Repolarizing Cardiac Potassium Channels: Multiple Sites and Mechanisms for CaMKII-Mediated Regulation

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Abstract

Marked reductions in the densities of the fast, transient voltage-dependent K^+ (Kv) current, $I_{to,f}$, and of the inwardly rectifying (Kir) K^+ current, I_{K1}, are routinely observed in the hypertrophied and failing human heart and in experimental models of pathological cardiac hypertrophy. Attenuation of these prominent repolarizing K^+ currents results in action potential prolongation and increased dispersion of repolarization, both of which are arrhythmogenic. Cardiac hypertrophy and failure are also associated with increased expression and activity of the multifunctional calcium (Ca^{2+}) calmodulin (CaM) dependent protein kinase II (CaMKII) and several lines of evidence suggest that CaMKII activation can (directly or indirectly) lead to changes in the functional cell surface expression and the biophysical properties of cardiac $I_{to,f}$ and I_{K1} channels.

Keywords

Kv Channels; Kir Channels; Kv4.3; Macromolecular Kv Channel Complexes; Post Translational Regulation/Modulation

Myocardial potassium (K^+) selective channels function to control resting membrane potentials, action potential waveforms, refractory periods and automaticity and, in most cardiac cells, multiple types of voltage-gated K^+ (Kv) and non-voltage-gated, inwardly rectifying, K^+ (Kir) channels, are co-expressed (1). The densities and the properties of these channels change during normal development and in response to altered cardiac output. In contrast with this 'physiological' remodeling, changes in Kv and Kir channel functioning associated with inherited or acquired cardiac disease can have dramatic effects on action potential waveforms, synchronization and propagation, predisposing individuals to potentially life threatening arrhythmias (2). Considerable evidence suggests that myocardial Kv and Kir channels function as components of macromolecular protein complexes, comprising pore-forming (α) and accessory (β) subunits, and linked to cytosolic, cytoskeletal and membrane-associated regulatory proteins (1,3). In addition, these channels are potential targets for the actions of a variety of transmitters, hormones and intracellular signaling pathways, suggesting that multiple, transcriptional, translational and

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Conflicts of Interest: None

Abbreviations: CaMKII, $I_{to,f}$; I_{Kr}; I_{Ks}; I_{K1}

posttranslational, mechanisms contribute to the physiological regulation of myocardial Kv and Kir channels and to the derangements in channel expression and functioning evident in the diseased myocardium.

Cardiac action potential amplitudes and durations are largely determined by Kv channels and, in most cells, two types of Kv currents, transient outward (I_{to}) and delayed rectifier (I_K), are distinguished. These are broad classifications, however, and there are multiple components of I_{to} and I_K, several of which are differentially expressed, contributing to regional heterogeneities in action potential waveforms and in the responses to transmitters and intracellular signaling pathways (1). Outward K^+ currents through Kir, particularly I_{K1}, channels also contribute to repolarization and to regional differences in action potential waveforms and response properties. Changes in cardiac K^+ channel densities, particularly changes in the densities of the K^+ channels that are differentially distributed, are expected to have profound effects on the waveforms of action potentials, the normal propagation of activity in the heart, and the propensity to develop and to sustain arrhythmias (1,2).

In the hypertrophied and failing human heart and in experimental models of (pathological) cardiac hypertrophy, membrane excitability and excitation-contraction coupling are altered (2). Although the molecular mechanisms underlying "electrical remodeling" in the hypertrophied and failing heart are poorly understood, marked reductions in the densities of the fast, transient Kv current, $I_{to,f}$, and of the Kir current, I_{K1}, are consistently reported (2). Downregulation of repolarizing $I_{to,f}$ and I_{K1} channels will lead to action potential prolongation and increased dispersion of repolarization, both of which are arrhythmogenic, motivating interest in defining the molecular determinants of $I_{to,f}$ and I_{K1} channel expression and remodeling (1,2). Cardiac hypertrophy and failure are also associated with increased expression and activity of the multifunctional calcium (Ca^{2+}) calmodulin (CaM) dependent protein kinase II (CaMKII), a serine-threonine kinase activated by binding of Ca^{2+}-bound calmodulin (Ca^{2+}/CaM) and autophosphorylation (4). These observations and studies in experimental models of hypertrophy and failure have led to suggestions that CaMKII might be a potential therapeutic target for the failing heart (4) and direct support for this hypothesis was provided recently with the demonstration that inhibition of CaMKII improves contractility in falling human heart (5).

