Regulation of Kv4.3 Current by KChIP2 Splice Variants
A Component of Native Cardiac \( I_{to} \)?

Isabelle Deschênes, PhD; Deborah DiSilvestre, MSc; George J. Juang, MD; Richard C. Wu, MD; W. Frank An, PhD; Gordon F. Tomaselli, MD

**Background**—The transient outward potassium current \( (I_o) \) encoded by the Kv4 family of potassium channels is important in the repolarization of cardiac myocytes. KChIPs are a recently identified group of Ca\(^{2+}\)-binding accessory subunits that modulate Kv4-encoded currents. KChIP2 is the only family member expressed in the heart.

**Methods and Results**—We previously cloned 2 novel splice variants of KChIP2 from human heart, named KChIP2S and KChIP2T. The transmural distribution of KChIP2 mRNA and protein in human and canine left ventricle was examined using kinetic RT-PCR and Western blots in the same tissues. A steep gradient of mRNA with greater KChIP2 expression in the epicardium was observed. However, no gradient of immunoreactive protein was observed. Immunocytochemistry reveals KChIP2 expression in the t-tubules and the nucleus. The predominant effects of all 3 KChIP2 splice variants on hKv4.3-encoded current are to increase the density, slow the current decay in a Ca\(^{2+}\)-dependent manner, and hasten recovery from inactivation in a splice variant–specific fashion.

**Conclusions**—A family of KChIP2 proteins is expressed in human hearts that exhibits differential modulation of hKv4.3 current in a Ca\(^{2+}\)-dependent fashion. The effect of KChIP2 on the biophysical properties of expressed Kv4.3 current and the absence of a gradient of protein across the ventricular wall suggest that KChIP2 is either not a requisite component of human or canine ventricular \( I_o \) or that its functional effect is being affected or additionally modified by other factors present in myocardial cells. (*Circulation. 2002;106:423-429.*)

**Key Words:** ion channels ▪ potassium ▪ proteins

The Ca\(^{2+}\)-independent transient outward potassium current \( (I_o) \) plays a significant role in the repolarization of cardiac myocytes.\(^1\) Recent studies have identified candidate genes underlying \( I_o \) in humans, most notably Kv4.3\(^2\) and Kv1.4.\(^3\) Expression of these \( \alpha \)-subunits in heterologous systems generates currents similar to \( I_o \) but does not replicate the native cardiac current with complete fidelity, suggesting that accessory subunits may play a significant role in the functional expression of members of the Kv4 family.

A few general themes have emerged from the study of accessory or \( \beta \) subunits of potassium channels. Coexpression of \( \beta \) subunits with potassium channel \( \alpha \) subunits typically increases current density and alters gating. At least 4 general classes of K channel ancillary subunits have been described; the type of interaction and effects on \( \alpha \) subunit function differ among the potassium channel ancillary subunits.

We sought to examine the role of a new family of Kv channel–interacting proteins (KChIPs) in regulating Kv4.3 channel expression. The KChIPs were initially cloned using a yeast 2-hybrid strategy with the amino acid terminus of rat Kv4.3 as bait.\(^4\) Three members of the KChIP family were found and named KChIP1, KChIP2, and KChIP3. KChIP2 is the only member of the family expressed in the heart. Coexpression of Kv4.x and KChIP2 splice variants increases peak current density and alters the voltage dependence and kinetics of gating compared with Kv4.x channels expressed alone.\(^4\)\(^-\)\(^6\)

We have cloned 2 unique splice variants of human KChIP2 and examined their electrophysiological effects on expressed human Kv4.3 current and their role in the formation of cardiac \( I_o \). KChIP2 splice variants are expressed in the human and canine ventricle; however, the previously described transmural gradient in mRNA\(^7\) is not observed at the level of immunoreactive protein. Furthermore, the functional effects of all of the KChIP2 splice variants on hKv4.3 current decay expressed in mammalian cells diminish rather than enhance the mimicry of cardiac \( I_o \). Although mouse KChIP2 was shown recently to be necessary for expression of \( I_o \) in mouse heart,\(^8\) our data cast doubt on the notion that KChIP2 alone is responsible for the density gradient and biophysical features of native human and canine cardiac \( I_o \).
Methods

Cloning of KChIP2 Splice Variants

We used 3 different sets of primers designed from the previously identified KChIP2 for reverse transcriptase–polymerase chain reaction (RT-PCR). Total human heart RNA was prepared from normal and failing human left ventricle with TRizol reagent (Life Technologies, Rockville, Md) according to the manufacturer’s instructions. The sequences for each of the splice variants have previously been deposited in Genebank (accession Nos. KChIP2S AF295530 and KChIP2T AF295076).

