cav currents have also been recorded in neonatal rat and rabbit ventricular myocytes (547, 548). In addition, it has been demonstrated that T-type Cav currents are expressed in ventricular myocytes in several animal models of ventricular hypertrophy (328, 383), findings consistent with the view that substantial remodeling occurs in the hypertrophied myocardium, reflecting a reversion to a fetal/neonatal pattern of gene expression (486). It is certainly possible that similar remodeling occurs in the hypertrophied human heart (49). Nevertheless, it is important to note that, to date, T-type LVA Cav channels have not been detected in normal or diseased human myocardial cells (49, 394).

In addition to marked differences in biophysical properties, the physiological role(s) of cardiac T-type LVA Cav channels appears to be quite different from the L-type HVA Cav channels. As noted previously, for example, Ca\(^{2+}\) entry through L-type channels in cardiac cells results in Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular (Ca\(^{2+}\)) stores and is the main trigger for excitation-contraction coupling (57, 154). Although Ca\(^{2+}\) entry through T-type channels also triggers Ca\(^{2+}\) release from intracellular stores, the coupling is less efficient (589), and it seems unlikely that T channels contribute importantly to excitation-contraction coupling. This may simply reflect the fact that LVA channel densities are low and that, owing to rapid inactivation, very little Ca\(^{2+}\) actually enters cells on depolarization. Alternatively, these functional differences may reflect the fact that HVA and LVA cardiac Cav channels are differentially localized, i.e., L-type, but not T-type, Cav channels are highly localized in the t tubules near the storage sites for intracellular Ca\(^{2+}\) sequestration/release (394, 589). Further experiments will be necessary to determine the mechanistic basis for the distinct functional roles of L- and T-type Ca\(^{2+}\) channels in regulating excitation-contraction coupling.

The finding that T-type LVA Cav currents activate at relatively hyperpolarized potentials and that these channels are expressed preferentially in pacemaker and conducting cells in the heart suggests the interesting possibility that there is a role for LVA channels in pacemaking (200). Although some experimental support for this hypothesis has been provided, rigorous testing is complicated by the paucity of highly selective LVA Cav channel blockers (394). In addition, it has been reported that (rabbit) SAN cells express rapidly activating Na\(^{+}\)-dependent inward currents that are blocked by Ca\(^{2+}\) channel blockers (582). These observations suggest that pacemaker cells may express additional novel inward currents that contribute to shaping action potential waveforms and to regulating normal cardiac rhythms. Additional studies, focused on further characterization of the properties of LVA Cav channels in SAN and AVN cells and determination of the functional roles of these channels in regulating pacemaking, will be needed to explore these possibilities directly.

III. MYOCARDIAL ACTION POTENTIALS AND REPOLARIZING VOLTAGE-GATED POTASSIUM CURRENTS

Voltage-gated K\(^{+}\) (Kv) channels are the primary determinants of action potential repolarization in the mammalian myocardium, and compared with cardiac Nav and Cav channels, there is considerable electrophysiological and functional cardiac Kv channel diversity (42, 373, 375). Based primarily on differences in time- and voltage-dependent properties and pharmacological sensitivities (42, 373, 375), two broad classes of repolarizing cardiac Kv currents have been distinguished (42): transient outward K\(^{+}\) currents (I\(_{to}\)) and delayed, outwardly rectifying K\(^{+}\) currents (I\(_{K}\)) (Table 1). The transient currents (I\(_{to}\)) activate and inactivate rapidly on membrane depolarizations to potentials positive to approximately −30 mV and underlie the early phase (phase 1) of repolarization in ventricular and atrial cells (Fig. 2). Cardiac delayed rectifiers (I\(_{K}\)) activate at similar membrane potentials and with variable kinetics, and these currents determine the latter phase (phase 3) of repolarization back to the diastolic potential (Fig. 2). Multiple types of myocardial I\(_{to}\) and I\(_{K}\) channels with distinct time- and voltage-dependent properties (Table 1), however, have been identified, and differences in the densities and the biophysical properties of these channels contribute to variations in the waveforms of action potentials recorded in different cardiac cell types (Fig. 1), as well as in different species (24, 374, 375, 436). The detailed pharmacological, time- and voltage-dependent properties of each of the various repolarizing Kv currents characterized in different cardiac cell types and species are quite similar, thereby allowing Kv channels to be classified based on these biophysical properties (Table 1). The observed similarities in Kv channel properties also suggest that the molecular correlates of the underlying Kv channels are also similar (42, 373), and considerable experimental evidence has now been provided to support this hypothesis (see sect. vi).

A. Transient Outward Kv Currents

Although cardiac transient outward currents were first described in (sheep) Purkinje fibers and thought to reflect Cl\(^{-}\) conductances (143, 175), subsequent work demonstrated the presence of two transient outward cur-
currents with distinct properties and referred to as $I_{to1}$ and $I_{to2}$ (112). Pharmacological studies revealed that $I_{to1}$ is blocked by 4-aminoypyridine (4-AP) and unaffected by changes in extracellular Ca$^{2+}$, whereas $I_{to2}$ is not blocked by 4-AP and is Ca$^{2+}$ dependent (257, 256). In further studies, it was shown that the Ca$^{2+}$-dependent $I_{toq}$ in Purkinje fibers and ventricular cells is a Cl$^-$ (not a K$^+$) current (593, 594). In contrast, the Ca$^{2+}$-independent component, $I_{to1}$, was shown to be K$^+$ selective (594), and transient outward K$^+$ currents, referred to by various different laboratories as $I_{to}$, $I_{to1}$, or $I_t$ (42, 83, 436), have now been described in many cardiac cell types and in most species. Comparison of the detailed biophysical properties of the transient outward K$^+$ currents described in various cell types/species, however, suggested there might actually be two types of transient outward K$^+$ currents (42), and electrophysiological and pharmacological studies have now provided considerable support for this hypothesis. In adult mouse ventricular myocytes, for example, two transient K$^+$ currents, termed $I_{to,fast}$ ($I_{to,f}$) and $I_{to,slow}$ ($I_{to,s}$), have been distinguished (562). On membrane depolarization, mouse ventricular $I_{to,f}$ channels activate and inactivate rapidly, and on membrane repolarization, these ($I_{to,f}$) channels recover rapidly from steady-state inactivation (562). In the adult mouse, $I_{to,f}$ channels contribute importantly to the rapid repolarization of action potentials (194, 562) that is likely necessary to maintain the very high resting heart rates (~700 beats/min) in these animals. In humans and other larger mammals, $I_{to,f}$ underlies the early phase (phase 1) of repolarization in ventricular and atrial cells (Fig. 2) and likely also contributes to determining the plateau (phase 2).

Similar to $I_{to,f}$ mouse ventricular $I_{to,s}$ channels activate and inactivate rapidly (562). In contrast to $I_{to,f}$ however, $I_{to,s}$ channels recover very slowly (time constants of seconds) from (steady-state) inactivation and are functionally distinct from $I_{to,f}$ channels (196, 562). In addition, $I_{to,f}$ is readily distinguished from other K$^+$ currents, including $I_{to,s}$ (562), using the spider K$^+$ channel toxins Heteropoda toxin-2 or -3 (449). The distinct properties of $I_{to,f}$ and $I_{to,s}$ suggested that these currents reflect the functioning of two molecularly distinct Kv channels, and considerable evidence has now been provided to support this hypothesis (see sect. viC). Detailed comparisons of the properties of the transient outward K$^+$ currents expressed in other species, whether termed $I_{to}$, $I_{to1}$, or $I_t$, suggest that, in each case, these currents could also be classified as $I_{to,s}$ or $I_{to,f}$ based on the kinetics of current inactivation and recovery from steady-state inactivation, as well as by the differential sensitivities of the channels to the Heteropoda toxins. Although the properties of the transient K$^+$ currents in different cell types and species are similar and are amenable to classification as either $I_{to,f}$ or $I_{to,s}$ (Table 1), there are differences in the detailed biophysical properties of the ($I_{to,f}$ and $I_{to,s}$) currents in different cells/species (15). These observations suggest that there may well be subtle, albeit potentially important, molecular heterogeneity among $I_{to,f}$ and $I_{to,s}$ channels in different cell types and/or in different species (see sect. viC).

Although originally identified in Purkinje fibers, $I_{to,f}$ is a prominent repolarizing current in atrial and ventricular myocytes in most species (26, 58, 65, 69, 75, 79, 160, 178, 264, 294, 297, 500, 530, 545, 546, 578), including humans. Nevertheless, there are exceptions. In guinea pig ventricular cells, for example, $I_{to,f}$ has not been detected except when extracellular Ca$^{2+}$ is removed (224). In addition, $I_{to,f}$ is not detected in rabbit atrial or ventricular cells (156, 160, 183, 530). Nevertheless, there are transient Kv currents in rabbit myocytes (typically referred to as $I_t$), which inactivate slowly and recover from (steady-state) inactivation very slowly (160, 530). The properties of these currents, therefore, more closely resemble mouse ventricular $I_{to,s}$ than $I_{to,f}$ (562). Similar to the mouse, however, two distinct transient outward Kv currents have been described in the ventricles of other mammals (77, 178, 294, 500), including humans (225, 264, 545, 546), and the properties of these currents are quite similar to those of mouse ventricular $I_{to,f}$ and $I_{to,s}$ (79, 196, 562), permitting their classification as such (Table 1). In ferret, the rates of inactivation and recovery (from steady-state inactivation) of the transient Kv currents in myocytes isolated from the left ventricular (LV) endocardium are significantly slower than the currents in cells from the epicardial surface of the LV, suggesting the presence of two distinct transient Kv currents that are differentially expressed (77). Examination of the reported biophysical properties (77) suggests that the endocardial and epicardial LV currents can be classified as $I_{to,s}$ and $I_{to,f}$ respectively (Table 1). The distinct transient Kv currents in the epicardial, midmyocardial, and endocardial layers of canine (294, 500) and human (264, 545, 546) ventricles can also be appropriately referred to as $I_{to,s}$ or $I_{to,f}$ (Table 1).

Transient Kv currents that can be classified as $I_{to,f}$ (Table 1) have also been shown to be expressed in (rabbit) SAN cells, although, similar to Nav currents, $I_{to,f}$ densities vary markedly among (SAN) cells (213, 283). $I_{to,f}$ densities are higher, for example, in the larger cells isolated from the periphery, compared with the smaller cells in the center, of the SAN (213, 283). In addition, when expressed, $I_{to,f}$ appears to play a role in shaping action potential waveforms and in regulating automaticity in SAN cells (72, 213, 283). Cells isolated from the (rabbit) AVN also express $I_{to,f}$ (349, 361, 371), and detailed kinetic analysis of the currents reveals the presence of two components with distinct rates of inactivation and recovery (349). It is unclear whether these findings reflect differences in the kinetic properties of a single type of $I_{to,f}$ channel or if two distinct types of $I_{to}$ channels are expressed in (rabbit) AVN cells. Similar to the (rabbit) SAN,

Physiol Rev • VOL 85 • OCTOBER 2005 • www.prv.org
there is considerable heterogeneity in $I_{to}$ densities among (rabbit) AVN cells (349). In contrast to SAN cells (213, 283), however, the differences in $I_{to}$ densities are not correlated with cell size in AVN cells (349). Interestingly, and similar to findings in guinea pig atrial and ventricular cells, $I_{to,f}$ is not detected in guinea pig AVN cells (579). It is presently unclear, however, whether currents with properties similar to ventricular $I_{to,s}$ are expressed in conducting tissues in guinea pig heart. Owing to the marked differences in inactivation and recovery kinetics of $I_{to,f}$ and $I_{to,s}$ channels, however, the differential expression of these two channel types would be expected to have profound functional effects on the regulation of rhythmicity in the normal heart, effects that will be augmented in the diseased myocardium.

B. Delayed Rectifier Kv Currents

Myocardial delayed rectifier Kv currents, $I_K$, also first described in (sheep) Purkinje fibers (379), have been characterized in atrial and ventricular myocytes, as well as in pacemaker cells, isolated from a variety of different species, and, in most cases, multiple components of $I_K$ are coexpressed (Table 1). In guinea pig ventricular and atrial myocytes, for example, two prominent components of $I_K$, $I_{Kr}$ ($I_{Kr,rapid}$) and $I_{Ks}$ ($I_{Ks,slow}$), were first distinguished, based on marked differences in time- and voltage-dependent properties (216, 445, 446). Both $I_{Kr}$ and $I_{Ks}$ are also coexpressed in guinea pig AVN cells (579). Although $I_{Kr}$ activates rapidly, inactivates very rapidly, and displays marked inward rectification, no inward rectification is observed in ventricular and atrial $I_{Kr}$ or $I_{Ks}$ expression is heterogeneous and variable, might also reflect the fact that functional cardiac Kv channel expression is labile and might well be affected by the isolation methods, which typically involve the use of enzymes (578). Detailed studies focused on current characterizations in intact preparations, as well as on examining the effects of specific enzymes and cell isolation methods on Kv current densities and properties, will be needed to explore these various possibilities further.

Although $I_{Kr}$ and $I_{Ks}$ are not prominent repolarizing Kv currents in rodent atria or ventricles, there are other components of delayed rectification with time- and voltage-dependent properties distinct from $I_{Ks}$ and $I_{Kr}$ (Table 1) in myocytes from these (and other) species. In rat ventricular myocytes, for example, there are multiple delayed rectifier Kv currents that are coexpressed, and these are referred to as $I_{Ks}$, $I_{Klate}$ and $I_{ss}$ (26, 210, 561). In adult mouse ventricular myocytes, three distinct delayed rectifier Kv currents have also been separated and characterized (168, 196, 263, 291, 303, 560, 562, 586, 587), and these are referred to as $I_{Ks,slow1}$, $I_{Ks,slow2}$, and $I_{ss}$ (Table 1).

Multiple components of delayed rectification have also been described in rodent atrial myocytes (68, 69, 74, 75, 497). In both rat and mouse, it has been demonstrated that all the various delayed rectifier Kv current components contribute, together with $I_{to}$ channels, to (ventricular and atrial) action potential repolarization (291, 303, 560, 586, 587). It is interesting to note that a steady-state, noninactivating K+ current, which resembles $I_{ss}$ in rodent atria and ventricles, has also been described in human atrial myocytes (58).

In rat (74, 75), canine (577), and human (531–533) atrial myocytes, a novel, very rapidly activating, and largely noninactivating, outward Kv current, now typically referred to as $I_{Koltrapid}$ or $I_{Kur}$ (479), has been described (Table 1). In most species, $I_{Kur}$ is not detected in ventricular cells, and it seems likely that the expression and the properties of $I_{Kur}$, together with $I_{to}$, contribute to determining the more rapid repolarization evident in atrial, compared with ventricular, myocytes (Fig. 2). Importantly, as in most other species, $I_{Kur}$ is not expressed in human ventricular myocytes or in Purkinje fibers, suggesting that $I_{Kur}$ channels might represent a therapeutic target for the treatment of atrial arrhythmias without complicating effects on impulse propagation, ventricular functioning, or cardiac output (444). The potential of this pharmacological strategy, however, will have to be deter-
mined by the atrial specificity/selectivity of the drugs that are developed. In contrast to rat, canine, and human, Kv currents have been described in guinea pig (576) and mouse (168, 291, 587) ventricular myocytes that have biophysical properties very similar to human (rat or canine) atrial $I_{\text{Kur}}$. Indeed, the properties of the rapidly activating, $I_{\text{Kur}}$-like, current in guinea pig ventricular myocytes, referred to as $I_{\text{Kp}}$ (576), and the micromolar 4-AP-sensitive component of mouse ventricular $I_{\text{Ks slow1}}$, referred to as $I_{\text{Ks slow1}}$ (69, 291, 302, 587), are indistinguishable from human (canine and rat) atrial $I_{\text{Kur}}$. These currents should, therefore, probably be renamed $I_{\text{Kur}}$ (Table 1) to reflect the similarities in properties, as well as molecular identities of the channels underlying $I_{\text{Kp}}$ and $I_{\text{Ks slow1}}$ and $I_{\text{Kur}}$ (see sect. viD).

C. Regional Differences in Kv Current Expression and Properties

Although the properties of $I_{\text{to,f}}$ in different cardiac cells are similar (Table 1), there are marked regional differences in current densities. In humans (160, 367, 527, 542, 543) and in rats (26, 74, 75), for example, $I_{\text{to,f}}$ densities are significantly higher in atrial, compared with ventricular, myocytes. Similarly, in the rabbit, $I_{\text{to,a}}$ densities are higher in atrial myocytes and Purkinje fibers than in ventricular cells (75, 514). In the mouse, however, $I_{\text{to,f}}$ density is significantly higher in ventricular (79, 562), than in atrial (69), myocytes. The density of $I_{\text{to,f}}$ is also quite variable in sheep Purkinje fibers (520) and in different regions of the ventricles in canine (294, 297, 522), cat (178), ferret (77), human (367, 545, 546), mouse (79, 196, 562), and rat (107, 545) hearts. In canine (522) and mouse (79, 196, 562) heart, for example, $I_{\text{to,f}}$ density is higher in the right ventricle (RV), compared with the left ventricle (LV), and $I_{\text{to,f}}$ densities are lower in the base of the LV than in the LV apex (79). In addition, in canine and in human heart, $I_{\text{to,f}}$ density varies throughout the thickness of the ventricular walls, being severalfold higher in the epicardial and midmyocardial, than in the endocardial, layers (297, 546). In large mammals, the regional and cellular heterogeneities in $I_{\text{to,f}}$ densities are directly reflected in the differences in action potential waveforms in Purkinje, ventricular, and atrial cells (75, 107, 366, 520, 551). Within the ventricles, for example, the differences in $I_{\text{to,f}}$ densities are revealed by the presence and the appearance and depth of the “notch” in the initial phase (phase 1) of action potential repolarization (24, 364, 374; see Fig. 2).