Clearly, these new findings further demonstrate that understanding the functional role(s) of CaMKII in the regulation of cardiac contractility and cardiac excitability is an important, and a timely, problem. Interestingly, transgenic mice overexpressing CaMKIIδ, the predominant cardiac CaMKII isoform, develop hypertrophy and display increased susceptibility to ventricular arrhythmias (6,7). Action potentials are prolonged in CaMKIIδ-overexpressing ventricular myocytes reflecting, at least in part, reductions in the densities of $I_{to,f}$ and I_{K1} (6). Chronic overexpression of CaMKIIδ also results in reduced expression of the transcripts (*Kcnd2* and *Kcnj2*) encoding the main pore-forming α subunits underlying the generation of mouse ventricular $I_{to,f}$ (Kv4.2) and I_{K1} (Kir2.1) channels (8), suggesting a potential role for CaMKII-mediated transcriptional regulation of these channels, at least under conditions of chronic upregulation of CaMKIIδ expression/activity. It has also been reported, however, that increased CaMKII activity results in down-regulation of *KCND3* (Kv4.3) transcript expression and reduced $I_{to,f}$ densities in canine ventricular myocytes (9).

In contrast, chronic inhibition of CaMKII in the mouse heart results in increased $I_{to,f}$ and I_{K1} densities, although, in this case, no measurable differences in *Kcnd2* and *Kcnj2* transcript expression, or in Kv4.2 or Kir2.1 protein expression, were observed (10). The results of several previous studies have also suggested potential roles for CaMKII in the acute regulation of cardiac $I_{to,f}$ channels. It has been reported, for example, that CaMKII co-immunoprecipitates from adult rat heart lysates with both Kv4.2 and Kv4.3 (11), suggesting

that CaMKII may be a component of native cardiac $I_{to,f}$ channel complexes. Interestingly, a number of cytosolic (KChIP/Kvβ) and transmembrane (DPP6/10, MinK/MiRPs) Kv channel accessory subunits have been shown to associate with Kv4 α subunits in heterologous expression systems and to modify the cell surface expression and/or the properties of Kv4-encoded channels (12), observations interpreted as suggesting that native cardiac $I_{to,f}$ channels function in macromolecular complexes (Figure 1) and, in addition, that each of the protein components of these $I_{to,f}$ channel complexes might well be a target for CaMKII-mediated regulation.

Chronic inhibition of CaMKII also reportedly accelerates $I_{to,f}$ inactivation in human atrial and in rat ventricular myocytes (11,18). In HEK-293 and CHO cells, inhibition of CaMKII also accelerates the rate of Kv4.3-encoded current inactivation (11,19). The opposite effect is produced by autophosphorylated (activated) CaMKII and has been suggested to be mediated by phosphorylation of a CaMKII consensus motif in the C-terminus (Figure 2) of Kv4.3 (19). The association of CaMKII with Kv4.3 reportedly occurs in the absence of increased intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), and the Kv4.3 protein is phosphorylated at low $[Ca^{2+}]_i$ (11), again suggesting that CaMKII may be a component of, or closely associated with, Kv-encoded cardiac $I_{to,f}$ channel complexes. In marked contrast to these results, it has also been reported that chronic and acute overexpression of CaMKIIδ in mouse (chronic) and in rabbit (acute) ventricular myocytes accelerates $I_{to,f}$ inactivation (8). Some of the differences in experimental observations may reflect the fact that different Kv4 α subunits underlie $I_{to,f}$ channels in rodents (Kv4.3) and in larger mammals (Kv4.3), including humans (1). It is certainly possible that some of the apparent differences reflect the molecular complexities of endogenous $I_{to,f}$ channels (Figure 1), which are likely not recapitulated in heterologous expression systems (12). Each of the various Kv4-accessory and regulatory proteins might also be a potential target for direct regulation, or indirect modulation, by CaMKII dependent phosphorylation.

In addition to effects on current kinetics, it has also been reported that co-transfection (in COS-7 cells) of activated CaMKII with Kv4.2 and the accessory subunit, KChIP3, results in increased total and cell surface Kv4.2 expression and that these effects reflect phosphorylation of two CaMKII consensus motifs in the C-terminus of Kv4.2, resulting in increased current amplitudes without measurable changes in gating (20). In HEK-293 cells, increasing $[Ca^{2+}]_i$ reportedly resulted in Kv4.2 phosphorylation and a slowing of inactivation (11). The latter effect is prevented by inhibition of CaMKII, an observation interpreted as suggesting that increased $[Ca^{2+}]_i$ results in the direct modulation of Kv4.2 channels by endogenous CaMKII (11). Given the likely critical roles of the various Kv4 channel accessory subunits (Figure 1) in determining the time-and voltage-dependent properties and the functional cell surface expression of native cardiac $I_{to,f}$ channels (12), however, the possibility that accessory subunits are also important targets of CaMKII-mediated effects on $I_{to,f}$ cannot be excluded. There are multiple putative consensus sites for CaMKII-mediated phosphorylation of KChIP2, as well as Kvβ (Figure 2), any of which might affect functional $I_{to,f}$ channel densities and/or properties. More importantly, this is a possibility that needs to be explored experimentally using Mass spectrometry-based proteomic approaches (21) that will allow basal and stimulated phosphorylation sites on each of the components of native $I_{to,f}$ channel complexes to be identified directly.