Heterologous Expression of hKv4.3 and KChIP2s in HEK293 Cells

Transient transfections of hKv4.3 expressed in pCDNA3.1 and of the KChIP2s expressed in pCMV-IRS were performed in human embryonic kidney cells (HEK293) using lipofectamine (Life Technologies, Rockville, Md), as previously described.

Electrophysiology

Cells were used for patch-clamp experiments 24 to 72 hours after transfection. Currents from transfected cells were recorded in the whole-cell configuration of the patch clamp. Cells that emitted green fluorescence and that expressed L- current-like current were considered to express both hKv4.3 and KChIP2. Currents were clamped using an Axopatch 200A amplifier (Axon Instruments) interfaced to a personal computer. The internal solution contained (in mmol/L) NaCl 140, MgCl2 1, N-2-HEPES 10, EGTA 1, and Mg-ATP 2 with pH adjusted to 7.2 with KOH. The bath solution contained (in mmol/L) NaCl 140, KCl 5, MgCl2 1, CaCl2 2, HEPES 10, and glucose 10 adjusted to pH 7.4. For the experiments with Ca2+-buffering, 0.1 mmol/L EGTA was added to the bath solution and 10 mmol/L BAPTA was added to the internal solution. Membrane currents were filtered at 5 kHz and digitized with 12-bit resolution. Experiments were performed at room temperature (22 to 23°C).

Thin sections of the left ventricle were dissected from the endocardium (~1 mm) and epicardium (1 to 2 mm), respectively, and frozen in liquid nitrogen. Similarly, sections from the mid portion of the ventricular wall were rapidly frozen in liquid nitrogen for later protein and RNA isolation. Cells were isolated from endocardial and epicardial sections of similar thickness and the mid ventricular wall from control and failing hearts after protease/collagenase digestion via the LAD, as previously described. The cells were stored at room temperature in Tyrode’s solution consisting of (in mmol/L) NaCl 140, KCl 5, MgCl2 1, HEPES 10, and glucose 10, pH 7.4, supplemented with 10 taurine, 5 Na pyruvate, and 20 BDM. The concentration of CaCl2 was gradually raised from 100 μmol/L to 1 mmol/L.

Whole-cell L currents in myocytes were recorded at 22 to 24°C in the presence of 0.3 mmol/L CaCl2 to eliminate voltage-activated Ca2+ and Ca2+-dependent currents, as previously described.

Real-Time PCR and Western Blotting

Nonfailing human cardiac tissue was obtained from explanted hearts deemed unsuitable for transplantation. Failing human cardiac tissue was obtained from explanted hearts of patients undergoing heart transplantation for terminal heart failure. Normal canine tissue was obtained from mongrel dogs, and failing canine tissues were obtained from dogs subjected to rapid ventricular pacing, as previously described.

Fluorescence-based kinetic real-time PCR was performed using a Perkin-Elmer Applied Biosystems Model 7900 sequence-detection system. Total RNA was isolated from canine and human ventricle using Qiagen RNAeasy with on-column DNase digestion. The 5′ nuclease activity of Taq DNA polymerase cleaves the probe, and a fluorescent signal is generated that is proportional to the amount of starting target template. Each reporter signal is then divided by the fluorescence of an internal reference dye (ROX) to normalize for non-PCR-related fluorescence.

Tissue lysates were prepared as previously described. All samples were run in duplicate on 12.5% Tris-HCl precast gels (Bio-Rad) in 25 mmol/L Tris, 192 mmol/L glycine, and 0.1% (wt/vol) SDS running buffer, as previously described. A standard control sample was run on all gels to facilitate comparisons across gels. Primary antibody incubations were performed overnight at 4°C using the previously described rabbit pan-KChIP polyclonal antibody or an antibody raised to an epitope (SYDQLTDSVDD) that spans the splice excision site in KChIP2 that is present in KChIP2S and 2T; this antibody will be referred to as anti-KChIP2 S/T. Bands on the Western blots were considered specific if they were not present when the gel was stained with preimmune serum and for anti-KChIP2S/T if peptide epitope reduced the band intensity. Relative band densities were quantified using ImageQuant software (Molecular Dynamics).