There are also marked regional differences in the expression/distribution of $I_{\text{to,a}}$ in adult rat, mouse, human, and canine ventricles (77, 79, 196, 366, 367, 551, 562). In mouse RV and LV, for example, $I_{\text{to,a}}$ is undetectable, whereas cells in the interventricular septum express only $I_{\text{to,a}}$ or both $I_{\text{to,f}}$ and $I_{\text{to,a}}$ (79, 196, 562). Even when expressed, however, $I_{\text{to,f}}$ density is significantly lower in septum, compared with ventricular (or atrial), cells (79, 562). The densities of the delayed rectifier Kv currents, $I_{\text{Ks slow1}}$, $I_{\text{Ks slow2}}$, and $I_{\text{Ks}}$, in contrast, are similar throughout adult mouse ventricles (79, 196, 562). The main determinant of action potential heterogeneity in the mouse, therefore, appears to be the differential expression of $I_{\text{to,f}}$ (79, 196).

In larger mammals, including humans, the differential expression of $I_{\text{to,f}}$ is also a primary determinant of action potential heterogeneity (24, 374). In human heart, however, it is clear that differences in the expression levels of the various delayed rectifier Kv currents, as well as the persistent component of the Nav current (see sect. uA), also play important roles in regulating action potential heterogeneity (24, 374). In canine heart, for example, $I_{\text{Ks}}$ density is higher in cells in the RV, compared with the LV, whereas $I_{\text{Kr}}$ densities are similar in both chambers (24, 522). $I_{\text{Ks}}$ density is also higher in canine LV epicardial and endocardial cells than in M cells (24, 296). In guinea pig heart, $I_{\text{Kr}}$ and $I_{\text{Ks}}$ densities are approximately twofold higher in atrial, than in ventricular, myocytes (37, 216, 442, 524). There are also regional differences in functional $I_{\text{Ks}}$ and $I_{\text{Ks}}$ expression within the ventricles (80, 316). In cells isolated from the (guinea pig) LV free wall, for example, $I_{\text{Ks}}$ density is higher in subepicardial, than in either midmyocardial or subendocardial, myocytes (316). At the base of the LV, however, the densities of both $I_{\text{Kr}}$ and $I_{\text{Ks}}$ are significantly lower in endocardial, than in either epicardial or midmyocardial, cells (80). These differences clearly contribute to the marked differences in action potential waveforms and frequency-dependent properties in cells through the thickness of the ventricular wall (24). In addition to having a major impact on action potential repolarization, it is now very clear that differences in functional $I_{\text{Kr}}$ and $I_{\text{Ks}}$ densities are also expected to influence the maintenance of normal cardiac rhythms and the susceptibility to rhythm disturbances (24).

IV. OTHER MYOCARDIAL POTASSIUM CURRENTS CONTRIBUTING TO REPOLARIZATION

In addition to the depolarization-activated Kv currents, non-voltage-gated inwardly rectifying K$^+$ (Kir) currents, through $I_{\text{K1}}$ channels, also contribute to myocardial action potential repolarization, particularly in ventricular cells (232, 306, 377, 380). There are also other types of Kir channels that are expressed and are important in the normal functioning of the heart, although these do not seem to play important roles in action potential repolarization under normal physiological conditions (306, 377). One example of a functionally important class of myocardial...
dial Kir channels is the $I_{KATP}$ channels, which are inhibited by intracellular ATP, activated by nucleotide diphosphates, and thought to provide a link between cellular metabolism and membrane potential (232, 380). In the ventricular myocardium, the opening of $I_{KATP}$ channels is thought to be important under conditions of metabolic stress, as occurs during ischemia or hypoxia, and to result in shortening action potential durations and minimizing K$^+$ efflux (165, 232). The opening of $I_{KATP}$ channels has also been suggested to contribute to the cardioprotection resulting from ischemic preconditioning (141, 188). Although $I_{KATP}$ channels appear to be distributed uniformly in the RV and LV and through the thickness of the ventricular wall, these channels are expressed at much higher density than other sarcolemmal K$^+$ channels, suggesting that action potentials could be shortened markedly when only very small numbers of $I_{KATP}$ channels are activated (463).

Another important cardiac Kir channel type is the $I_{K(ACh)}$ channels, which are gated through a G protein-coupled mechanism mediated by muscarinic acetylcholine receptor activation (275, 564). Physiologically, $I_{K(ACh)}$ channels are activated by the binding of G protein βγ subunits in response to the acetylcholine released on vagal stimulation (414). Although $I_{K(ACh)}$ channels are expressed in AVN, SAN, atrial, and Purkinje cells, and are activated by acetylcholine released on vagal stimulation, these channels are not thought to contribute appreciably to action potential repolarization under normal physiological conditions. Consistent with this hypothesis, targeted deletion of one of the Kir subunits (Table 6) encoding $I_{K(ACh)}$ channels, Kir3.4, does not measurably affect resting heart rates (552). Interestingly, however, atrial fibrillation is not evident in Kir3.4 null mice exposed to the acetylcholine receptor agonist carbachol, suggesting that activation of $I_{K(ACh)}$ channels is involved in the cholinergic induction of atrial fibrillation (269).

As the “inward rectifier” terminology implies, Kir channels carry inward K$^+$ currents better than outward K$^+$ currents (306, 377). Nevertheless, it is the outward K$^+$ currents through these channels that are important physiologically because myocardial membrane potentials never reach values more negative than the K$^+$ reversal potential (approximately −90 mV). As a result, there is never an opportunity for the inward movement of K$^+$ currents through Kir (or any other K$^+$ selective) channels. At the macroscopic level, $I_{K_1}$ channels have been characterized in human (206, 514), guinea pig (133, 514), and rabbit (183, 381, 468) atrial and ventricular myocytes and in rabbit SAN cells (381). The properties of the $I_{K_1}$ channels in each of these preparations are similar in that all are K$^+$ selective, blocked by extracellular Ba$^{2+}$ and intracellular Cs$^+$ and strongly inwardly rectifying (183, 206, 223, 514). The strong inward rectification evident in cardiac $I_{K_1}$ channels is attributed to block by intracellular Mg$^{2+}$ (506), Ca$^{2+}$ (335), and polyamines (164, 307, 308). Removal/depletion of intracellular polyamines, Mg$^{2+}$ and/or Ca$^{2+}$, eliminates the steep inward rectification of (cardiac) $I_{K_1}$ channels and converts to a linear current-voltage relation (164, 307, 308, 335, 506).

The expression of $I_{K_1}$ is clearly reflected in the negative slope region (between approximately −50 and −10 mV) of the (total steady-state) myocyte conductance-voltage relation, which is prominent in ventricular myocytes, but is small or undetectable in atrial cells (183). The fact that the strongly inwardly rectifying $I_{K_1}$ channels conduct at negative membrane potentials suggests that these channels will play a role in establishing the resting membrane potentials of Purkinje fibers, as well as of atrial and ventricular myocytes. Direct experimental support for this hypothesis was provided with the demonstration that ventricular membrane potentials are depolarized in the presence of Ba$^{2+}$ (377), which blocks $I_{K_1}$ channels. In addition, action potentials are prolonged, and phase 3 repolarization is slowed in the presence of extracellular Ba$^{2+}$ (306), suggesting that $I_{K_1}$ channels also contribute to repolarization, particularly in the ventricular myocardium. The voltage-dependent properties of $I_{K_1}$ channels (306, 377), however, are such that the conductance is low at potentials positive to approximately −40 mV. Nevertheless, because the driving force on K$^+$ is markedly increased at depolarized potentials, these channels should contribute outward K$^+$ current during the phase 2 plateau and during phase 3 repolarization (Fig. 2). In contrast to atrial, ventricular, and Purkinje cells, $I_{K_1}$ density is low or undetectable in SAN and AVN cells (244, 381, 468). These observations, as well as the fact that pacemaker currents are expressed and functional in SAN and AVN cells, likely explain the findings that resting membrane potentials in these (SAN/AVN) cells are depolarized (significantly) and that the rising phases of the action potentials in these cells are less steep, relative to resting membrane potentials/action potentials in atrial and ventricular cells (Fig. 1).

Similar to the Kv channels, $I_{K_1}$ densities and the detailed biophysical properties of the currents do vary in different myocardial cell types. In human heart, for example, $I_{K_1}$ density is more than twofold higher in ventricular, than in atrial, cells (514). In guinea pig, the properties of the atrial and ventricular $I_{K_1}$ currents are also distinct in that ventricular $I_{K_1}$ inactivates during maintained depolarizations, whereas atrial $I_{K_1}$ does not (133, 223). In addition, changes in extracellular K$^+$ modulate the magnitude of ventricular $I_{K_1}$, but have little effect on atrial $I_{K_1}$ (223). At the macroscopic level, (guinea pig) atrial and ventricular $I_{K_1}$ channels are also distinct. Mean channel open times of ventricular $I_{K_1}$ channels, for example, are approximately five times longer than those of atrial $I_{K_1}$ channels, whereas the single atrial and ventricular $I_{K_1}$ channel conductances are indistinguishable (223). Taken
together, these observations suggested the interesting possibility that distinct molecular entities underlie ventricular and atrial $I_{K1}$ channels, and experimental support for this hypothesis has now been provided (133).

V. MOLECULAR COMPONENTS OF MYOCARDIAL NAV AND CAV CHANNELS

A. Nav Channel Pore-Forming α-Subunits

Voltage-gated Na$^+$ (Nav) channel pore-forming (α) subunits (Fig. 3A) belong to the “S4” superfamily of voltage-gated ion channel genes (93, 172, 174, 573). Nav α-subunits have four homologous domains (I to IV), each of which contains six transmembrane-spanning regions (S1-S6), and these four domains come together to form the Na$^+$-selective pore. Structure-function studies have revealed many of the important features of voltage-dependent Nav channel gating (91, 93). The cytoplasmic linker between domains III and IV, for example, has been shown to play a pivotal role in voltage-dependent Nav channel inactivation (392), and a critical isoleucine, phenylalanine, methionine (IFM) motif within this linker (91, 444) has been identified as an important molecular component of the inactivation gate (516, 517, 544). Voltage-dependent inactivation of Nav channels is attributed to the rapid block of the inner mouth of the channel pore by the cytoplasmic linker between domains III and IV that occurs within milliseconds of membrane depolarization (483). Consistent with the functional electrophysiological data, solution NMR analysis of this cytoplasmic linker peptide revealed a rigid helical structure positioned to block the pore (427).

Although there are a number of homologous Nav α-subunits (Table 2), Nav1.5 (SCN5A) is the prominent Nav α-subunit expressed in the mammalian myocardium, and this subunit encodes the rapidly activating and inactivating, tetrodotoxin (TTX)-insensitive Nav channels that underlie rapid (phase 0) depolarization in atrial and ventricular myocytes and in Purkinje fibers (Fig. 1). Nevertheless, several studies have demonstrated that mRNAs encoding other Nav α-subunits, notably Nav1.1, Nav1.3 (120, 426, 471), and Nav1.4 (590), which are typically considered the Nav α-subunits encoding brain and skeletal muscle Nav channels, respectively, are also expressed in the myocardium. In contrast to the Nav channels formed by Nav1.5, however, Nav1.1-, Nav1.3- and Nav1.4-encoded Nav channels are blocked by nanomolar concentrations of TTX (120, 426, 471, 590). In addition, although cardiac Nav currents are generally considered relatively TTX insensitive (174, 573), application of nanomolar concentrations of TTX has been reported to shorten canine Purkinje fiber action potential durations (113). These findings suggest a possible role for TTX-sensitive Nav channels in the generation of the persistent component of cardiac Nav currents, at least in canine Purkinje fibers. Nevertheless, there have been very few reports documenting the presence of TTX-sensitive inward Nav current components in cardiac cells, raising some concern about the functional significance of the expression data, in spite of the fact that the (message) expression levels of

---

**Table 2. Diversity of voltage-gated Na$^+$ (Nav) channel α- and β-subunits**

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Protein</th>
<th>Gene</th>
<th>Human</th>
<th>Mouse</th>
<th>Cardiac Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Navα1</td>
<td>Nav1.1</td>
<td>SCN1A</td>
<td>2q24</td>
<td>2C13</td>
<td>$I_{Na}$ (TTX)$^*$$^??$</td>
</tr>
<tr>
<td>Nav1.2</td>
<td>SCN2A</td>
<td>2q23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nav1.3</td>
<td>SCN3A</td>
<td>2q24</td>
<td>2C13</td>
<td>$I_{Na}$ (TTX)$^*$$^??$</td>
<td></td>
</tr>
<tr>
<td>Nav1.4</td>
<td>SCN4A</td>
<td>17q21</td>
<td>11E1</td>
<td>$I_{Na}$ (TTX)$^*$$^??$</td>
<td></td>
</tr>
<tr>
<td>Nav1.5</td>
<td>SCN5A</td>
<td>3p21</td>
<td>9F3</td>
<td>$I_{Na}$ (TTX-resistant)$^*$$^??$</td>
<td></td>
</tr>
<tr>
<td>Nav1.6</td>
<td>SCN6A</td>
<td>2q13</td>
<td>15F2</td>
<td>$I_{Na}$ (TTX)$^*$$^??$</td>
<td></td>
</tr>
<tr>
<td>Nav1.7</td>
<td>SCN9A</td>
<td>2q24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nav1.8</td>
<td>SCN10A</td>
<td>3p22</td>
<td>9F3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nav1.9</td>
<td>SCN11A</td>
<td>3p21</td>
<td>9F3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NavαX</td>
<td>Nav2.1</td>
<td>SCN6A</td>
<td>2q21-23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SCN7A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navβ</td>
<td>$β_1$</td>
<td>SCN1B</td>
<td>19p11</td>
<td>7A3</td>
<td>??</td>
</tr>
<tr>
<td></td>
<td>$β_2$</td>
<td>SCN2B</td>
<td>11q24</td>
<td></td>
<td>$I_{Na}$ (TTX-resistant)$^*$$^??$</td>
</tr>
<tr>
<td></td>
<td>$β_3$</td>
<td>SCN3B</td>
<td>11q26</td>
<td>9F3</td>
<td>$I_{Na}$ (TTX-resistant)$^*$$^??$</td>
</tr>
<tr>
<td></td>
<td>$β_4$</td>
<td>SCN4B</td>
<td>11q24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Boxes denote cardiac expression. * Major cardiac (TTX-resistant) Nav current in atria, ventricles, Purkinje fibers, and nodal cells. † TTX-sensitive, neuronal-like, Nav current.
Nav1.1, Nav1.3, and Nav1.4 in the myocardium appear to be quite high (120, 426, 471, 590).

It has also been reported that there are several Nav1 α-subunit proteins in addition to Nav1.5 in adult (mouse) myocardium (314). These include Nav1.1, Nav1.3, and Nav1.6 (314). The immunolocalization data also suggest that the Nav1.1, Nav1.3, and Nav1.6 α-subunits are localized in the t tubules in adult mouse ventricles (314), whereas Nav1.5 appears to be localized preferentially to intercalated disks in mouse, as well as in rabbit and rat, hearts (270, 315, 400). The subcellular localization of Nav1.5-encoded myocardial Nav channels at the intercalated disks has been interpreted as suggesting that these (Nav) channels play a major role in regulating conduction (270). Although the functional role(s) of t-tubular Nav channels in cardiac functioning has not been established, voltage-clamp studies have clearly demonstrated that TTX-sensitive Nav currents can be measured in whole cell recordings from adult mouse ventricular myocytes treated with β-scorpion toxin, which shifts the voltage dependence of activation of brain Nav channels, but does not affect cardiac (i.e., SCN5A-encoded) Nav channels (314). These observations have been interpreted as suggesting a distinct role for the neuronal Nav channels localized to the t tubules, i.e., linking depolarization of the sarcolemmal membrane with the t tubules, thereby coupling depolarization with excitation-contraction coupling (314). This hypothesis has important functional implications and certainly warrants further direct experimental testing.

Immunohistochemical studies have also provided evidence suggesting that Nav1.1 and Nav1.3, but not Nav1.5, are expressed in rat and mouse SAN (314). These findings suggest a substantive molecular difference between the SAN and the remainder of the myocardium in terms of Nav channel expression. Given the primary role of the SAN in regulating heart rate, it would seem certain that modulating the (TTX-sensitive) Na⁺ current in the SAN should impact heart rate. Nevertheless, exposure to TTX reportedly has no effect on heart rate in the mouse (314). In mice in which one copy of the SCN5A gene has been disrupted, however, conduction defects, as well as ventricular dysfunction, are evident (389), suggesting that SCN5A encodes most, if not all, of the cardiac Nav current. It seems reasonable to suggest, therefore, that additional studies focused on exploring the functioning of Nav1.1-, Nav1.3- and Nav1.6-encoded channels in the heart are warranted.

During the plateau phase of the action potential in human ventricular myocytes, ~99% of the Nav channels are in an inactivated, nonconducting state with the inactivation gate occluding the inner mouth of the conducting pore through specific interactions with sites on either the S6 segment (345) or the S4-S5 loop (346) of domain IV. Mutations in the linker between domains III and IV in SCN5A, linked to the LQT3 syndrome (Fig. 3A), disrupt Nav channel inactivation (52, 346). These “gain of function” mutations lead to an increase in the amplitude of the sustained component of the Na⁺ current (Fig. 4B) and to action potential prolongation (Fig. 4A). The enhanced...
inward current can be measured during sustained depolarizations and appears to reflect a change in (Nav channel) gating that results in channel “bursting” (52). As illustrated in Figure 4A, the increase in late inward Na\(^+\) current (due to Nav channel bursting) prolongs “modeled” cardiac action potentials (103, 105). Interestingly, action potential prolongation is also evident in genetically modified mice expressing human LQT3-associated mutant SCN5A Nav channels (384). In mice heterozygous for an SCN5A deletion at residues (KPQ) 1505–1507, a modification linked to LQT3, premature ventricular beats and pacing-induced ventricular tachycardia are also evident (384). Mutations in SCN5A are also linked to another (rare) inherited rhythm disorder, the Brugada syndrome (23, 41) and, similar to LQT3 mutations, a number of Brugada mutations in SCN5A have been identified (Fig. 4A). In contrast to long QT3, however, Brugada syndrome mutations are “loss of function” mutations in Nav1.5 and result in reduced Nav current (Fig. 4D) and lead to slowing of the action potential upstroke (Fig. 4C). Reductions in Nav current can also influence action potential amplitudes (Fig. 4C), as well as phase 1 repolarization. In addition, owing to intrinsic electrical heterogeneity of the heart (Fig. 1), the impact of Brugada and LQT3 mutations in SCN5A would be expected to be variable in different regions/cell types, an effect which may contribute further to arrhythmogenesis.