Considerable evidence suggests a role for CaMKII in the functional regulation of cardiac I_{K1} channels. In ventricular myocytes from mice overexpressing CaMKIIδ, I_{K1} is attenuated and expression of *Kcnj2* (which encodes Kir2.1) is markedly reduced (8). In contrast, acute overexpression of CaMKIIδ in rabbit ventricular myocytes results in increased I_{K1} densities and this enhancement is blocked by inhibitors of CaMKII (8). It has also been reported that Kir2.× subunits are regulated by a variety of protein kinases (22) and examination of the C

terminus of Kir2.1 reveals three putative CaMKII phosphorylation sites. Experiments focused on isolating endogenous cardiac I_{K1} channels complexes and identification of basal and stimulated CaMKII-dependent phosphorylation sites (23) on Kir2.1, as well as of other components of I_{K1} channel complexes, are needed to delineate the mechanisms involved in the acute and the chronic CaMKII-mediated regulation of native cardiac I_{K1} channel expression and functioning.

Summary and Future Directions

The discussion here was focused on $I_{to,f}$ and I_{K1} channels, primarily because the expression/ properties of these channels are affected in cardiac hypertrophy and in the failing myocardium and the expression of Kv4-encoded $I_{to,f}$ and Kv3-encoded I_{K1} channels are regulated by CaMKII activity. Nevertheless, it is clear that there are multiple other K^+ channels expressed in cardiac cells and that many of these are also potential targets for CaMKII-mediated regulation. Sequence scanning, for example, reveals a number of putative CaMKII phosphorylation sites in several other repolarizing K^+ channel pore-forming α subunits, including Kv1.5, Kv7.1 (KvLQT1) and Kv12.1 (HERG), which encode Kv channels that underlie other critical repolarizing cardiac K^+ currents, I_{Kur}, I_{Kr} and I_{Ks}, in atrial and ventricular myocytes. In Kv1.5, for example, one (T133) putative CaMKII phosphorylation site in the N-terminus and three (T490, S557 and S580) in the C terminus. The number of potential CaMKII sites in Kv12.1 (HERG) is even larger, totaling 20 (9T and 11S) in the cytosolic N- and C-termini, although at least two of these may be mutually exclusive. Similar to cardiac $I_{to,f}$ and I_{K1} channels, proteomic approaches (23) hold the promise of identifying CaMKII-mediated phosphorylation sites on native Kv1.5 , Kv7.1 and Kv12.1 α subunits and/or on the accessory/regulatory proteins that contribute to the functioning of the repolarizing K^+ channels encoded by these α subunits in the mammalian myocardium.

Acknowledgments

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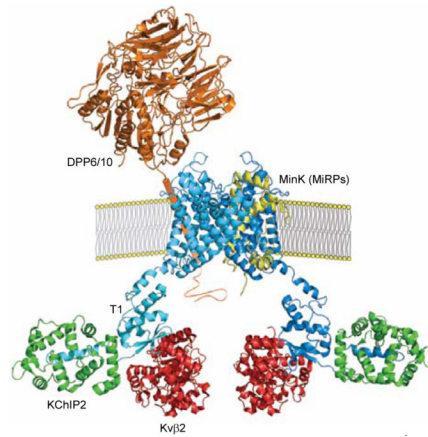
Figure 1. Schematic of putative Kv4.3-encoded channel $I_{to,f}$ macromolecular complex
Cross section of Kv4.3 channel in the membrane showing two Kv4.3 α subunits (blue), each interacting with a KChIP2 (green) and Kvβ2 (red) cytosolic accessory subunit (1:1:1: stoichiometry) through distinct, non-overlapping N-terminal domains (12). The transmembrane accessory subunits, DPP6/10 and MinK/MiRPs, have also been proposed to interact with Kv4.3 α subunits in a 1:2 stoichiometry and contribute to the formation of functional $I_{to,f}$ channels (12). All protein structures were generated based on published structural data (13–17) using PyMOL.
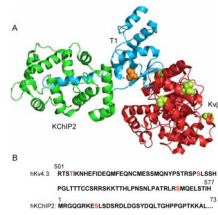
Figure 2. Putative CaMKII phosphorylation sites in Kv4.3, Kvβ2, and KChIP2
(**A**) The T1 domain of one Kv4.3 α subunit associated with Kvβ2 and KChIP2 accessory subunits. Putative CaMKII phosphorylation sites are shown in orange for Kv4.3 (residue T53) and green for Kvβ2 (residues S112, S132, T174, and S192). (**B**) Linear sequences of the hKv4.3C terminus and the hKChIP2N terminus with additional putative CaMKII phosphorylation sites in red