Immunohistochemistry

Ventricular myocytes were plated on laminin-coated coverslips before permeabilization and staining. Cells were incubated overnight at 4°C with pan-KChIP, mouse monoclonal anti-a-actinin antibody (Chemicon International, Temecula, Calif), or rabbit polyclonal anti-Kv4.3 antibody (Chemicon International). Incubation with secondary antibodies was performed at room temperature for 1 hour using anti-rabbit Alexa 488 (Molecular Probes, Eugene, Ore) or anti-mouse Rhodamine Red-X (Jackson Immunoresearch, West Grove, Penn). Imaging was performed on a Nikon Diaphot 300 inverted epi-fluorescence microscope attached to a PCM-2000 laser scanning confocal microscope system (Nikon, Inc).

Statistical Analysis

Comparisons of the biophysical properties of expressed hKv4.3 current in the presence and absence of the different KChIP2 splice variants were performed using an unpaired t test. Data were considered significantly different at P<0.05.

Results

Sequence Analysis of KChIP2 Splice Variants

At least 3 different splice variants of KChIP2 are present in the human heart. In addition to KChIP2, which is 759 bp and encodes a protein of 253 amino acids, we cloned 2 additional splice variants from human cardiac tissue that we called KChIP2S and KChIP2T. KChIP2S and KChIP2T have a deletion of 96 nucleotides starting at position 74 in the KChIP2 sequence. This results in the loss of the 32 amino acids amino terminal to the first of 4 Ca2+-binding EF-hand motifs. A single nucleotide change results in a substitution of Asn in KChIP2 for Asp at position 57 in KChIP2S. The remaining portion of KChIP2S is identical to KChIP2. KChIP2T contains a 21-bp insertion at position 295 encoding the amino acid sequence PGALFSQ immediately amino terminal to the first EF hand.

Electrophysiological Recordings of hKv4.3 in the Presence of KChIP2 Splice Variants

We examined the effect of the KChIP2 splice variants on the electrophysiology of hKv4.3-encoded currents expressed in HEK293 cells (Figure 1). Coexpression of any of the KChIP2 variants altered the biophysical properties of the hKv4.3 current. The current density was increased by 2- to 3-fold by KChIP2 coexpression compared with hKv4.3 expressed alone (Figures 1 and 2A). None of the KChIP2 variants altered the current-voltage relationship (Figure 2F) or the steady-state availability of Kv4.3 (Figure 2E) (Table 1).

The most prominent effect of all of the KChIP2 variants was a slowing of the current decay of hKv4.3 at all voltages.
between 0 and +60 mV (Figures 1 and 2B). At +60 mV, the time constants of the current decays (τ) were well fit by single exponentials; the time constant was 70.3 ± 11.4 ms for hKv4.3 alone, and coexpression of KChIP2 and KChIP2T slowed the decay 2-fold and KChIP2S nearly 3-fold (Table 1) despite similar effects on the current density.

KChIP2 and KChIP2S altered recovery from inactivation. The time constant for recovery from inactivation (τrec) of hKv4.3 was 75.1 ± 2.1 ms. In the presence of KChIP2 and KChIP2S, the current recovered significantly faster than hKv4.3 alone (Table 1). However, coexpression of KChIP2T had no significant effect on the rate of recovery from inactivation (Table 1, Figure 2C).

Each of the KChIP2 splice variants contains 4 EF-hand motifs, suggesting a role for Ca²⁺ in the functional effects of these ancillary subunits. We buffered both intracellular and extracellular Ca²⁺ (to <0.1 nmol/L free Ca²⁺) to determine if any of the effects of KChIP2 on Kv4.3 current were Ca²⁺ dependent. Buffering Ca²⁺ did not affect the biophysical properties of Kv4.3 expressed alone. Furthermore, neither the augmented current density nor hastening of recovery from inactivation was affected by buffering Ca²⁺. Buffering Ca²⁺ had 2 splice variant–specific effects on the current decays. The slowing of hKv4.3 current decay by all 3 KChIP2 splice variants was attenuated when intracellular and extracellular Ca²⁺ was buffered: KChIP2S-containing currents exhibiting the largest change (Figure 2D and Table 1). When Kv4.3 was coexpressed with KChIP2 and KChIP2S, the current decays significantly hastened over the range of test potentials from 0 to +60 mV with Ca²⁺ buffering. In contrast, KChIP2T-containing currents exhibit minimal voltage dependence of the current decay with Ca²⁺ buffering. Indeed, at +60 mV, the time constants of the current decay in the presence and absence of the KChIP2 splice variants converged (Table 1, Figure 2D).