Other identified SCN5A mutations, linked to both the LQT3 and Brugada syndromes, are found in several other regions of SCN5A, most notably in the COOH terminus (Fig. 3A), that also lead to altered channel inactivation (60, 105, 358, 422, 519, 540). These findings should probably have been expected, given that structure-function studies suggest that multiple domains in Nav \(\alpha\)-subunits contribute to the regulation of channel gating (8, 48, 91, 105, 114, 174, 248, 254, 299, 357, 358, 521). A role for the COOH-terminal tail of Nav1.5 in the regulation of channel inactivation, for example, has been demonstrated (36, 51, 248, 251, 521). In addition, point mutations in the COOH terminus affect the kinetics and the voltage dependence of channel inactivation and recovery from inactivation and promote sustained channel activity (8, 48, 254, 299, 357). Single-channel studies have also demonstrated that the proximal portion of the COOH terminus has pronounced effects on repetitive channel openings during prolonged depolarization (114). Modeling studies of the COOH terminus of Nav1.5, assuming homology with the NH\(_2\) terminus of calmodulin, predict that the proximal region (of the COOH terminus) adopts an \(\alpha\)-helical structure, a prediction verified in circular dichroism studies on a purified COOH-terminal protein (114). The distal region of the COOH-terminal tail, in contrast, is largely unstructured and does not appear to affect channel gating measurably (114). These observations suggest that interactions occur between the structured (proximal) region of the COOH terminus of Nav1.5 and other components of the channel protein complex and that these interactions stabilize channels in the inactivated state (114). In addition, biochemical studies provide strong support for a model in which there is a direct physical interaction between the III-IV linker of Nav1.5 and the proximal, structured portion of the COOH terminus (358). Taken together, these findings suggest the formation of a molecular complex between these domains that is pivotal for channel inactivation and further that mutations in either the III-IV linker or the COOH-terminal tail disrupt this interaction and destabilize inactivation. In addition, the homology with calmodulin suggests that there may be structural similarities in the control of Nav and Cav channel gating, a hypothesis that clearly warrants direct experimental testing.

Analyses of additional mutations in SCN5A linked to LQT3, Brugada, and conduction system defects have provided further molecular insights into Nav channel functioning and arrhythmia mechanisms. One of the well-described LQT3 mutations, I1768V, for example, does not result in increased channel bursting, but rather, accelerates the rate of recovery of Nav channels from inactivation at diastolic membrane potentials (106). Computational analysis predicted that this mutation would have a substantial effect under nonequilibrium conditions, e.g., during action potential repolarization (106). Subsequent experiments confirmed this prediction, revealing a novel mechanism by which mutation-altered Nav channel gating can prolong cardiac action potentials (7). Interestingly, it has also been demonstrated that a common polymorphism (that results in an S/Y switch) at residue 1102 in SCN5A is associated with elevated arrhythmia risk in African Americans (481). Expression studies revealed that this variant results in very subtle changes in Nav1.5 channel activation and inactivation. Modeling studies suggest, however, that these changes are not likely to alter cellular electrical activity in carriers unless they are treated with drugs that block (cardiac) Kv channels (481). Additional polymorphisms in SCN5A have also been identified that affect, at least in heterologous expression systems, the trafficking of functional cell surface Nav channels (318, 570). In principle, the presence of these polymorphisms could, like the S1102Y polymorphism (481), impact arrhythmia susceptibility in the context of other factors (e.g., disease or drugs) that affect membrane excitability, action potential durations, and rhythmicity (318). Mutations in SCN5A and changes in Nav1.5-channel gating have also been linked to sudden infant death syndrome (358). In addition, mutations in two of the neuronal Nav channel \(\alpha\)-subunits, SCN1A and SCN2A, are associated with epilepsies (204, 220, 249, 359). It will be interesting to determine if the molecular mechanisms linking these mutations, as well as the mutations in the skeletal muscle Nav channel SCN4A, to disorders in membrane

Physiol Rev • VOL 85 • OCTOBER 2005 • www.prv.org
excitability are similar to those evident for SCN5A in the LQT3 and Brugada syndromes.

B. Nav Channel Accessory Subunits and Other Interacting Proteins

Although molecular and functional studies of cardiac Nav channels have focused primarily on the pore-forming Nav1.5 α-subunit, it is now quite clear that functional Nav channels in cardiac (and other) cells reflect the assembly of multimeric protein complexes comprising accessory subunits, as well as a variety of other auxiliary, interacting and regulatory proteins. All available evidence suggests, for example, that functional Nav channels in cardiac (and other) cells are multisubunit proteins consisting of a central pore-forming Nav α-subunit (Fig. 5) and one to two auxiliary Nav β-subunits (229). In brain, the functional stoichiometry appears to be one Nav α to two Nav β-subunits (229); the α/β-subunit composition of cardiac Nav changes is probably similar. Three different Nav β-subunit genes, SCN1b (230, 320), SCN2b (231, 241), and SCN3b (354) encoding Navβ1, Navβ2 and Navβ3 proteins, respectively, have been identified, and it appears that all three Nav β-subunits are expressed in heart (Table 2). The functional role(s) of the Nav β-subunits in the generation of cardiac Nav currents, however, is not well understood (20). Expression studies suggest that SCN2b plays a role in controlling the Ca\(^{2+}\) permeability of Nav channels (450). The targeted deletion of SCN2b (in Navβ2 \(-/-\) mice) markedly affects neuronal Nav channel expression and properties and has profound neurological consequences (95). No cardiac phenotype, however, has been described in Navβ2 \(-/-\) mice (95), suggesting that Navβ1 or Navβ3 more likely contributes to the formation of the SCN5A-encoded cardiac Nav channels. Consistent with this hypothesis, heterologous coexpression of Navβ1, which markedly affects Nav1.4-encoded skeletal muscle Nav channels (319), alters the inactivation kinetics and the densities of Nav1.5-encoded (cardiac) Nav channels (20, 134, 155). Heterologous coexpression of SCN3b with SCN5A also reportedly increases the cell surface density and modifies the inactivation kinetics of Nav1.5-encoded currents (155).

In addition to modifying the cell surface expression and the kinetic properties of Nav channels, Nav β-subunits also appear to be multifunctional cell adhesion molecules of the IgG superfamily (228) that target channels to the plasma membrane and mediate channel interactions with a variety of signaling molecules. It has been demonstrated, for example, that Nav β-subunits interact with cell adhesion molecules (252, 413), components of the extracellular matrix (413, 481, 559), and mediate the re-

![Molecular assembly of cardiac Cav (Nav), Kv, and Kir channels.](http://physrev.physiology.org/)

**FIG. 5.** Molecular assembly of cardiac Cav (Nav), Kv, and Kir channels. **Top:** the four domains (I–IV) of individual Cav (and Nav) α-subunits contribute to the formation of individual Cav (Nav) channels, whereas four Kv (or Kir) α-subunits combine to form tetrameric Kv (or Kir) channels. **Bottom:** schematic illustrating functional cardiac Cav, Nav, and Kv channels, composed of the pore-forming α-subunits and a variety of channel accessory subunits.
recruitment of the actin-binding protein ankyrin to the plasma membrane at points of cell-cell contact (321). The ankryns are (cytosolic) cytoskeletal proteins that have been suggested to function in regulating the trafficking of a variety of plasma membrane proteins (53, 54, 350). It has been demonstrated directly that the intracellular COOH terminus of Navβ1 mediates the interaction with ankyrin and that Navβ1 and ankyrin B associate in transfected cells and in rat brain membranes (135, 322). Interestingly, the intracellular COOH-terminal domain of Navβ1 has also been shown to be required for interaction with Nav1, specifically Nav1.2, α-subunits (347). Taken together, these observations suggest an important functional role for the cytosolic COOH-terminal domain of Navβ1 in the regulation of Nav channel trafficking and/or Nav channel localization, perhaps through ankyrin B (Fig. 6) and/or interactions with other components of the actin cytoskeleton (135).

It has long been recognized that the proper functioning of myocardial Nav channels requires an intact actin cytoskeleton (324, 504). Disruption of actin polymerization on treatment with cytochalasin D, for example, results in marked (~20%) reductions in peak Nav current densities in isolated rat and rabbit ventricular myocytes (504). Single-channel recordings from excised membrane patches from cytochalasin D-treated cells, however, revealed that, in addition to reduced open probability, channel “bursting” is increased (504). The latter effect is attributed to a change in Nav channel gating and is functionally similar to the alterations in channel activity seen with some long QT3 mutations (52, 60, 105, 346, 358, 419, 519, 540). These results suggest the interesting possibility that multiple pathways (mechanisms) may be important in mediating Nav channel gating in the normal and in the diseased myocardium.

An important functional role for the actin cytoskeleton in the regulation of Nav channel gating was directly revealed with the demonstration that mice heterozygous for a targeted deletion of ankyrin B, ankyrin B +/-, have abnormal cardiac electrical activity attributed to altered Nav channel gating and cell surface expression levels (40). Single-channel studies on ankyrin B +/- ventricular cells revealed increased channel bursting, consistent with a LQT channel phenotype (94). These observations were interpreted as suggesting that mutations in ankyrin, which would lead to Nav channel dysfunction, might well be important in familial LQT syndromes or other inherited cardiac rhythm disturbances (50). Consistent with this hypothesis, molecular genetic studies subsequently revealed a loss-of-function mutation in ankyrin B (E1425G) that is causally linked to variant 4 of familial long QT syndrome, LQT4 (351). In addition to providing fundamentally important new insights into the molecular defect underlying LQT4 (351), these findings demonstrate a novel, physiologically important, mechanism for regulating Nav functioning involving interactions between channel proteins and the cytoskeleton that are coordinated by the adaptor protein ankyrin B. It is possible that the ankyrin B mutations interfere directly with Navβ1-Nav1 interactions, or perhaps indirectly, through other regulatory and/or signaling molecules. In this context, it is also interesting to note that the cell surface expression of the Na+/K+-ATPase, the Na+/Ca2+ exchanger, and inositol 1,4,5-trisphosphate (IP3) receptors, each of which also interacts with ankyrin B, are all affected in ankyrin B +/- ventricular myocytes (351). It seems certain that the

FIG. 6. Schematic illustrating the complexity of protein-protein interactions that likely are involved in regulating/modulating the expression, distribution, and functioning of myocardial ion channels. The α- and β-subunits of Nav channels interact with the actin cytoskeleton through syntrophin-dystrophin and ankyrin B and with the extracellular matrix through the sarcoglycan complex. Interactions between Kv channel α (and/or β) subunits and the actin cytoskeleton are mediated by the actin binding proteins filamin and α-actinin and through PDZ domain-containing scaffolding proteins.
ankyrin B-mediated interactions between each of these molecules and the actin cytoskeleton are important for the normal functioning of each of these ion transport proteins, as well as the intracellular pathways coupled to these proteins. The physiological import of these interactions and the impact of the disruption of each of these interactions on the generation of normal cardiac rhythms is potentially staggering.

Extrapolating this concept further, it is interesting to note that there is now a growing body of evidence in the cardiovascular (and other) system that functional Nav (and other) channels interact directly and/or indirectly with the actin cytoskeleton and with a variety of regulatory and signaling molecules (Fig. 6) which might well play a role in the regulation of channel trafficking and channel expression and/or in the modulation of channel properties and functioning (418). It has, for example, been reported that syntrophins, proteins thought to provide a link between the actin cytoskeleton and other membrane-associated proteins, interact directly with Nav α-subunits, including Nav1.5 and the skeletal muscle Nav channel α-subunit Nav1.4 (182, 212). Because the syntrophins also interact directly with dystrophin and dystrobrevin (117) and, therefore, with the entire dystrophin-associated complex (151) in cardiac and skeletal muscle, it seems reasonable to suggest further that alterations in the properties of any of the protein components of this macromolecular complex (Fig. 5) could alter myocardial Nav channel functioning or expression. It is of further interest to note that cardiomyopathy is often evident in patients with congenital (skeletal) muscular dystrophies, attributed to mutations in the dystrophin gene (343), as well as to mutations in other genes that are part of the dystrophin-sarcoglycan protein complex (144). With the assumption that the model of functional cardiac Nav channels proposed in Figure 6 is at least qualitatively correct, it would seem reasonable to speculate that the mutations in any of the genes encoding any of the components of the depicted macromolecular complex could lead to altered Nav channel functioning and cardiac arrhythmias alone or in the background of other myocardial disease, particularly structural heart disease. If complexes such as those illustrated in Figure 6 are indeed shown to be the physiologically important units of cardiac Nav functioning, exploring the molecular mechanisms involved in mediating the many protein-protein interactions important in controlling the assembly, trafficking and functioning of these macromolecular channel protein complexes might well provide insights into the link between structural heart disease and the electrophysiological abnormalities that are linked to the generation of life-threatening cardiac arrhythmias in a wide variety of myocardial disease states.

Other potentially important signaling molecules in cardiac physiology and pathophysiology are also linked to syntrophin and, therefore, to Nav1.5 channels (Fig. 6). Nitric oxide synthase (NOS), for example, which is also part of the dystrophin-proteoglycan complex (189), and thought to play a role in myocardial ischemia (466), appears to bind directly to syntrophin, as well as to caveolin-3 (115, 207, 265), the muscle-specific caveolin in plasmalemmal caveoli. Nearly all of the endothelial NOS activity can be immunoprecipitated from cardiac muscle using an anti-caveolin-3 antibody (161, 162). The caveolins are also important regulators of NOS activity (161, 415), and it is of interest to note that overexpression of caveolin-3 results in cardiomyopathy and the downregulation of NOS and other components of the dystrophin-dystroglycan complex (27). Given that it has also been demonstrated that caveolin-3 binds directly to the cytoplasmic tail of β-dystroglycan (477), these observations suggest additional links between cardiac Nav channels, the actin cytoskeleton, and the extracellular matrix (Fig. 6). Caveolin-3 is thought to play a direct role in regulating the interaction of caveolins with the sarcolemmal membrane and, therefore, to function to facilitate the transfer of (Nav or other) channels to the cell surface membrane from the intracellular compartment (161). It seems reasonable to suggest here that alterations in the interactions between any of the individual components of the proposed functional Nav channel complex (Fig. 6), through acquired or inherited disease, could have profound effects on the properties and/or the functional cell surface expression of Nav channels, effects that in turn will impact the generation of normal cardiac rhythms and the likelihood that rhythm disturbances will occur.

In cardiac myocytes, functional cell surface Nav channel expression is also regulated directly by β-adrenergic receptor occupancy and the activation of the stimulatory G protein (G_s) pathway (310, 334). In addition, it has now been demonstrated that G_sα functions through binding to caveolin-3, increasing the presentation of caveolins to the sarcolemmal membrane which could lead to increased cell surface expression of Nav channels (569). It has also been reported that caveolin-3 expression is increased and that nitric oxide signaling is augmented in a (canine) pacing-induced model of heart failure (203). The relationship between these biochemical changes and the observed structural and electrical changes evident in this model and the relevance of this model and these changes to human heart failure remain to be established. As for heterologously expressed Nav channels, there may well also be additional regulatory molecules, including growth factors (295, 555) and membrane lipid-anchoring proteins (460), that play a role in regulating the properties and the functional cell surface density of cardiac Nav channels. Clearly, this possibility warrants direct experimental testing.
C. Cav Channel Pore-Forming α-Subunits

Similar to Nav channels, voltage-gated Ca\textsuperscript{2+} (Cav) channel pore-forming α-subunits belong to the “S4” superfamily of voltage-gated ion channel genes (92, 395), and functional voltage-gated Ca\textsuperscript{2+} (Cav) channels reflect the multimeric assembly of one Cav α-subunit (α\textsubscript{1}) and auxiliary Cavβ and Cavα\textsubscript{2}δ, as well at least in some cases, as Cavγ\textsubscript{3} subunits (Fig. 5). Also similar to Nav channels, Cav α-subunits comprise four homologous domains (domains I–IV), each of which is composed of six transmembrane segments (S1–S6), with an “S4” voltage-sensing domain and a Ca\textsuperscript{2+}-selective pore region between S5 and S6 (92, 395). Four distinct subfamilies of Cav channel pore-forming α-subunits, Cav1, Cav2, Cav3, and Cav4 (47), each with many subfamily members (Table 3), and alternately spliced transcripts (476) have been identified. The Cav α\textsubscript{1}-subunits are differentially expressed, and studies in heterologous expression systems have revealed that the various Cav α\textsubscript{1}-subunit genes encode Cav channels with distinct time- and voltage-dependent properties and pharmacological sensitivities. Heterologous expression of any one of the four members of the Cav subfamily, Cav1.1, Cav1.2, Cav1.3, or Cav1.4 (Table 3), for example, reveals L-type HVA Cav channel currents (92, 395), whereas heterologous expression of Cav3 α-subunits produces T-type LVA Cav channel currents (92, 395).