Immunohistochemistry of KChIP2
The subcellular distribution of KChIP2 in human ventricular myocytes was examined by immunocytochemistry of normal and failing human myocytes using a polyclonal antibody to KChIP1 (pan-KChIP) that recognizes all 3 variants. KChIP2 colocalizes with hKv4.3 in a t-tubular distribution in human ventricular myocytes and prominently stains the nucleus (Figure 3). Costaining of myocytes with α-actinin, which stains between the Z-lines, reveals a close proximity between KChIP2 and α-actinin without colocalization (Figures 3A

Figure 1. Currents were recorded from HEK293 cells transfected with either hKv4.3 alone (A) or hKv4.3 coexpressed with KChIP2 (B), KChIP2S (C), and KChIP2T (D).

Figure 2. The effect of KChIP2 on hKv4.3 currents. A, Bar graph of the current densities (±5 cells in each group, at least 2 transfections for each isoform) reveals at least a doubling of the density for all of the splice variants compared with hKv4.3 alone. There is no statistically significant difference in the effect of the splice variants on current density. B, Plot of the time constants of inactivation in absence of Ca²⁺ buffering from a single exponential fit over the entire range of test voltages. The time constants of current decay are significantly greater in the presence of KChIP2 splice variants compared with hKv4.3 expressed alone. Currents encoded by KChIP2S-hKv4.3 exhibited significantly slower decays compared with the currents encoded by hKv4.3 and either KChIP2 or KChIP2T over the range of test potentials from 0 to +60 mV. C, Recovery curves after a 500-ms test pulse (P1) in absence of Ca²⁺ buffering. The data are fit with single exponentials. D, Plot of time constants at different voltages as in panel B but in the presence of intracellular and extracellular Ca²⁺ buffering. Currents encoded by Kv4.3-KChIP2S or KChIP2 exhibited voltage-dependent speeding of the current decay over the range of test potentials from 0 to +60 mV. Steady-state availability (E) and current-voltage relationships (F) in absence of Ca²⁺ buffering are not affected by KChIP2 variants.
Functional Effects of KChIP2 Splice Variants on hKv4.3 Currents Expressed in HEK293 Cells

<table>
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<tr>
<th>Biophysical Parameters</th>
<th>hKv4.3</th>
<th>hKv4.3 + KChIP2</th>
<th>hKv4.3 + KChIP2S</th>
<th>hKv4.3 + KChIP2T</th>
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</thead>
<tbody>
<tr>
<td>Inactivation time constant, $\tau_{in}$ in ms at $-60$ mV</td>
<td>$70 \pm 11$</td>
<td>$148 \pm 17^*$</td>
<td>$78 \pm 16$</td>
<td>$209 \pm 20^*$</td>
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<tr>
<td>Recovery from inactivation, $\tau_{re}$ in ms</td>
<td>$75.1 \pm 2.1$</td>
<td>$41.2 \pm 1.1^*$</td>
<td>$45.9 \pm 2.6^*$</td>
<td>$45.8 \pm 6.2^*$</td>
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<td>Steady-state inactivation, $V_{1/2}$ in mV</td>
<td>$-45.2 \pm 1.6$</td>
<td>$-44.7 \pm 1.4$</td>
<td>$-46.5 \pm 1.9$</td>
<td>$-45.6 \pm 1.5$</td>
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<td>No. of cells</td>
<td>6</td>
<td>5</td>
<td>5</td>
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*Significantly different from hKv4.3 + Ca$^{2+}$ (P<0.05).
†Significantly different from hKv4.3 + KChIP2 and hKv4.3 + KChIP2T + Ca$^{2+}$ (P<0.05).
‡Significantly different from hKv4.3−Ca$^{2+}$ (P<0.05).
§Significantly different from hKv4.3 + KChIP2 and hKv4.3 + KChIP2S + Ca$^{2+}$ (P<0.05).
∥Significantly different from hKv4.3 + KChIP2 and hKv4.3 + KChIP2S−Ca$^{2+}$ (P<0.05).

Expression of KChIP2 mRNA and Protein

We measured KChIP2 mRNA levels using kinetic real-time PCR with a fluorescent probe designed to recognize all of the splice variants. A steep gradient of KChIP2 mRNA has previously been measured across the left ventricular wall. We confirmed this result with real-time PCR in normal human left ventricle myocardium normalized to 18S rRNA, demonstrating a 14-fold increase in KChIP2 mRNA in epicardium compared with endocardium (Figure 4A).