Numerous studies, exploiting both heterologous expression systems in vitro and transgenic strategies in vivo (363), have provided important (and new) molecular insights into Cav channel composition and functioning in cardiac (and other) cells. In the past decade, a number of mutations in Cav channel α- and β-subunit genes have also been identified in both humans and mice that result in disorders of excitability, such as epilepsy, ataxia, periodic paralysis, and migraine (153, 245, 388, 402, 410). Until very recently, there have been no established links between inherited disorders of myocardial membrane excitability and mutations in the subunits encoding cardiac Cav channels. It has now been demonstrated, however, that a de novo point mutation in the CACNA1C gene, which encodes the Cav1.2 channel α-subunit, underlies Timothy syndrome, a multisystem disorder with sporadic inheritance (479). Individuals with Timothy syndrome have profound cardiac arrhythmias, as well as dysfunc-

### Table 3. Diversity of voltage-gated Ca\textsuperscript{2+} (Cav) channel α- and β-subunits

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Protein</th>
<th>Gene</th>
<th>Human</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav1.1 (α\textsubscript{1.1}) (α\textsubscript{1I})</td>
<td>CACNA1S</td>
<td>1q31-32</td>
<td>1E4</td>
</tr>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav1.2 (α\textsubscript{1.1-2}) (α\textsubscript{1II})</td>
<td>CACNA1C</td>
<td>12p13.3</td>
<td>6E3</td>
</tr>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav1.3 (α\textsubscript{1.3}) (α\textsubscript{1III})</td>
<td>CACNA1D</td>
<td>3p14.3</td>
<td>14A3</td>
</tr>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav1.4 (α\textsubscript{1.4}) (α\textsubscript{1IV})</td>
<td>CACNA1F</td>
<td>Xp11.23</td>
<td>XA1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Protein</th>
<th>Gene</th>
<th>Human</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav2.1 (α\textsubscript{2.1}) (α\textsubscript{1a})</td>
<td>CACNA1A</td>
<td>19p13</td>
<td>8C3</td>
</tr>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav2.2 (α\textsubscript{2.2}) (α\textsubscript{1b})</td>
<td>CACNA1B</td>
<td>9q34</td>
<td>2A3</td>
</tr>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav2.3 (α\textsubscript{2.3}) (α\textsubscript{1c})</td>
<td>CACNA1E</td>
<td>1q25-31</td>
<td>1G1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Protein</th>
<th>Gene</th>
<th>Human</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav3.1 (α\textsubscript{3.1}) (α\textsubscript{1a})</td>
<td>CACNA1G</td>
<td>17q21</td>
<td>11D</td>
</tr>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav3.2 (α\textsubscript{3.2}) (α\textsubscript{1b})</td>
<td>CACNA1H</td>
<td>16p13.3</td>
<td>17A3.3</td>
</tr>
<tr>
<td>Cav\textsubscript{α}\textsubscript{1}</td>
<td>Cav3.3 (α\textsubscript{3.3}) (α\textsubscript{1c})</td>
<td>CACNA1I</td>
<td>22q13</td>
<td></td>
</tr>
</tbody>
</table>

**Boxes** denote cardiac expression.
dysfunction is seen in mice with a targeted disruption. Interestingly, however, it has been reported that sinus node HVA channels throughout the myocardium (92, 395). Invariant and 6 alternative exons (474), and the Cav1.2 subunit gene encodes the CACNA1C protein, including Cav1.2a, Cav1.2b, and Cav1.2c, are generated (6, 348, 474, 475). Interestingly, although nearly identical (>95%) in amino acid sequence, the various α1C splice variants appear to be expressed in different cells/tissues, and the cardiac specific isoform is Cav1.2a, which encodes cardiac L-type HVA Cav channels (6, 92). Thus similar to cardiac Nav channels and consistent with the similarities in the properties of the L-type cardiac Cav channel currents, it appears that a single pore-forming α1-subunit (Cav1.2a) is responsible for the generation of HVA channels throughout the myocardium (92, 395). Interestingly, however, it has been reported that sinus node dysfunction is seen in mice with a targeted disruption (363) of the Cav1.3 α-subunit gene, CACNAID (585), suggesting that SA nodal HVA Cav channels are encoded by Cav1.3, rather than by Cav1.2 (585). These observations raise the interesting possibility that, with the right tools, one would be able to manipulate selectively the functioning of atrial/ventricular (Cav1.2) and/or nodal (Cav1.3)-encoded myocardial Cav channels.

The recently identified linkage between Timothy syndrome and a point mutation in the CACNAIC gene encoding Cav1.2a (479) demonstrates, as likely would have been expected, that defective Cav channel (like defective Na and Kv channel) functioning can lead to cardiac arrhythmias. The Timothy syndrome mutation is a missense mutation that results in a single amino acid change, glycine (G) to arginine (R), at residue 406 (479), which is in the cytoplasmic loop between domains I and II, immediately C terminal to S6 transmembrane segment in domain I (Fig. 3). Heterologous expression studies reveal that the G406R mutation in Cav1.2a markedly reduces voltage-dependent channel inactivation, resulting in increased persistent inward Ca2+ current (479). The biophysical consequence of the Timothy syndrome mutation in Cav1.2a, therefore, is highly reminiscent of several Nav1.5 channel mutations associated with long QT and Brugada syndromes that result in increased persistent inward Na+ currents (and in action potential prolongation). Interestingly, however, in contrast to the LQT and Brugada syndromes, Timothy syndrome is a multisystem disorder (479). This latter observation is consistent with the hypothesis that Cav1.2a, unlike Nav1.5, is expressed widely and that mutations in Cav1.2a result in phenotypic consequences in many different organ systems.

D. Cav Channel Accessory Subunits and Other Interacting Proteins

There are a number of Cav accessory subunits that coassemble with Cavα1 (Fig. 5) and play a role in the generation of functional Cav channels in cardiac, as well as in other, cells. Three distinct types of Cav channel accessory subunits, Cavβ, Cavαδ, and Cavγ (Table 3), for example, have been identified (28, 110). Of these subunits, only the accessory Cavβ and Cavαδ subunits appear to be expressed in the myocardium (Table 3) and to contribute to the formation of functional cardiac Cav channels (110). The accessory Cav β-subunits are cytosolic proteins that assemble with Cav1 α-subunits and regulate the expression of functional cell surface HVA Cav channels, including cardiac L-type Cav channels. Four different Cavβ subunit-encoding genes, CACNB1, CACNB2, CACNB3, and CACNB4, which encode the Cavβ1 (408, 434), Cavβ2 (222, 396), Cavβ3 (89, 222, 396), and Cavβ4 (89, 505) proteins, respectively, have been identified (Table 3). It appears, however, that Cavβ3 is most prominently expressed in the heart (222, 396). In each Cavβ subunit, there are three variable regions flanking two highly conserved domains (89, 222, 396, 408, 403, 505). The variable regions are in the COOH termini, the NH2 termini, and a small (~100 amino acids) region in the center of the linear protein sequence between the two conserved domains (89, 222, 396, 408, 434, 505). The conserved domains of the Cav β-subunits mediate interactions with the pore-forming Cav α-subunits, whereas the variable domains influence the functional effects of Cav β-subunit coexpression on the properties of the resulting Cav channels (407). In heterologous expression systems, coexpression of Cav β-subunits with Cav α-subunits markedly increases Cav channel current amplitudes and densities (59, 187, 541, 565), effects which could reflect increased cell surface channel expression, increased channel open probability, and/or the stabilization of the Cavα1-Cavβ1 channel complexes in the cell membrane (98, 99, 565). In addition to increasing current amplitudes, coexpression of Cav β-subunits also modifies the kinetics and the voltage dependences of Cav current activation and inactivation (70, 242, 279, 353).

A highly conserved sequence motif in Cav α-subunits, called the alpha subunit interaction domain, appears to mediate α-subunit interaction(s) with accessory Cav β-subunits (407). The Cavα1 interaction domain (QxxExxxLxGYYxxWxxxE) is located in the cytoplasmic loop between domains I and II, exactly 24 amino acids from the S6 transmembrane region of domain I (63, 64, 132, 211). Interestingly, the Timothy syndrome mutation, G406R, is close to this subunit interaction domain (479), suggesting that the (G406R) mutations might disrupt Cavα1-Cavβ subunit-subunit interactions. Regions outside of this interaction domain, including low-affinity binding sites in
the COOH termini of the Cav α1-subunits, however, have also been suggested to participate in Cavβ-Cavα1 subunit-subunit interactions (412, 493, 523). Indeed, it now appears that these COOH-terminal regions in Cav α1-subunits also interact specifically with a second, highly conserved domain in the Cav β-subunits to produce the observed modulatory effects of accessory Cav β-subunit coexpression (131, 181).

In addition to the Cav β-subunits, another type of accessory subunit, referred to as Cavαβδ, has also been shown to be part of functional Cav channel complexes (146). Unlike Cavβ, the Cavαβδ subunits are transmembrane accessory subunits (Fig. 5), the first of which, Cavαβδ-1, was cloned from skeletal muscle (146). At least four different Cavαβδ-1 subunit-encoding genes, CACNA2D1, CACNA2D2, CACNA2D3, and CACNA2D4, have been identified (Table 3), and all produce heavily glycosylated proteins that are cleaved posttranslationally to yield αδ and δ proteins that then become linked via disulfide bridges (Fig. 5). In each of the Cavαβδ-1 complexes, the Cavαδ domain is located extracellularly, whereas the Cavδ domain, which has a large hydrophobic region, inserts into the membrane (Fig. 5) and serves as an anchor to secure the entire (Cavαδ) complex (197, 198, 554). In contrast to the accessory Cavβ subunits, the functional roles of accessory Cavαδ subunits are variable and appear to depend, at least in part, on the identities of the coexpressed Cavα1 and Cavβ subunits, as well as on the expression environment. In general, however, coexpression of Cavαβδ-1 shifts the voltage dependence of activation of Cavα/Cavβ-encoded channels, accelerates current activation and inactivation, and increases current amplitudes, compared with the channels/currents produced on expression of the Cavα1 and Cavβ subunits alone (38, 158, 197, 198, 260, 472). The increase in functional cell surface Cav current densities on coexpression of Cavαβδ-1 (and Cavβ) subunits appears to reflect improved targeting of Cav α1-subunits to the plasma membrane (469). This effect (improved targeting) is produced through interactions with Cavαδ, whereas the changes in channel kinetics are attributed to the presence of the Cavδ protein (469).

A distinct type of Cav channel accessory subunit was revealed with the identification of the Cavγ subunit, Cavγ1, that is expressed in mammalian skeletal muscle, and that contributes to the formation of functioning of skeletal muscle Cav channels (462). A number (seven) of additional Cavγ-encoding genes, CACNG1-CACNG8 (Table 3), have now been identified in skeletal muscle and in brain (250). All Cavγ subunits have four transmembrane spanning domains with the COOH and NH2 termini predicted to be intracellular (462). Coexpression of γ-subunits with various combinations of Cavα1 and Cavβ subunits has been shown to affect both the time- and the voltage-dependent properties of the resulting Cav currents (250). In addition, the Cavγ-subunits that are expressed in the nervous system, Cavγ2, Cavγ3, Cavγ4, and Cavγ5, all have COOH-terminal PDZ-binding domain motifs (250). These observations suggest the interesting possibility that the Cavγ-subunits play a role in controlling the localization and/or trafficking of functional Cav channels (250). Interestingly, it has also been reported that Cavγ2 interacts with the AMPA subtype of neuronal glutamate receptors, suggesting that the Cav γ-subunits may, like other channel accessory subunits, be multifunctional proteins (96). Although Cav γ-subunits appear to be widely expressed in the nervous system (250), it is not clear at present whether one or more of the CACNG genes is expressed in the heart and/or if these subunits play a functional role(s) in the generation of cardiac L-type Cav channels. Clearly, further studies focused on exploring this topic and determining directly the role(s) of the various Cav channel accessory subunits in the generation of cardiac Cav channels are warranted.

As noted in section μβ, it is now very well documented that HVA myocardial Cav channels undergo rapid Ca2+- and voltage-dependent inactivation (44, 166, 281, 326). Fundamentally important insights into the likely molecular mechanism underlying the Ca2+-dependent component of inactivation were revealed with the demonstration that the EF-hand domain containing protein, calmodulin, that binds Ca2+ and modulates a variety of Ca2+-dependent processes, is associated with the COOH-terminal cytoplasmic domain of L-type HVA channels (318). Several subsequent studies have provided many of the molecular details of the calmodulin/Cav channel α1-subunit association and interaction domains (17, 149, 293, 355, 428). In addition, the generality of the calmodulin-mediated mechanism of Ca2+-dependent inactivation of Cav channels was documented with the demonstration that P/Q-type neuronal HVA channels are also regulated by calmodulin binding (128).

In addition to the regulation of channel gating by Ca2+ and calmodulin, the properties and the functional expression of myocardial Cav channels are also regulated by a variety of extracellular signals and intracellular signaling pathways. Prominent among these are the rather well studied β-adrenergic G protein-coupled receptor-mediated augmentation of cardiac L-type Cav channel currents, increased Ca2+ entry, and positive inotropy (255, 509). Considerable experimental evidence suggests that the pore-forming Cav1.2 α-subunit and Cav β-subunits are targets of posttranslational modifications by a variety of protein kinases that impact the functional cell surface expression and the properties of cardiac L-type Cav channels (255, 509). It has also been reported that cardiac HVA Cav channels are actually associated with β-adrenergic receptors in macromolecular complexes that likely also include heterotrimeric G proteins, adenylate cyclase, protein kinases, phosphatases and protein kinase A binding.

Physiol Rev • VOL 85 • OCTOBER 2005 • www.prv.org
proteins, or AKAPs (18, 122), which appear to subserve a scaffolding function (18). Unlike Nav channels, however, no direct links between cardiac Cav channel subunits and actin or actin-binding proteins have been demonstrated to date. Nevertheless, a number of Ca\textsuperscript{2+}-binding proteins, including calmodulin, have been shown to be linked both directly and indirectly to the actin cytoskeleton (452). In addition, it has been reported that the time- and voltage-dependent properties of L-type HVA channels are altered in skeletal muscle from dystrophin-deficient animals, an effect interpreted as resulting from remodeling of the subcellular actin cytoskeleton (111). Similarly, neuronal HVA Cav channel inactivation is differentially affected by agents that stabilize and destabilize the actin cytoskeleton (239). In retinal ganglion neurons, for example, stabilization or disruption of the actin cytoskeleton affects functional cell surface Cav channel expression (454). It seems reasonable to suggest, therefore, that Cav channels interact directly or indirectly with the actin cytoskeleton (556) and that, similar to cardiac Nav channels, the functioning of myocardial Cav channels likely also depends importantly on interactions with the actin cytoskeleton. In support of this hypothesis, targeted deletion of endothelial NOS eliminates the muscarinic modulation of myocardial L-type Cav channels (202). Further investigations focused on delineating the molecular mechanism controlling functional Cav channel expression will be needed to define the role of the cytoskeleton in regulating myocardial Cav (and Nav) channel expression and functioning.

VI. MOLECULAR COMPONENTS OF MYOCARDIAL KV CHANNELS

A. Kv Channel Pore-Forming \( \alpha \)-Subunits

Similar to Nav and Cav \( \alpha \)-subunits, voltage-gated K\textsuperscript{+} channel (Kv) pore-forming (\( \alpha \)) subunits (Fig. 3) belong to the “S4” superfamily of voltage-gated channels (405). In contrast to Nav and Cav channel \( \alpha \)-subunits, however, Kv \( \alpha \)-subunits are six transmembrane-spanning domain proteins (Fig. 3, B and C), and functional Kv channels comprise four \( \alpha \)-subunits (Fig. 5). A very large number of Kv \( \alpha \)-subunit genes have been identified, and a systematic terminology for naming these subunits (Table 4) has been developed (199). Heterologous expression of Kv \( \alpha \)-subunits in the Kv1-Kv4 subfamilies reveals functional Kv channels with distinct time- and voltage-dependent properties (116), whereas Kv \( \alpha \)-subunits of the Kv5–9 subfamilies (Table 4) are electrically silent (88, 142, 221, 441). Coexpression of Kv5-Kv9 subunits with Kv2 \( \alpha \)-subunits, however, attenuates the amplitudes of the Kv2-encoded K\textsuperscript{+} currents (441). Nevertheless, the functional roles of the “silent” Kv \( \alpha \)-subunits in the generation of myocardial Kv channels remains to be determined.

Further functional Kv channel diversity in cardiac and other cells could, in principle, arise through alternative splicing of transcripts (29), as well as through the formation of heteromultimeric channels (116) between two or more Kv \( \alpha \)-subunit proteins in the same Kv subfamily. Kv channel assembly, as well as the properties of the resulting channels, are largely determined by the intracellular NH\textsubscript{2}- and COOH-terminal \( \alpha \)-subunit domains (100). Molecular and biochemical studies have revealed that, of the many Kv1-Kv9 \( \alpha \)-subunits identified, only a small subset is expressed in the heart (Table 4). Although many studies have characterized the detailed time- and voltage-dependent properties of the various Kv \( \alpha \)-subunit-encoded Kv channels in heterologous expression systems, these studies have provided little insight into the molecular correlates of functional cardiac Kv channels. The difficulties encountered in these studies probably reflect the fact that Kv channel properties depend on the expression environment (397), likely owing to cell-type specific differences in posttranslational processing of the Kv channel \( \alpha \)-subunit proteins and/or the expression of Kv channel accessory subunits or other Kv channel interacting, regulatory proteins (397).

Additional subfamilies of Kv \( \alpha \)-subunit genes in the KCNQ and KCNH subfamilies (Table 4) have been identified, and one member of each of these subfamilies, KCNQ1 and KCNH2, has been shown to be the loci of mutations leading to congenital long QT syndromes, LQT1 (Fig. 3C) and LQT2 (Fig. 3B), respectively (40, 119, 447, 448, 499, 528). Heterologous expression of human KCNH2, which encodes the ether-a-go-go-related protein ERG1, reveals Kv currents (448, 499) that are similar to cardiac I\textsubscript{Kr} (Table 4). Similar to SCN5A mutations linked to the LQT3 and Brugada syndromes (Fig. 3A), LQT2 mutations in KCNH2 are found throughout the ERG1 protein sequence (Fig. 3B). These (LQT2) mutations are all “loss of function” and result in reduced functional I\textsubscript{Kr} channel expression owing to dominant negative effects or to alterations in channel processing or trafficking (23, 126, 220, 246, 253, 273, 584). Interestingly, a novel “gain of function” mutation in KCNH2, which results in increased I\textsubscript{Kr} channel densities, has recently been identified and linked to one form of short QT syndrome (78).

There are six additional members of the KCNH subfamily, KCNH3–KCNH8 (Table 4). Two of these, KCNH3 and KCNH4, appear to be nervous system specific (465), and it is presently unclear whether any of the others are present in the myocardium. Alternatively processed forms of KCNH2, with unique NH\textsubscript{2} and COOH termini, however, have been cloned from both mouse and human heart cDNA libraries and postulated to contribute to the generation of functional cardiac I\textsubscript{Kr} channels (272, 282, 304). Indeed, coexpression of the NH\textsubscript{2}-terminal splice variant ERG1b with the full-length ERG1a produces Kv currents that more closely resemble cardiac I\textsubscript{Kr} than the currents
produced on expression of ERG1a alone (304). Although it has been reported that only the full-length ERG1 protein(s) are detected in adult rat, mouse, and human hearts (404), raising some doubts about the functional significance of alternative splicing of \( \text{KCNH2} \) transcripts, more recent studies have identified ERG1b protein in adult rat, human, and canine heart (240). Presumably, these disparate results reflect the fact that different anti-ERG1 antibodies were used (240, 404). Further studies will be needed to explore this and other possible explanations.

Using antibodies targeting the specific ERG1 isoforms, it was also demonstrated that ERG1a and ERG1b coimmunoprecipitate from heart, suggesting that functional cardiac \( I_{Kr} \) channels reflect the heteromeric assembly of ERG1a and ERG1b subunits (240). The expression, distribution, and functioning of the COOH-terminal variant of ERG, ERG-USO (272), in contrast, remains to be explored.

As noted previously, mutations in the \( \text{KCNQ1} \) gene have been linked to LQT1 (253, 329). Heterologous expression of KvLQT1 (\( \text{KCNQ1} \)) alone yields rapidly activating and noninactivating outward \( \text{K}^+ \) currents, whereas coexpression with the \( \text{K}^+ \) channel accessory subunit,

### Table 4. Diversity of voltage-gated \( \text{K}^+ \) (\( \text{Kv} \)) channel \( \alpha \)-subunits

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Protein</th>
<th>Gene</th>
<th>Locus</th>
<th>Human</th>
<th>Mouse</th>
<th>Cardiac Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Kv}1 )</td>
<td>( \text{Kv}1.1 ) ( \text{KCNA1} )</td>
<td>12p13</td>
<td>6F2</td>
<td>( I_{\text{K}<em>{\text{slow}}} ) ( (I</em>{\text{K},\text{DTX}}) )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}1.2 )</td>
<td>( \text{KCNA2} )</td>
<td>1p11</td>
<td>3F2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}1.3 )</td>
<td>( \text{KCNA3} )</td>
<td>1p21</td>
<td>3F2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}1.4 )</td>
<td>( \text{KCNA4} )</td>
<td>11p14</td>
<td>2E2</td>
<td>( I_{\text{to,as}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}1.5 )</td>
<td>( \text{KCNA5} )</td>
<td>12p13</td>
<td>6F2</td>
<td>( I_{\text{Kur}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}1.6 )</td>
<td>( \text{KCNA6} )</td>
<td>12p13</td>
<td>6F2</td>
<td>( I_{\text{K}_{\text{slow1}}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}1.7 )</td>
<td>( \text{KCNA7} )</td>
<td>19q13</td>
<td>7B3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}1.10 )</td>
<td>( \text{KCNA10} )</td>
<td>1p11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}2 )</td>
<td>( \text{Kv}2.1 ) ( \text{KCNB1} )</td>
<td>20q13.1</td>
<td>2H3</td>
<td>( I_{\text{K}_{\text{slow2}}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}2.2 )</td>
<td>( \text{KCNB2} )</td>
<td>8q13</td>
<td>??</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}3 )</td>
<td>( \text{Kv}3.1 ) ( \text{KCNC1} )</td>
<td>11p15</td>
<td>7B3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}3.2 )</td>
<td>( \text{KCNC2} )</td>
<td>12q21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}3.3 )</td>
<td>( \text{KCNC3} )</td>
<td>19q13.4</td>
<td>7B2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}3.4 )</td>
<td>( \text{KCNC4} )</td>
<td>1p11</td>
<td>3F2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}4 )</td>
<td>( \text{Kv}4.1 ) ( \text{KCND1} )</td>
<td>Xp11.2</td>
<td>??</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}4.2 )</td>
<td>( \text{KCND2} )</td>
<td>7q32</td>
<td>6A2</td>
<td>( I_{\text{to,f}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}4.3 )</td>
<td>( \text{KCND3} )</td>
<td>1p11</td>
<td>3F2.2</td>
<td>( I_{\text{to,f}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}5 )</td>
<td>( \text{Kv}5.1 ) ( \text{KCNF1} )</td>
<td>2p25</td>
<td>??</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}6 )</td>
<td>( \text{Kv}6.1 ) ( \text{KCNG1} )</td>
<td>20q13.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}6.2 )</td>
<td>( \text{KCNG2} )</td>
<td>18q23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}6.3 )</td>
<td>( \text{KCNG3} )</td>
<td>2p21</td>
<td>17E3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Kv}6.4 )</td>
<td>( \text{KCNG4} )</td>
<td>16q24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Boxes denote cardiac expression.
minK (see sect. vB), produces slowly activating K+ currents that resemble the slow component of cardiac delayed rectification, I_{Ks} (40, 447). Similar to the SCN5A mutations linked to the LQT3 and Brugada syndromes (Fig. 3A) and KCNH2 mutations linked to LQT2 (Fig. 3B), KCNQ1 mutations linked to LQT1 have been identified throughout the protein sequence (Fig. 3C). Expression studies suggest that the various LQT1-associated mutations in KCNQ1 are all loss of function, resulting in reductions in functional I_{Ks} cell surface channel expression. Simulations demonstrate that reductions in I_{Ks} density (Fig. 7B) result in markedly prolonged ventricular action potential waveforms (Fig. 7A). Given the intrinsic heterogeneities in I_{Ks} (and other) channel densities and action potential waveforms throughout the myocardium (Fig. 1), the effects of LQT1 mutations in KCNQ1 might also be heterogeneous, further impacting the arrhythmogenic potential of these mutations. Importantly, a novel “gain of function” mutation in KCNQ1 (V307L) was identified and linked to short QT interval syndrome (46). Heterologous expression studies revealed that expression of KCNQ1 V307L, alone or with wild-type KCNQ1, in the presence of KCN2, produces I_{Ks}-like currents with markedly altered activation kinetics and voltage-dependent properties (46) relative to the channels produced by wild-type KCNQ1 and KCN2. A gain of function mutation (S140G) in KCNQ1 has also been identified in a family with hereditary persistent atrial fibrillation (97). Computer simulations incorporating the KCNQ1 short QT mutant channels reveal that I_{Ks} densities are increased (Fig. 7D) and that action potentials are shortened markedly (Fig. 7C). As noted above for LQT1 mutations, the impact of gain of function mutations in KCNQ1 on different cell types and regions of the heart will likely be heterogeneous, owing to the existing heterogeneity in I_{Ks} (and other current) densities and action potential waveforms, an effect which may acerbate the arrhythmogenic potential of alterations in I_{Ks} densities.

Similar to the multiplicity of α-subunits in the Kv and the KCNH subfamilies, there are a number (four) of additional members of the KCNQ subfamily (Table 4), although none of these appears to be expressed in heart. Two of the KCNQ subfamily members, KCNQ2 and KCNQ3, however, are expressed in the nervous system and have been identified as loci of mutations leading to benign familial neonatal convulsions (65, 329, 453, 526). Heterologous expression of KCNQ2 or KCNQ3 results in the generation of slowly activating, noninactivating K^+-selective channels that also deactivate very slowly on membrane repolarization (329, 453, 526). Interestingly, the properties of the heteromeric KCNQ2/KCNQ3 channels closely resemble neuronal muscarinic acetylcholine receptor regulated ion channel currents, typically referred to as “M” currents/channels (329, 526).

FIG. 7. Simulations reveal the effects of loss of function (LQT1) and gain of function (short QT) mutations in KCNQ1. Steady-state action potential waveforms (A and C) and outward I_{Ks} currents (B and D) were simulated (103, 105). Control action potential and current waveforms simulated for wild-type KCNQ1-encoded I_{Ks} currents are depicted as the solid black lines in A–D. The corresponding simulated voltage and current waveforms depicting the effects of KCNQ1 mutations are illustrated by the dashed purple (LQT1) and red (short QT) lines in A–D.

“Loss of function” LQT1 mutations (Fig. 3C), resulting in a decrease in the maximum amplitude and a slowing of the time to peak of I_{Ks} (B), lead to marked action potential prolongation (A). In contrast, “gain of function” short QT mutations in KCNQ1 increase the maximal amplitude of I_{Ks} (D) and shorten action potential durations.

B. Kv Channel Accessory Subunits

Similar to the Nav and Cav channels, a number of different types of Kv channel accessory subunits have been identified (Table 5) and postulated to contribute to the generation of functional myocardial Kv channels. The
Cardiac and non-car diac cell lines generally express multiple subunits of the fast and slow Kv channels. The combination of subunits determines the properties of the channels, including their voltage dependence, pharmacology, and sub-cellular localization. Biochemical studies have suggested that the physiological significance of this subunit in the regulation of myocardial membrane currents was clearly demonstrated with the identification of mutations in KCNE1 that are associated with one type of inherited long QT syndrome, LQT5 (62, 478, 481).

Several additional members of the minK-related peptide, MiRP (KCNE), subfamily (Table 5) have also been identified and characterized in coexpression studies with Kv α-subunits (1–3, 339). One of these, MiRP1 (KCNE2), has been suggested to function as an accessory subunit of ERG1 (KCNH2) to generate cardiac Ikr (1, 5). As noted above, however, it has previously also been suggested that minK associates with ERG1 to produce IKr channels (341). Although the resolution of this seeming controversy must await further experimentation, particularly biochemical studies, it is reasonable to conclude that MiRP1 (KCNE2) is functionally important in the regulation of myocardial membrane excitability, as evidenced by the fact that KCNE2 variants are associated with sporadic and drug-induced long QT syndromes (5, 227, 457, 478). It is also interesting to note that studies in heterologous systems have revealed that the MiRP subfamily of Kv channel accessory subunits can assemble with Kv α-subunits in several different subfamilies (4, 583) to modify the properties and/or the cell surface expression of Kv α-subunit-encoded channels. Heterologous expression studies, for example, have shown that MiRP1 also interacts with Kv4 α-subunits (583). In addition, it has been demonstrated that MiRP2 (KCNE3) forms Kv channels with Kv3.4 α-subunits in skeletal muscle and that mutations in MiRP2 result in reduced Kv3.4-encoded Kv current densities, membrane depolarization, and periodic paralysis (4). Biochemical and coexpression studies have also suggested that MiRP1 can associate with hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels, suggesting a distinct function for the MiRP1 subunit in the regulation of myocardial pacemaker currents, rather than, or in addition to, the regulation of myocardial Kv channels (574). Taken together, these observations suggest the interesting possibility that members of the KCNE subfamily might be multifunctional proteins, coassembling with several different Kv and/or other (e.g., HCN) ion channel pore-forming α-subunits and contributing to the formation of multiple types of cardiac Kv (and other Kv?) channels. Experiments focused on testing this hypothesis and on defining the functional roles of the various MiRP (KCNE) subunits in the generation of myocardial Kv (and other) channels will be of considerable interest.

Another type of Kv channel accessory subunit was revealed with the biochemical identification (360) and

**Table 5. Auxiliary Kv channel subunits**

<table>
<thead>
<tr>
<th>Family</th>
<th>Subunit</th>
<th>Gene</th>
<th>Human</th>
<th>Mouse</th>
<th>Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kvβ</td>
<td>Kvβ1</td>
<td>KCNAB1</td>
<td>3q25</td>
<td>3D</td>
<td>??</td>
</tr>
<tr>
<td></td>
<td>Kvβ2</td>
<td>KCNAB2</td>
<td>1p36.3</td>
<td>4E2</td>
<td>??</td>
</tr>
<tr>
<td></td>
<td>Kvβ3</td>
<td>KCNAB3</td>
<td>17p13</td>
<td>11B3</td>
<td></td>
</tr>
<tr>
<td>KCNE</td>
<td>Mink</td>
<td>KCNE1</td>
<td>21q22</td>
<td>16C4</td>
<td>I_k</td>
</tr>
<tr>
<td></td>
<td>MiRP1</td>
<td>KCNE2</td>
<td>21q22</td>
<td>16C4</td>
<td>I_kr,??, I_lo,??</td>
</tr>
<tr>
<td></td>
<td>MiRP2</td>
<td>KCNE3</td>
<td>11q13</td>
<td>7E1</td>
<td>??</td>
</tr>
<tr>
<td></td>
<td>MiRP3</td>
<td>KCNE4</td>
<td>2q36.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MiRP4</td>
<td>KCNE5</td>
<td>Xq22</td>
<td>XF1</td>
<td></td>
</tr>
<tr>
<td>KChAP</td>
<td>KChAP</td>
<td>PIAS3</td>
<td>1q12</td>
<td>3F1</td>
<td>I_lo,??, I_kr??</td>
</tr>
<tr>
<td>KCNP</td>
<td>ChhP1</td>
<td>KCNIP1</td>
<td>5q35</td>
<td>11A4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ChhP2</td>
<td>KCNIP2</td>
<td>10q25</td>
<td>19C3</td>
<td>I_lo, others??</td>
</tr>
<tr>
<td></td>
<td>ChhP3</td>
<td>KCNIP3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ChhP4.2</td>
<td>CSEN</td>
<td>2q11.1</td>
<td>2F1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ChhP4.3</td>
<td>KCNIP4</td>
<td>4p15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCS</td>
<td>NCS-1</td>
<td>FREQ</td>
<td>9q34</td>
<td>2A3</td>
<td>I_lo, others??</td>
</tr>
</tbody>
</table>

Boxes denote cardiac expression.

First of these was cloned from human (362), and later from rat (170), heart and was referred to as minK, i.e., “minimal K+” channel subunit. MinK, which is encoded by the KCNE1 gene on chromosome 21 in human (Table 5), is a small (130 amino acids) protein with a single transmembrane spanning domain (170, 288, 362). Although initial characterization of minK in Xenopus oocytes suggested that this small protein could produce functional Kv channels when expressed alone (170, 362), subsequent studies demonstrated the presence of a KCNQ1 homolog in oocytes that combines with the heterologously expressed minK to generate Kv channels that very closely resemble cardiac IKs (447). As noted above, these observations have led to suggestions that minK coassembles with the KvLQT1 protein to produce functional cardiac IKs channels (40, 447). It has also been reported, however, that minK coassembles with the ERG1 protein in heterologous expression systems, observations interpreted as suggesting a role for minK in the generation of cardiac Ikr channels (341). It has become increasingly clear, however, that accessory Kv subunits, such as minK, can, at least in heterologous expression systems, associate with multiple different Kvα subunits. It is not a given, however, that these interactions occur in intact tissues. As a result, it is presently unclear whether minK subunits actually associate with both KvLQT1 and ERG1 (and other Kv?) α-subunits in the myocardium and contribute to the function of both cardiac IKs and Ikr (and other Kv?) channels. Biochemical studies focused on exploring these questions are clearly warranted. Although the details of minK functioning remain to be clarified, it is important to emphasize that the physiological significance of this subunit in the generation of cardiac membrane currents was clearly demonstrated with the identification of mutations in KCNE1 that are associated with one type of inherited long QT syndrome, LQT5 (62, 478, 481).
subsequent cloning (419) of low molecular mass (<45 kDa) cytosolic Kv β-subunits from brain. Three distinct, but homologous, Kv β-subunits, Kv β1, Kv β2, and Kv β3, encoded on three different (KCNAB) genes (Table 5), as well as alternatively spliced transcripts (337), have now been identified (90, 124, 147, 148, 317, 352). Of these, Kvβ1.1, Kvβ1.2, Kvβ1.3, and Kvβ2 have been shown to be expressed in the heart (12, 90, 124, 147, 148, 278, 317, 352). The Kv β-subunits share a conserved COOH-terminal “core” region, related in amino acid sequence to aldotetone reductases, members of the triose phosphate isomerase enzyme family (101, 338). The NH2-terminal domains of the Kv β-subunits are unique, and members of the Kvβ1 subfamily, as well as Kvβ3.1, have long NH2-terminal sequences that are structurally similar to the Shaker (Kv1) channel inactivation gate that functions in a “ball and chain”-like mechanism (217) to accelerate Kv1 channel inactivation (300 301). Although the core region of the Kv β-subunits contains a NAPH/NADPH binding site (101, 338) and Kv β-subunits crystallize with NADPH bound (191), the role of this binding in regulating the functional interactions between Kv β- and Kv α-subunits and/or in controlling the expression/properties of the resulting Kv channels has not been defined.

Previous studies have demonstrated that the Kv β-subunits interact with the intracellular T1 tetramerization domain of the Kv α-subunits of the Kv1 subfamily, combining in a 1:1 stoichiometric ratio (190, 192). Heterologous coexpression studies have revealed that Kv β-subunits affect the functional properties and the cell surface expression of Kv1 α-subunit-encoded channels (9, 10, 90, 147, 148, 317, 352, 464). In some cases, the functional consequences of Kvβ coexpression have been shown to be Kv1 α-subunit specific (10). Coexpression of Kvβ2, for example, increases the expression of Kv1.2-encoded channels and decreases the expression of Kv1.5-encoded channels (9, 10). Because Kv1α and Kvβ subunits coassemble in the endoplasmic reticulum (369), the observed increases in functional cell surface Kv1-encoded channel expression suggest that the Kv β-subunits affect Kv1 channel assembly, processing or stability or, possibly, function as chaperone proteins.

The facts that the Kv β-subunits were originally identified in association with Kv1 α-subunits (360) and that heterologous expression studies suggest that Kvβ1 and Kvβ2 interact only with the Kv1 α-subunits (370, 458) have been interpreted as suggesting that the Kv β-subunits function as specific accessory subunits in the generation of Kv channels encoded by Kv1 α-subunits. In expression systems, however, Kvβ3 has also been shown to interact specifically with Kv2 subunits (167). Biochemical studies also suggest that Kv β-subunits might well be functionally more diverse (370, 393, 458). Both Kvβ1.1 and Kvβ1.2, for example, communoprecipitate with Kv4 α-subunits following coexpression in COS-1 cells (370).
(81). Unlike other NCS-1 proteins, however, KChIP2 and KChIP3 lack NH$_2$-terminal myristoylation sites, and the NH$_2$ termini of each of the KChIP proteins are unique (21). Nevertheless, KChIP2 and KChIP3 have several potential palmitoylation (on cysteine residues) sites, and metabolic labeling studies suggest that these sites are palmitoylated in situ (491). It is now clear that KChIP3 is identical to the previously identified protein calsenilin, which is a Ca$^{2+}$-binding protein that interacts with presenilin-1 and presenilin-2 and regulates the proteolytic processing of these two proteins (82). In addition, however, KChIP3 is also identical to another previously identified protein called DREAM, which is a Ca$^{2+}$-regulated transcriptional repressor (87). The DREAM protein has been shown to bind to the downstream regulatory elements (DRE) of several genes in the absence of Ca$^{2+}$ and to dissociate from the DRE sequence when Ca$^{2+}$ is elevated (87). Thus DREAM is thought to act as an activity-dependent regulator of gene expression (87). An additional member of the KChIP family, KChIP4, also referred to as calsenilin-like-protein or CALP, was subsequently identified in biochemical studies focused on identifying the binding partners of the presenilin proteins (356). The interactions between KChIP3 (calsenilin) and KChIP4 (CALP) and the presenilin proteins are also Ca$^{2+}$ dependent (356).

Of the four KChIP genes, only KChIP2 appears to be expressed in the heart (21, 431). There are, however, numerous splice variants of KChIP2 that have now been identified (33, 125, 129, 390, 391, 430, 431). Studies in heterologous systems have revealed that coexpression of any one of the (full-length) KChIP proteins with Kv4 $\alpha$-subunits increases the functional cell surface expression of Kv4.x-encoded Kv channels, slows current inactivation, speeds recovery from inactivation and shifts the voltage dependence of channel activation (21, 193, 195). In contrast, KChIP expression reportedly does not affect the properties or the densities of the K$^+$ currents produced on expression of other Kv $\alpha$-subunits, including Kv1.4 and Kv2.1 (21). These observations were interpreted as suggesting that the modulatory effects of the KChIP proteins are specific for $\alpha$-subunits of the Kv4 subfamily (21). In addition, although the binding of the KChIP proteins to Kv4 $\alpha$-subunits is not Ca$^{2+}$ dependent, mutations in EF hand domains 2, 3, and 4 of KChIP1 reportedly eliminate the modulatory effects of KChIP1 on Kv4.2-encoded K$^+$ currents in CHO cells (21). It has, however, also been reported that a splice variant of KChIP2, KChIP2d, which lacks three of the four EP hand domains of full-length KChIP2, modifies the inactivation kinetics of heterologously expressed Kv4.3-encoded K$^+$ currents, but does not alter the kinetics of channel recovery from steady-state inactivation (390). Taken together, these findings suggest that distinct regions of the (full-length) KChIP proteins underlie the various modulatory effects of KChIPs on the properties and cell surface expression of Kv4-encoded channels. Structural analysis has revealed that Kv4$\alpha$ and KChIP accessory subunits assemble in a 4:4 stoichiometry and provide new insights into the intracellular interactions between the NH$_2$ termini of Kv4 subunits and the EF hand domains of the KChIPs (258, 588). In addition, it had been shown that myristoylation of KChIP1 appears necessary for the normal trafficking of newly synthesized (KChIP1) protein to the endoplasmic reticulum where the association with Kv4 $\alpha$-subunits occurs (385). It may be that palmitoylation of KChIP2 and KChIP3 plays a similar functional role. Mutagenesis and structural studies have also revealed that two regions in the NH$_2$ termini of Kv4 subunits are necessary for KChIPx interaction with (and modulation of) Kv4-encoded channels and that residues 71–90 (in Kv4.x) form a “contact loop” that mediates the interaction with the KChIP protein(s) (451).

Biochemical methods were exploited in efforts that led to the identification of another Kv channel accessory subunit, DPPX or DPP6, that also appears to interact specifically with Kv4 $\alpha$-subunits (368). A novel protein of previously unknown function, DPP6 is structurally related to CD26, which is a dipeptidyl aminotransferase and a cell adhesion protein (368). Interestingly, DPP6 actually belongs to a family of nonclassical serine proteases, although DPP6 itself has no enzymatic activity (368). In contrast to the KChIPs, DPP6 is an integral membrane glycoprotein with a rather large extracellular COOH-terminal domain (368). Coexpression of DPP6 with Kv4 $\alpha$-subunits affects the trafficking and the membrane targeting of Kv4 $\alpha$-subunits and modifies the kinetic properties of expressed cell surface Kv4-encoded channels (368). Although expressed in brain and thought to function in the generation of neuronal Kv4-encoded transient outward Kv currents, DPP6 does not appear to be expressed in heart and, therefore, cannot contribute to the formation of functional cardiac Kv channels. Another member of the dipeptidyl transferase family, DPP10, has also been shown to associate with Kv4 $\alpha$-subunits in heterologous expression systems and to modify the biophysical properties of Kv4.4.x-encoded channels (235). The effects of DPP10 on Kv4 channels are qualitatively similar to the effects of DPP6 (235), although, like DPP6, DPP10 also appears to be expressed predominantly in the brain (235). It also seems unlikely, therefore, that DPP10 plays a role in the generation of cardiac Kv channels. It is certainly possible, however, that there are additional members of this family that are expressed in the myocardium and that remain to be identified and characterized.

C. Molecular Correlates of Cardiac Transient Outward Kv Channels

All available evidence suggests that Kv $\alpha$-subunits of the Kv4 subfamily encode rapidly activating, inactivating,
and recovering cardiac transient outward Kv channels referred to as $I_{to,f}$ (Table 1). In rat and mouse ventricular myocytes exposed to antisense oligonucleotides (AsODNs) targeted against Kv4.2 or Kv4.3, for example, $I_{to,f}$ density is reduced by $\sim 50\%$ (169, 193). Significant reductions in ventricular $I_{to,f}$ density are also seen in cells exposed to an adenoviral construct encoding a truncated Kv4.2 subunit (Kv4.2ST) that functions as a dominant negative (238). In addition, it has been reported that $I_{to,f}$ is eliminated in ventricular myocytes isolated from transgenic mice expressing a pore mutant of Kv4.2, Kv4.2W362F, that functions as a dominant negative, Kv4.2DN (43). Taken together, these results demonstrate that members of the Kv4 subfamily underlie $I_{to,f}$ in mouse and rat ventricles. Biochemical studies have also shown that Kv4.2 and Kv4.3 are associated in adult mouse ventricles, suggesting that functional mouse ventricular $I_{to,f}$ channels reflect the heteromeric assembly of the Kv4.2 and Kv4.3 $\alpha$-subunits (193). Given that the properties of the currents classified as $I_{to,f}$ in other species (Table 1) are very similar to mouse (and rat) $I_{to,f}$, it seems reasonable to suggest that Kv4 $\alpha$-subunits also underlie $I_{to,f}$ in other species. In dog and human myocardium, however, Kv4.2 appears not to be expressed (266), suggesting that only Kv4.3 contributes to $I_{to,f}$ in larger mammals. Direct biochemical and/or molecular evidence to support this hypothesis, however, has not been provided to date. In addition, multiple splice variants of Kv4.3 have been identified in human (387) and rat (490) heart, although the functional roles of these variants in the generation of cardiac $I_{to,f}$ channels remain to be determined.

It has been demonstrated that KChIP2 coimmunoprecipitates with Kv4.2 and Kv4.3 $\alpha$-subunits from adult mouse ventricles, consistent with a role for this subunit in the generation of functional Kv4-encoded mouse ventricular $I_{to,f}$ channels (193). An important structural role of KChIP2 in the generation of myocardial $I_{to,f}$ channels is suggested by the observation that $I_{to,f}$ is eliminated in ventricular myocytes isolated from mice with a targeted disruption of the KChIP2 locus (271). In both canine and human heart, it has been demonstrated that there is a gradient in KChIP2 message expression across the thickness of the (left and right) ventricular walls (429, 431), observations interpreted as suggesting that KChIP2 underlies the observed differences in $I_{to,f}$ densities in myocytes isolated from the epicardial, midmyocardial, and endocardial layers of the (human and canine) ventricles (429, 431). Although this point remains somewhat controversial (129), it has been reported that KChIP2 protein expression in canine ventricles parallels KChIP2 message expression (429), lending further support to the hypothesis that KChIP2, not Kv4.3, underlies the gradient in canine (and human) ventricular $I_{to,f}$ densities. In rat and mouse, however, there is no detectable gradient in KChIP2 message or protein expression in the ventricles (193, 431), and it appears that differences in Kv4.2 expression underlie the regional variations in $I_{to,f}$ densities in rodents (137, 193). Thus there seem to be two potentially important differences between $I_{to,f}$ in rodents and $I_{to,f}$ in large mammals, including humans. In rat and mouse, $I_{to,f}$ channels reflect the heteromeric assembly of Kv4.2, Kv4.3, and KChIP2, and differences in Kv4.2 expression underlie regional differences in $I_{to,f}$ densities. In large mammals, however, $I_{to,f}$ channels appear to be produced by the coassembly of Kv4.3 and KChIP2, and KChIP2 appears to be the primary determinant of the observed regional differences in $I_{to,f}$ densities.

Although the Kv$\beta$ accessory subunits were originally identified based on association with Kv1 $\alpha$-subunits and have been considered to be Kv1 $\alpha$-subunit specific, recent studies suggest a functional role for Kv$\beta$1 subunits in the generation of cardiac $I_{to,f}$ channels (12). Electrophysiological studies for example, have revealed that $I_{to,f}$ densities are decreased in ventricular myocytes isolated from mice bearing a targeted deletion of the KCNAB1 gene, which encodes Kv$\beta$1 subunits (12). In addition, biochemical studies revealed that Kv4.2 and Kv4.3 coimmunoprecipitate with the Kv$\beta$1 splice variants, Kv$\beta$1.1 and Kv$\beta$1.2, from adult mouse ventricles (12). Taken together, these observations suggest that (mouse) ventricular $I_{to,f}$ channels function as multimeric protein complexes comprising the Kv4.2 and Kv4.3 pore-forming $\alpha$-subunits and the accessory Kv$\beta$1.1, Kv$\beta$1.2, and KChIP2 subunits (Fig. 5). The targeted disruption of Kv$\beta$1 reduces the cell surface membrane expression of Kv4 $\alpha$-subunits (12), further suggesting that Kv$\beta$1 functions to regulate the assembly and/or the trafficking of mouse ventricular $I_{to,f}$ channels from the endoplasmic reticulum to the cell surface (12).

The Kv$\beta$1 COOH-terminal “core” domain has been shown to interact with NH$_2$-terminal tetramerization (T1) domains in Kv1 $\alpha$-subunits (191), and recent studies suggest that Kv4 $\alpha$-subunit NH$_2$-terminal domains structurally resemble Kv1 T1 domains (451). It seems reasonable to suggest, therefore, that Kv4 NH$_2$ termini are likely involved in mediating the interaction with Kv$\beta$1 subunits. As noted above, however, it has also previously been demonstrated that Kv4 $\alpha$-subunit NH$_2$-terminal domains are also important in mediating the interactions with KChIPs (21, 451). Taken together, these observations suggest that Kv4 NH$_2$-terminal domains are multifunctional, mediating $\alpha$-subunit/$\alpha$-subunit interactions, as well as the associations with the accessory KChIP2 and Kv$\beta$1 subunits. It has also been reported, however, that Kv$\beta$1 subunits regulate the cell surface expression of Kv4.3 subunits in heterologous expression systems through interactions with the COOH, not the NH$_2$, terminus (566). It is not clear if Kv$\beta$1 subunits play a role in the generation of $I_{to,f}$ (and/or other) channels in large mammals, including humans, primarily because this possibility has not been explored directly. Given the heterogeneity of subunits...
that can affect Kv4 channel properties in heterologous expression systems (130), it seems reasonable to suggest that additional accessory subunits or regulatory proteins might be involved in mediating the interaction(s) between Kv4α and Kvβ1 subunits. It is certainly also possible that the interactions between Kv4α and Kvβ1 subunits are indirect, mediated, for example, by other accessory subunits, such as KChIP2 or KChAP (278) or through scaffolding proteins (39) or components of the actin cytoskeleton (401, 529). In addition, there could well be further complexity in the subunit composition of \(I_{\text{to,f}}\) channels, as well as in \(I_{\text{to,s}}\) channel regulation and posttranslational processing in some cell types/species. Further studies focused on defining all of the molecular components of functional myocardial \(I_{\text{to,f}}\) (and other) channels are needed to provide insights into the detailed molecular mechanisms involved in the regulation and modulation of these channels in the normal and in the diseased myocardium.

Electrophysiological studies on atrial myocytes isolated from Kv4.2DN mice revealed that, similar to the findings in ventricular cells (43), \(I_{\text{to,f}}\) is eliminated (563). There are some differences, however, in the properties of mouse (and rat) ventricular and atrial \(I_{\text{to,f}}\) (26, 43, 68, 69, 75, 79, 194, 562, 563), differences that may reflect variations in the subunit composition of the channels and/or in posttranslational processing of these subunits. Further studies focused on detailing the molecular compositions and the mechanisms controlling the expression and functioning of \(I_{\text{to,f}}\) channels in other cell types, particularly atrial, nodal, and Purkinje cells, in rodents and in large animals, are needed to define definitively the similarities/differences in the molecular compositions of \(I_{\text{to,f}}\) channels in different cell types/species.

The kinetic and pharmacological properties of slow transient outward myocardial Kv currents, referred to as \(I_{\text{to,s}}\) (Table 1), are different from \(I_{\text{to,f}}\) observations interpreted as suggesting that the molecular correlates of \(I_{\text{to,s}}\) and \(I_{\text{to,f}}\) channels are also distinct. Direct support for this hypothesis was provided in studies (196) completed on myocytes isolated from (Kv1.4 null) mice with a targeted disruption of the KV1.4 (Kv1.4) locus (305). The waveforms of the outward currents in cells isolated from the interventricular septum of Kv1.4 null animals are indistinguishable from those recorded in wild-type ventricular cells (196). In cells isolated from the interventricular septum of Kv1.4 null animals, however, \(I_{\text{to,s}}\) is undetectable, thereby demonstrating directly that Kv1.4 underlies \(I_{\text{to,s}}\) (196). Given the similarities in the time- and voltage-dependent properties of \(I_{\text{to,s}}\) (Table 1) in other species (77, 83, 178, 294, 545, 546), it seems reasonable to suggest that Kv1.4 also encodes \(I_{\text{to,s}}\) in ferret, rabbit, canine, and human atrial and ventricular myocytes.

Interestingly, it has also been reported that \(I_{\text{to,s}}\) and the Kv1.4 protein are upregulated in the right and left ventricles of Kv4.2DN-expressing transgenic animals (43, 194, 196), suggesting that electrical remodeling occurs in the myocardium when \(I_{\text{to,f}}\) is eliminated. When the Kv4.2DN transgene is expressed in the Kv1.4 null background, however, both \(I_{\text{to,f}}\) and \(I_{\text{to,s}}\) are eliminated and, interestingly, no further electrical remodeling is evident (194). Indeed, electrophysiological recordings from Kv4.2DN-expressing Kv1.4 null cells revealed that the waveforms of the Kv currents in RV, LV, and interventricular septum cells are indistinguishable (194). Although these observations suggest that the molecular mechanisms underlying the observed electrical remodeling in Kv4.2DN ventricles is highly specific for Kv1.4, it is presently unclear which of the many possible transcriptional, translational, and/or posttranslational mechanisms (430) might be operative. Future studies focused on delineating the molecular mechanisms involved in the regulation of ion channel remodeling in this and other mouse models will likely provide important new mechanistic insights.

D. Molecular Correlates of Cardiac Delayed Rectifier Kv Channels

As noted above, \(KCNH2\) has been identified as the locus of mutations underlying one form of familial long QT syndrome, LQT2 (119). Heterologous expression of \(KCNH2\) cRNA in Xenopus oocytes reveals voltage-gated, inwardly rectifying \(K^+\)-selective channels with properties similar to cardiac \(I_{\text{Kr}}\) channels (448, 499), observations interpreted as suggesting that \(KCNH2\) encodes \(I_{\text{Kr}}\) (448). Subsequent studies identified NH2- and COOH-terminal splice variants of the ERG1 protein (272, 282, 304), and recent biochemical studies suggest that an NH2-terminal ERG1 splice variant, ERG1b, coassembles with the full-length ERG1α protein to form heteromeric \(I_{\text{Kr}}\) channels in rat, human, and canine heart (240). The role of the COOH-terminal variants of ERG1, ERG1-USO (272) in the generation of functional cardiac \(I_{\text{Kr}}\) channels, however, is presently unclear. It has been reported that heterologously expressed \(KCNH2\) and minK (\(KCNH2\)) coimmunoprecipitate (341) and that antisense oligodeoxynucleotides targeted against minK attenuate \(I_{\text{Kr}}\) amplitudes in AT-1 (an atrial tumor line) cells (567). It has also been reported that heterologous coexpression of another member of the KCNE subfamily of accessory subunits, MiRP1 (\(KCN1\)) (\(KCN1\)) coimmunoprecipitate (5). It is presently unclear, however, whether the minK or MiRP1 (or both) accessory subunits associate with ERG1α and/or ERG1β in adult human heart and contribute to the generation of functional cardiac \(I_{\text{Kr}}\) channels. The availability of specific anti-ERG1 antibodies that can be exploited to immunoprecipitate ERG1 proteins from heart (240) should make it possible to explore directly the association between the minK/MiRP accessory subunits.
and the pore-forming ERG1 subunits and the functional roles of these interactions in the generation of myocardial $I_{Kr}$ channels.

Biochemical studies have now revealed that the heat shock proteins, Hsp70 and Hsp90, coimmunoprecipitate with heterologously expressed ERG1 and that geldanamycin, a specific inhibitor of Hsp90, prevents the maturation (posttranslational processing) and increases the proteosomal degradation of the ERG1 protein (163). Interestingly, the interactions between the ERG1 protein and Hsp70/Hsp90 are increased in LQT2 trafficking deficient KCNH2 mutants, such as ERG1G601S (180). In addition, the mutant ERG1G601S protein is retained in the endoplasmic reticulum (163). Importantly, it has also been demonstrated that inhibition of Hsp90 decreases functional $I_{Kr}$ densities in isolated ventricular myocytes (163). Taken together, these results suggest that Hsp70 and Hsp90 function as chaperones, bringing mature proteins to the cell surface to generate functional $I_{Kr}$ protein complexes to the cell surface (163). Importantly, it has also been demonstrated that inhibition of Hsp90 decreases functional $I_{Kr}$ densities in isolated ventricular myocytes (163). Taken together, these results suggest that Hsp70 and Hsp90 function as chaperones, bringing mature ERG1 protein complexes to the cell surface to generate functional $I_{Kr}$ channels (163). It is certainly possible that there are additional components of myocardial $I_{Kr}$ channels that influence the properties and/or the functional cell surface expression of these channels, and further studies are needed to test these hypotheses directly.

Heterologous expression of KCNQ1, the locus of mutations in LQT1 (522), reveals rapidly activating, noninactivating Kv currents, whereas coexpression with KCNE1 (minK) produces slowly activating Kv currents similar to cardiac $I_{Kr}$ (40, 447). These observations, together with biochemical data demonstrating that heterologously expressed KvLQT1 and minK proteins associate (447), have been interpreted as suggesting that minK coassembles with KvLQT1 to form functional cardiac $I_{Kr}$ channels (40, 447). In addition, the finding that mutations in the transmembrane domain of minK alter the properties of the KCNQ1 encoded Kv channels was interpreted as suggesting that the transmembrane segment of minK contributes to the channel pore (185, 487, 492, 527). Nevertheless, and similar to the suggested interaction between ERG1 and MiRP1 (and/or minK), there is presently no direct biochemical/molecular evidence demonstrating a functional interaction between the minK and KvLQT1 proteins and/or that minK/KvLQT1 interactions play a role in the generation of cardiac $I_{Kr}$ channels.

A yeast two-hybrid screen, using the intracellular cytoplasmic COOH terminus of minK as the bait, led to the identification of a novel LIM-domain-containing protein, fh12 (274). Heterologous expression studies further suggest that fh12 is required for the generation of functional cell surface KvLQT1/minK ($I_{Kr}$) channels (274). These observations suggest that fh12 is required for the proper assembly of the KvLQT1 and minK subunits, the trafficking of assembled channels, and/or the cell surface expression of functional KvLQT1/minK ($I_{Kr}$) channels. Nevertheless, direct biochemical evidence for coassembly of the KvLQT1 protein with minK, with fh12 and/or with any other Kv channel accessory subunits (Table 5) in the myocardium has not been provided, and the subunit stoichiometry of functional myocardial $I_{Kr}$ channels remains to be determined. Similar to KCNH2, splice variants of KCNQ1, which exert a dominant negative effect when coexpressed with the full-length KvLQT1 protein (236), have also been described, although the roles of these variants in the generation of $I_{Kr}$ channels in vivo remain to be determined.

A variety of experimental strategies, primarily in mice, have been exploited in studies focused on defining the molecular correlates of several of the other types of cardiac delayed rectifier Kv currents (Table 1). A role for Kv1 $\alpha$-subunits in the generation of mouse ventricular $I_{K,slow}$, for example, was revealed with the demonstration that $I_{K,slow}$ is selectively attenuated in ventricular myocytes isolated from transgenic mice expressing a truncated Kv1.1 $\alpha$-subunit, Kv1.1N2067Tag, that functions as a dominant negative (303). It was subsequently shown, however, that $I_{K,slow}$ is also reduced in ventricular myocytes expressing a dominant negative mutant of Kv 2.1, Kv2.1N216 (560). Further analyses revealed that there are actually two distinct components of wild-type mouse ventricular $I_{K,slow}$: one that is sensitive to micromolar concentrations of 4-AP and encoded by Kv1 $\alpha$-subunits, and another that is sensitive to TEA and encoded by Kv2 $\alpha$-subunits (263, 560, 587). These currents are now referred to as $I_{K,slow1}$ and $I_{K,slow2}$, respectively (263, 291, 587). Subsequent studies revealed that $I_{K,slow1}$ is selectively eliminated in ventricular myocytes isolated from mice in which Kv1.5 has been deleted, suggesting that Kv1.5 encodes the micromolar 4-AP-sensitive mouse ventricular $I_{K,slow}$ (302). These findings, together with the previous results obtained on cells isolated from Kv1.4 --/-- animals (305), in which $I_{to,s}$ is eliminated (106), suggest that, in contrast to the Kv4 $\alpha$-subunits (193), the Kv1 $\alpha$-subunits, Kv1.4 and Kv1.5, do not associate in adult mouse ventricles in situ. Rather, functional Kv1 $\alpha$-subunit-encoded Kv channels in mouse ventricular myocytes appear to be homeric, composed of Kv1.4 $\alpha$-subunits ($I_{to,s}$) or Kv1.5 $\alpha$-subunits ($I_{K,slow1}$). The roles of Kv channel accessory subunits in the generation of these myocardial Kv1 $\alpha$-subunit-encoded Kv channels, however, remain to be defined.

Electrophysiological studies completed on isolated rat atrial myocytes (74, 75), and later on canine (577), human (19, 531), and mouse (69) atrial myocytes, demonstrated the presence of a novel component of delayed rectification, referred to as $I_{Kur}$ ($I_{Kaltrapid}$), with time- and voltage-dependent properties that are quite distinct from $I_{Kr}$ and $I_{Kr}$ (479). Although $I_{Kur}$ appears to be an atrial specific current in large mammals, the properties of $I_{Kur}$ in mouse ventricular myocytes are indistinguishable from (rat, human, and canine) atrial $I_{Kur}$ (168, 562,
586). Human, rat, and canine atrial \( I_{\text{Kur}} \) like mouse ventricular \( I_{\text{Ks,slow1}} \), activates rapidly and undergoes little or no inactivation during brief depolarizations, properties similar to those seen on heterologous expression of several different Kv α-subunits, including Kv1.2, Kv1.5, Kv3.1, and others. In addition, \( I_{\text{Kur}} \), like \( I_{\text{Ks,slow1}} \), is sensitive to micromolar concentrations of 4-AP (168, 562, 586). The later finding led to the hypothesis that Kv1.5 likely encodes human and rat atrial \( I_{\text{Kur}} \) (75, 533). Direct experimental support for this hypothesis was provided with the demonstration that exposure to antisense oligodeoxynucleotides targeted against Kv1.5 selectively attenuates \( I_{\text{Kur}} \) in isolated adult human (159) and rat (68) atrial myocytes. The important physiological role for Kv1.5 in human atria is suggested by the finding that \( I_{\text{Kur}} \) densities and Kv1.5 protein expression are reduced markedly in the atria of patients with chronic atrial fibrillation (511).

Although it was reported that Kv3.1, rather than Kv1.5, functions in canine atria to encode \( I_{\text{Kur}} \) (479), subsequent work demonstrated that Kv3.1 is not detectable in canine atria, whereas Kv1.5 (message and protein) is robustly expressed (157). It seems reasonable to conclude, therefore, that, similar to other Kv channels, the molecular correlate of cardiac \( I_{\text{Kur}} \) (Kv1.5) is similar across species. At present, it is unclear if Kv accessory subunits play a role in the generation of atrial (mouse, rat, canine, or human) \( I_{\text{Kur}} \). Unexpectedly, however, it has now been demonstrated that Kvβ1 subunits do not associate with Kv1.5 in adult mouse ventricles and that the targeted deletion of Kvβ1 has no detectable effect on mouse ventricular \( I_{\text{Kur}} \) (12). It may well be, however, that other Kv channel accessory subunits contribute to the generation of \( I_{\text{Kur}} \) channels. Further studies, focused on defining the molecular composition of \( I_{\text{Kur}} \) and the roles of accessory subunits, are needed to define the underlying molecular mechanisms involved in the regulation of \( I_{\text{Kur}} \) channels in the normal and diseased myocardium.

**VII. MOLECULAR COMPONENTS OF OTHER CARDIAC POTASSIUM CHANNELS**

A. Inwardly Rectifying Cardiac \( K^+ \) (Kir) Channel Pore-Forming α-Subunits

Similar to the Kv channels, functionally distinct types of myocardial inwardly rectifying \( K^+ \) channels (378) are formed by the association of diverse inward rectifier \( K^+ \) (Kir) channel pore-forming α-subunit genes (140). Several Kir subunit subfamilies, Kir1 through Kir6, most with several members, have been identified (Table 6) and, like Kv α-subunits, Kir α-subunits also assemble as tetramers to form functional \( K^+ \) selective channels (Fig. 5). Also similar to Kv channel α-subunits, a unifying terminology has been developed for naming the Kir α-subunit proteins (Kir1.x–Kir6.x) and the genes (\( KCNJ1–KCNJ15 \)) encoding these proteins (199). In contrast to the Kv α-subunits, however, the Kir α-subunits have two (not six) transmembrane domains (Fig. 5).

Based on the properties of heterologously expressed Kir subunits, it was suggested that α-subunits of the Kir2 subfamily likely encode the strongly inwardly rectifying Kir channels, \( I_{\text{K1}} \), in cardiac cells (140, 378), and all (three) members of the Kir2 subfamily (Table 6) are expressed in the myocardium (298, 488). Interestingly, the \( KCNJ2 \) gene, which encodes Kir2.1, has been identified as the locus of mutations in Andersen’s syndrome (243, 403), an inherited disorder that is often life-threatening owing to QT prolongation and cardiac (ventricular) arrhythmias. Similar to Timothy’s syndrome (479), however, Andersen’s syndrome is actually a multisystem disorder involving the cardiovascular, skeletomuscular, and other systems, and typically, Andersen’s syndrome patients present initially with developmental abnormalities (494). The mutations in \( KCNJ2 \) that are associated with Andersen’s syndrome that have been described to date appear to result in mutant Kir2.1 proteins that function in a dominant negative fashion to suppress Kv2.x-encoded \( I_{\text{K1}} \) currents (11, 280, 409). Individuals carrying Andersen’s syndrome mutations in \( KCNJ2 \) can display QT prolongation (Long QT7, periodic paralysis, as well as craniofacial malformations (11, 22, 494, 498), alone or in combination. Because only the \( KCNJ2 \) gene appears to be affected in Andersen’s syndrome, the multisystem nature of this disorder likely reflects the fact that Kir2.x-encoded channels are expressed and are functional in a variety of cells/tissues. Myocardial \( I_{\text{K1}} \) (and other \( K^+ \) channels) have been shown to be regulated directly by phosphatidylinositol bisphosphate (PIP2) (209, 219, 470, 489). Interestingly, many of the Andersen’s mutations are in the PIP2 binding region of Kir2.1 (139), suggesting that the regulation/modulation of \( I_{\text{K1}} \) channels by PIP2 is altered and that it is the alterations in the modulatory effects of PIP2 that underlie the phenotypic consequences of the Andersen’s syndrome mutations.

The first direct molecular evidence that Kir2 α-subunits encode cardiac \( I_{\text{K1}} \) channels was provided in studies completed on myocytes isolated from mice bearing a targeted disruption of the coding region of Kir2.1 (Kir2.1 \(-/-\)) or Kir 2.2 (Kir2.2 \(-/-\)) (580, 581). Although the Kir2.1 \(-/-\) mice have cleft palate and die shortly after birth, thereby precluding electrophysiological studies on adult cells (580), experiments completed on isolated newborn Kir2.1 \(-/-\) ventricular myocytes revealed that \( I_{\text{K1}} \) is absent (581). Interestingly, however, an inwardly rectifying current, with properties distinct from the wild-type \( I_{\text{K1}} \), is evident in Kir2.1 \(-/-\) myocytes (581), suggesting either that an additional Kir current component is present, but difficult to resolve in wild-type cells in the...
presence of $I_{K1}$ or, alternatively, that a novel $I_{K1}$ is up-regulated in Kir2.1/H11002 hearts. In contrast to the findings in Kir2.1/H11002 cells, voltage-clamp recordings from adult Kir2.2/H11002 ventricular myocytes revealed that $I_{K1}$ densities are reduced, compared with $I_{K1}$ densities in wild-type cells, and that the properties of the residual $I_{K1}$ currents (in Kir2.2/H11002 cells) are indistinguishable from wild-type $I_{K1}$ (581). These results were interpreted as suggesting that both Kir2.1 and Kir2.2 contribute to (mouse) ventricular $I_{K1}$ channels, and subsequent studies provided biochemical and molecular evidence to support this hypothesis (342, 592). The observation that the inwardly rectifying channels remaining in the absence of Kir2.1 have properties distinct from the endogenous $I_{K1}$ channels further suggests that functional cardiac $I_{K1}$ channels are heteromeric. Consistent with this hypothesis, detailed comparisons of the properties of heterologously expressed Kir2.1, Kir2.2, and Kir2.3 \(\alpha\)-subunits and endogenous guinea pig and sheep atrial and ventricular myocytes suggests marked regional and cell type specific differences in the molecular composition of $I_{K1}$ channels (133). Further studies focused on defining the molecular compositions of myocardial $I_{K1}$ channels in different cell types and in different species, including humans, are needed to define the molecular diversity and the functioning of these channels.

In the heart, weakly inwardly rectifying $I_{KATP}$ channels are thought to play a role in both myocardial ischemia and preconditioning (232, 377, 380). In heterologous systems, $I_{KATP}$ channels can be reconstituted by coexpression of Kir6.2 subunits with ATP-binding cassette proteins that encode the sulfonylurea receptors, SURx (31, 456). Although previous pharmacological and molecular studies suggest that cardiac sarcolemmal $I_{KATP}$ channels reflect the heteromeric assembly of Kir6.2 and SUR2A subunits, Kir6.1 is also expressed in the heart (406), and exposure of isolated (rat neonatal) ventricular myocytes to antisense oligodeoxynucleotides against

---

**Table 6. Diversity of inwardly rectifying $K^+$ (Kir) and two-pore domain $K^+$ (K2P) channel \(\alpha\)-subunits**

<table>
<thead>
<tr>
<th>Family</th>
<th>Subfamily</th>
<th>Protein</th>
<th>Gene</th>
<th>Location</th>
<th>Human</th>
<th>Mouse</th>
<th>Cardiac Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kir1</td>
<td></td>
<td>$KCNJ1$</td>
<td>11q25</td>
<td>9A4</td>
<td>??</td>
<td></td>
<td>TWIK</td>
</tr>
<tr>
<td>Kir2</td>
<td></td>
<td>$KCNJ2$</td>
<td>17q23</td>
<td>11E1</td>
<td>$I_{K1}$</td>
<td></td>
<td>TWIK-3 $KCNK7$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ12$</td>
<td>17p11.2</td>
<td>11B1.3</td>
<td>$I_{K1}$</td>
<td></td>
<td>TWIK-4 $KCNK8$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ4$</td>
<td>22U</td>
<td>??</td>
<td></td>
<td></td>
<td>TRED-1 $KCNK2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ14$</td>
<td>19q13.4</td>
<td>7B3</td>
<td>??</td>
<td></td>
<td>TRED-2 $KCNK10$</td>
</tr>
<tr>
<td>Kir3</td>
<td></td>
<td>$KCNJ3$</td>
<td>2C1.1</td>
<td>$I_{Kach}$</td>
<td></td>
<td></td>
<td>TASK-1 $KCNK3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ6$</td>
<td>21q22</td>
<td>16C4</td>
<td></td>
<td></td>
<td>TASK-2 $KCNK5$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ7$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TASK-3 $KCNK9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ9$</td>
<td>1q21</td>
<td>1H2.3</td>
<td></td>
<td></td>
<td>TASK-4 $KCNK14$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TASK-5 $KCNK15$</td>
</tr>
<tr>
<td>Kir4</td>
<td></td>
<td>$KCNJ5$</td>
<td>11q25</td>
<td>9A4</td>
<td>$I_{Kach}$</td>
<td></td>
<td>TRAAK-1 $KCNK4$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ10$</td>
<td>1q21</td>
<td>1H2.3</td>
<td></td>
<td></td>
<td>THIK-1 $KCNK13$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ15$</td>
<td>21q22</td>
<td>16C4</td>
<td></td>
<td></td>
<td>THIK-2 $KCNK12$</td>
</tr>
<tr>
<td>Kir5</td>
<td></td>
<td>$KCNJ16$</td>
<td>17q25</td>
<td></td>
<td></td>
<td></td>
<td>TALK-1 $KCNK16$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ8$</td>
<td>12p11.1</td>
<td>6G2</td>
<td>??</td>
<td></td>
<td>TALK-2 $KCNK17$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$KCNJ11$</td>
<td>11p15</td>
<td>783</td>
<td>$I_{KATP}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Boxes denote cardiac expression.
SUR1 reduces $I_{\text{KATP}}$ channel densities (572). These observations suggest the interesting possibility that there may be some molecular heterogeneity among cardiac $I_{\text{KATP}}$ channels. The absolute requirement for the Kir6.2 subunit in the generation of cardiac $I_{\text{KATP}}$ channels, however, was unequivocally demonstrated in studies completed on mice in which the Kir6.2 gene was disrupted by homologous recombination (292, 456, 484). Voltage-clamp recordings from ventricular myocytes isolated from these (Kir6.2 −/−) animals revealed no detectable $I_{\text{KATP}}$ channel activity (292, 484). These findings clearly suggest that Kir6.1 alone (i.e., in the absence of Kir6.2) cannot generate functional myocardial $I_{\text{KATP}}$ channels. Nevertheless, it is certainly still possible that Kir6.1 coassembles with Kir6.2 to form Kir6.1/Kir 6.2 heteromeric cardiac $I_{\text{KATP}}$ channels.

The suggestion that SUR2 plays a pivotal role in the generation of cardiac $I_{\text{KATP}}$ channels is supported by the finding that $I_{\text{KATP}}$ channel density is reduced in myocytes from animals (SUR2 −/−) in which SUR2 has been deleted (411). In contrast, there are no measurable cardiac effects of the targeted disruption of SUR1 (455, 456). Nevertheless, it is interesting to note that $I_{\text{KATP}}$ channel density is reduced, i.e., the channels are not eliminated, in SUR2 −/− ventricular myocytes, and the properties of the residual $I_{\text{KATP}}$ channels in SUR2 −/− ventricular myocytes are similar to those of the channels produced on heterologous coexpression of Kir6.2 and SUR1 (411). These findings strongly suggest that SUR1 likely also coassembles with Kir 6.2 in ventricular myocytes to produce functional SUR2–Kir6.2/Kir6.2 heteromeric $I_{\text{KATP}}$ channels (473). Similar to Kir channels, myocardial $I_{\text{KATP}}$ channels are also modulated by the binding of PIP2 and other membrane lipids (209, 470).

The waveforms of action potentials recorded from isolated Kir6.2 −/− ventricular myocytes are indistinguishable from those recorded from wild-type cells (484). These observations clearly suggest that $I_{\text{KATP}}$ channels do not play a role in shaping action potential waveforms (in mouse ventricles) under normal physiological conditions. The action potential shortening typically observed in wild-type ventricular cells during ischemia or metabolic blockade, however, is abolished in Kir6.2 −/− ventricular cells (484). In addition, the protective effect of ischemic preconditioning is abolished in Kir6.2 −/− hearts (456, 485), and infarct size in Kir6.2 −/− animals, with and without preconditioning, is the same (485). These observations are consistent with the hypothesis that cardiac $I_{\text{KATP}}$ channels play an important role under physiopathological conditions, particularly those involving metabolic stress (74, 577). Interestingly, however, it has been demonstrated that action potential durations are also largely unaffected in transgenic animals expressing mutant $I_{\text{KATP}}$ channels with markedly (40-fold) reduced ATP sensitivity (268). The mutant $I_{\text{KATP}}$ channels would be expected to be open (owing to the reduced sensitivity to closure by ATP) at rest and to markedly affect cardiac membrane excitability. The fact that action potentials are unaffected in ventricular myocytes expressing mutant $I_{\text{KATP}}$ channels clearly suggests that additional inhibitory regulatory mechanisms play a role in the physiological control of cardiac $I_{\text{KATP}}$ channel activity in vivo (268). Further studies focused on defining and characterizing these regulatory mechanisms will be of considerable interest.

B. Two-Pore Domain K+ (K2P) Channel Pore-Forming α-Subunits

In addition to the many Kv (Table 4) and Kir (Table 6) channel α-subunits, a novel type of K+ pore-forming α-subunit with four transmembrane spanning regions and two pore domains (K2P) was identified with the cloning of TWIK1 (289), now referred to as KCNK1 (199). Studies in heterologous systems suggest that functional K2P channels, unlike Kv and Kir channels that assemble as tetramers, reflect the dimeric assembly of (two) K2P α-subunits and that each of the (two) pore domains in each α-subunit contributes to the formation of the K+–selective pore (286). Subsequent to the identification of TWIK1, a rather large number of K2P α-subunit genes, KCNK1– KCNK17, were identified, and a subset of these appears to be expressed in the myocardium (Table 6). Similar to the Kv and Kir channels, a systematic terminology has been developed for naming the (KCNK) genes encoding K2P α-subunits.

Heterologous expression studies have demonstrated that the members of various K2P subunit subfamilies give rise to K+–selective currents with distinct time- and voltage-dependent properties and differential sensitivities to a variety of modulators, including pH, fatty acids, and anesthetics (286, 287). It seems likely, therefore, that K2P subunit-encoded K+ channels could be important in regulating the normal physiological functioning of the adult mammalian heart, as well, perhaps, as influencing myocardial responses to pathophysiological stimuli. Direct experimental support for this hypothesis was provided with the demonstration that the pathophysiological effects of platelet activating factor on the myocardium are directly linked to inhibition of KCNK3 (TASK-1)-encoded (or closely related) K+ channels in ventricular myocytes (39). Interestingly, the effect of platelet activating factor is dependent on protein kinase C (39), although the underlying molecular mechanisms have not been detailed. Al-
though it has been demonstrated that different KCNK-encoded K2P α-subunits coassemble to form heteromorphic channels (55), it is presently unclear whether heteromeric K2P α-subunit assembly is physiologically relevant in the myocardium. Similarly, there is very little information presently available about the role(s) of accessory subunits and/or other regulatory molecules in the generation of K2P α-subunit-encoded myocardial channels.

The facts that there are so many K2P α-subunits (Table 6), that many of them are ubiquitously expressed, and that the properties of K2P α-subunit-encoded channels are regulated by a variety of potentially relevant physiological (and pathophysiological) stimuli suggest that channels encoded by K2P α-subunits likely subserve a variety of important physiological functions. Experimental support for this hypothesis was provided with the demonstration that mice bearing a targeted disruption of the KCNK2 gene (which encodes TREK-1, Table 6) display increased sensitivity to epilepsy and ischemia (208). It is unclear, however, whether there is a cardiac phenotype in the KCNK2−/− mice, primarily because this possibility appears not to have been addressed (208). The physiological roles of TREK-1 and of each of the other K2P channel α-subunits expressed in the myocardium, as well as in other cell types, therefore, remain largely unknown. Both TREK-1 and TASK-1 are expressed in the heart, and heterologous expression of either of these subunits alone gives rise to instantaneous, nonactivating K+ currents that display little or no voltage dependence (286). These observations have led to suggestions that these subunits contribute to myocardial “background” or “leak” K+ currents (32), although presently, there is no direct experimental evidence to support this hypothesis. Interestingly, however, the properties of the currents produced on expression of TREK-1 or TASK-1 are similar to those of the current referred to as Ik, identified in guinea pig ventricular myocytes (576), as well as to Ias in mouse ventricular myocytes (79, 562). Further studies focused on defining the roles of each of the K2P α-subunits in the generation of myocardial K+ channels, and the roles of these channels in the physiological, as well as the pathophysiological, functioning of the heart, are needed to clarify these issues.

VIII. MYOCARDIAL POTASSIUM CHANNELS AND THE ACTIN CYTOSKELETON

Similar to myocardial Nav channels, considerable evidence has now accumulated to suggest that several different types of plasmalemmal K+ channels in cardiac cells also interact with components of the actin cytoskeleton and that these interactions play important roles in regulating the properties, the trafficking, and/or the anchoring of these channels. It has been shown, for example, that myocardial IKATP channels likely are linked to, and regulated by, the actin cytoskeleton (179, 336). Exposure to cytochalasin D, which disrupts/destabilizes actin filaments, for example, accelerates the rundown of cardiac IKATP channels, whereas actin filament stabilizers inhibit channel rundown (179). It appears that cytochalasin D exerts its effects by interfering with the interaction between SUR2A and Kir6.2 subunits, thereby modifying SUR-mediated regulation of the IKATP channels (76, 571). These observations suggest that the biophysical properties, as well as the cell surface expression of IKATP channels, are affected by cytoskeletal interactions. Similarly, the biophysical properties, including rectification and Ca2+ (but not Mg2+) sensitivity, of myocardial IK1 channels are affected by treatment with cytochalasin D (336).

There is also experimental evidence suggesting that the functioning of Kv channels in myocardial (and other) cells is regulated and/or modulated through interactions with the actin cytoskeleton. It has been demonstrated, for example, that exposure to phalloidin, which stabilizes actin filaments, markedly reduces action potential durations, whereas treatment with cytochalasin D (or cytochalasin B) prolongs action potential durations, in hypertrophied rat ventricular myocytes (568). Voltage-clamp studies revealed that the cytochalasin- and phalloidin-mediated effects on action potentials reflect the specific attenuation or augmentation, respectively, of IfK (568). These observations suggest that functional cardiac Kv4 α-subunit-encoded Itof channels are regulated/modulated directly or indirectly through interactions with the actin cytoskeleton. Interestingly, experiments in heterologous expression systems also suggest that the modulation of Kv1.5, which encodes cardiac IKur channels, by protein kinases and phosphatases, requires an intact cytoskeleton (333).

Similar to cardiac Nav channels, the interactions between functional myocardial Kir and Kv channels and the actin cytoskeleton are assumed to be mediated through association with actin-binding proteins and/or other scaffolding proteins, suggesting that Kir and Kv channels also function as multimeric protein complexes (Fig. 6). Consistent with this hypothesis, it was recently demonstrated that Kir2.x α-subunits associate with several scaffolding proteins, including CASK, veli-3, mint-1, and SAP-97 (285). Interestingly, Kir subunits in brain also interact with a very large number and variety of PDZ domain-containing proteins (285, 372). In addition, it has been reported that Kir6.x subunits interact directly with the 14–3–3 protein and that this interaction is requisite for the functional cell surface expression of assembled Kir6.x-SUR-encoded IKATP channels (575). It has also been reported that Kv α-subunits in several subfamilies bind to PDZ-containing proteins, including PSD-95 and SAP-97 (145, 237, 557, 558), although the physiological significance of these observations in terms of the expression and/or the function-
ing of cardiac Kv channels has not been determined. The interactions between Kv1 and Kv4 α-subunits and PSD-95 impact the recruitment of Kv1- and Kv4-encoded channels into lipid rafts (558). This finding may explain early observations suggesting that the expression of Kv4 α-subunits alone fails to reveal targeting to lipid rafts (327). More importantly, these observations suggest that specific associations between Kv α-subunits and PDZ-containing scaffolding proteins play an important role in the targeting of functional Kv channels to specific subcellular domains. The targeting of Kv channels to specific subcellular compartments would be expected to have rather profound effects on the regulation of membrane excitability and conduction through the myocardium (270, 463). In addition, Kv channel targeting could facilitate specific interactions between Kv channels and modulatory/regulatory proteins, including protein kinases and phosphatases, as has been clearly demonstrated in the protein kinase A-mediated regulation of cardiac I_{Ks} channels, that appears to be mediated by an A-kinase anchoring protein or AKAP (330, 331).

Interestingly, it has also been demonstrated that Kv1 α-subunits contain PDZ-binding domains (145) and that Kv1.5 binds directly to the actin-binding protein α-actinin-2 (329). Further studies revealed that members of three subfamilies of Kv α-subunits, Kv1.5, Kv2.1, and Kv4.2, bind to α-actinin-2, interactions that affect the properties and the functional expression of the resulting Kv α-subunit-encoded K⁺ currents (329). These observations suggest that several Kv α-encoded Kv channels likely also interact directly with the actin cytoskeleton via α-actinin-2 (Fig. 6). It has also been reported that Kv4 α-subunits interact directly with filamin (401) and that this interaction regulates the functional cell surface expression and the localization of Kv4-encoded channels (401). Subsequent studies revealed that actin depolymerization modulates the cell surface expression of heterologously expressed Kv4-encoded channels (529). Similar to Nav channels, these observations suggest the interesting and potentially important hypothesis that myocardial Kv channels also function as components of macromolecular complexes containing the channel components and a variety of scaffolding and regulatory proteins linked to the actin cytoskeleton (Fig. 6).

Recently, it was also suggested that Kir3-encoded channels interact with integrin (344), suggesting that myocardial K⁺ channel expression and functioning may also be linked to the extracellular matrix. Although clearly in the very early stages, it seems reasonable to suggest that the link between the cytoskeleton (as well, perhaps, as the extracellular matrix) in the regulation of myocardial K⁺ channel expression, localization, and functioning has been made. Further studies, aimed at exploring the roles of the cytoskeleton and the extracellular matrix in regulating myocardial K⁺ channel expression, distribution, and functioning, as well as those focused on probing the underlying molecular mechanisms, will likely provide important new insights into the physiological and pathophysiological roles of these interactions.

IX. SUMMARY AND CONCLUSIONS

Electrophysiological studies have identified multiple types of voltage-gated inward and outward currents expressed in cardiac cells (Table 1). The outward K⁺ currents are more numerous and more diverse than the inward (Na⁺ and Ca²⁺) currents, and most cardiac myocytes express multiple voltage-gated, as well as inwardly rectifying, K⁺ channels (Table 1). These (K⁺) channels are the primary determinants of myocardial action potential repolarization, and regional differences in K⁺ channel densities and properties underlie observed variations in action potential waveforms and contribute to the generation of normal cardiac rhythms. Voltage-gated inward Ca²⁺ channel currents and the Na⁺ channel “window” current, however, also contribute to myocardial action potential repolarization. The pivotal role played by the Nav channel “window” current, for example, has been elegantly demonstrated in electrophysiological studies characterizing mutations in SCN5A that underlie long QT3, as well as in computer-based simulations (Fig. 4) of cellular electrical activity (73, 90, 141, 188).

Molecular cloning has revealed an unexpected diversity of ion channel pore-forming α-subunits (Tables 2, 4, and 6) and accessory subunits (Tables 3 and 5) that contribute to the formation of the various inward and outward current-carrying channels (Table 1) identified electrophysiologically in myocardial cells. Similar to the electrophysiological diversity of myocardial K⁺ channels (Table 1), the molecular analysis has revealed that multiple voltage-gated (Kv) (Table 4) and inwardly rectifying (Kir) (Table 6) K⁺ channel pore-forming α-subunits, as well as a number of accessory subunits of these (Kir and Kv) channels (Table 5), are expressed in the myocardium. A variety of in vitro and in vivo experimental approaches have been exploited to probe the relationship(s) between these subunits and functional myocardial K⁺ channels, and important insights have been provided through molecular genetics and the application of techniques that allow functional channel expression to be manipulated directly. In contrast to the progress made in defining the roles of the various Kv and the Kir α-subunits in the generation of functional myocardial Kv and Kir channels, there is very little known about the functional roles of the K2P α-subunits (Table 6). In addition, the specific and/or multiple functional roles of most of the known K⁺ channel accessory subunits (Table 5) remain to be clarified. Defining the molecular correlates/compositions of the various myocardial K⁺ channels will facilitate future ef-
forts focused on delineating the molecular mechanisms controlling the properties and the functional expression of these channels.

In addition to the diversity of pore-forming and accessory channel subunits (Tables 2–6), several recent studies, exploiting a combination of molecular, biochemical, and electrophysiological approaches, have revealed a rather staggering array of proteins that seem likely to contribute to regulating the properties, the cell surface expression, and/or the subcellular localization of functional myocardial membrane ion channels (Fig. 6). Taken together, the results of these studies suggest that myocardial ion channels function as macromolecular protein complexes. Interestingly, several components of these macromolecular protein channel complexes provide links to the actin cytoskeleton and the extracellular matrix (Fig. 6), suggesting important functional links between different ion channel complexes in cardiac cells, as well as between myocardial structure and electrical functioning. Understanding this molecular complexity clearly demands that novel experimental approaches, such as proteomics and genomics, be exploited to identify the various components of functional ion channel complexes, the sites of protein-protein interactions, and the underlying mechanisms involved in mediating these interactions. These areas represent important areas for further research focus in efforts directed towards defining the detailed mechanisms involved in the regulation/modulation of myocardial membrane excitability and the generation of normal cardiac rhythms.

Numerous studies have documented changes in functional ion channel expression during normal cardiac development, as well as in damaged or diseased heart. It has also been demonstrated that electrical remodeling occurs in the heart in response to changes in electrical activity or cardiac output, and this remodeling is directly attributed to changes in the functional expression and/or the properties of the various ion channels that underlie myocardial action potential generation. Although there are numerous possible (transcriptional, translational, and posttranslational) mechanisms that could be involved in regulating the functional expression and the properties of these channels, very little is presently known about the underlying molecular mechanisms that are important in mediating the changes in channel expression evident during normal development, as well as in conjunction with myocardial damage, disease, and/or electrical remodeling. An important focus of future research efforts will almost certainly be on exploring these mechanisms in detail.

ACKNOWLEDGMENTS

We thank past and present members of our laboratories for their contributions to the understanding of the molecular basis of cardiac repolarization. In addition, we are indebted to Rick Wilson for his expert assistance with the generation of the tables and figures presented in this review and to Dr. Kevin Sampson for conducting the simulations illustrated in Figures 4 and 7.

Address for reprint requests and other correspondence: J. M. Nerbonne, Dept. of Molecular Biology and Pharmacology, Washington University Medical School, 660 South Euclid Ave., St. Louis, MO 63110 (E-mail: jnerbonne@msnotes.wustl.edu).

REFERENCES

16. Akar FG, Yan GX, Antzelevitch C, and Rosenbaum DS. Unique topographical distribution of M cells underlies reentrant mecha-


Molecular Physiology of Cardiac Repolarization


Molecular Physiology of Cardiac Repolarization


Molecular Physiology of Cardiac Repolarization


349. Mitcheson JS and Hancock JC. Characteristics of a transient outward current (sensitive to 4-amino pyridine) in Ca2+ tolerant myocytes isolated from the rabbit atrioventricular node. Pflügers Arch 438: 68–78, 1999.


and episodic ataxia type-2 are caused by mutations in the Ca\textsuperscript{2+} channel gene CACNA1A. Cell 87: 543–552, 1996.


Pate1 SP, Campbell DL, and Strauss HC. Elucidating KChIP effects on Kv4.3 inactivation and recovery kinetics with a minimal KChIP2 isoform. J Physiol 545: 5–11, 2002.


Rosati B, Grau F, Rodriguez S, Li H, Nerbonne JM, and McKinnon D. Concordant expression of KChIP2 mRNA, protein and...


455. Sills MN, Xu YC, Baracchin E, Goodman RH, Cooperman SS, Mandel G, and Chien KR. Expression of diverse Na+ channel


Molecular Physiology of Cardiac Repolarization