Western blots of human and canine tissues using a pan-KChIP antibody reveals several specific bands between 25 to 35 kDa, with the predominant band in both species running at ~34 kDa (Figures 4B and 4C). With prolonged exposure, 2 other lower molecular weight bands are apparent. The additional bands (eg, Figure 4B) could be attributable to protein degradation but are consistent with expressed KChIP2 splice variants. We therefore generated an antibody that recognizes a sequence in KChIP2S and 2T that straddles the deleted segment in KChIP2 before the first EF hand (anti-KChIP2 S/T). Anti-KChIP2 S/T recognizes only KChIP2S and KChIP2T expressed in HEK293; no specific bands appear in naive cells or cells transfected with KChIP2 (Figure 4E). The band below 25 kDa is nonspecific and seen with exposure to preimmune serum. The pan-KChIP antibody recognizes all 3 variants expressed in HEK293 cells (data not shown). Western blots of canine tissues using our specific KChIP2S/2T antibody revealed a single band of ~28 kDa (Figure 4D), suggesting that one or more of the splice variants is expressed in the canine heart.

Interestingly, despite the steep gradient in mRNA (Figure 4A), no such protein gradient is observed with the pan-KChIP antibody in either human or canine heart (Figures 4B and 4C). Similarly, the anti-KChIP2 S/T antibody does not reveal a gradient of expression of the KChIP2S or 2T splice variants across the left ventricular wall (Figure 4D).

Gradient of Functional Expression of KChIP2

Is there any evidence for a function gradient of KChIP2 expression? A steep gradient of expression of functional...
KChIP2 at the cell surface membrane would predict distinct biophysical profiles of the $I_{\text{to}}$ currents expressed in cells isolated from the different layers of the canine heart. We have examined $I_{\text{to}}$ recorded from the endocardial, midmyocardial, and epicardial layers of the canine left ventricle. The density of $I_{\text{to}}$ current is generally smaller in endocardial compared with epicardial or midmyocardial cells. The kinetics of the current decay does not differ significantly in the 3 layers of the left ventricle. The single exponential fits to the falling phase of the $I_{\text{to}}$ exhibit no voltage dependence over a range of test voltages from $-110$ to $-60$ mV (Figure 5). There are no significant differences in the voltage dependence of the steady-state availability or the fast component of recovery from inactivation (data not shown). Thus, there seems to be neither a protein nor functional gradient of KChIP2 expression across the wall of the left ventricle.

**Discussion**

**Splice Variants of KChIP2**

We have cloned 3 splice variants of KChIP2 from human heart, the previously described KChIP2, and 2 splice variants we named KChIP2S and KChIP2T, which have also been referred to as KChIP2.2 and KChIP2.3, respectively. KChIP2S is similar to the rat KChIP2S clone described by Ohya et al; however, their rat KChIP2L is distinct from our KChIP2T. The KChIP2S and 2T splice variants are homologous to rat (NM020094) and mouse (AB044571) KChIP2 sequences, respectively. Western blots with both polyclonal KChIP and 2S/T-specific antibodies suggest that all 3 of the splice variants are present in human and canine ventricles. The predominant form of KChIP2 in both human and canine ventricle is $\sim 34$ kDa, with a band at 25 to 30 kDa that is also recognized by the smaller splice variant–specific antibody anti-KChIP2 S/T (Figure 4).
By immunocytochemical staining using a pan-KChIP antibody, we have localized KChIP2 to the t-tubules of human myocytes. This localization and the cloning of KChIP2 by yeast 2-hybrid interaction with rKv4.3 are consistent with a physical association of KChIP2 and Kv4.3. However, our data suggest that KChIP2 does not exert a functional effect on \(I_{Na}\) in ventricular myocytes.

Notably, KChIP2 antibody also stains the nucleus; this is unlike any of the K channels or cytosolic proteins (\(\alpha\)-actinin) studied (Figure 3). The nuclear localization and homology of KChIPs to calsenilin and the transcriptional modulator DREAM\(^{16,17}\) suggest roles for KChIP other than functional regulation of Kv4 channels in the heart.

**Modulation of Kv4.3 by KChIP2 Splice Variants**

The KChIP2 splice variants have differential effects on expressed hKv4.3 currents. Each of the KChIP2 splice variants at least doubled the current density of heterologously expressed hKv4.3 (Figures 1 and 2A). The change in current density is qualitatively consistent with previous reports, but the augmentation of expression that we observed was not as robust as previously reported.\(^4,5\) The reason for this difference is unclear; however, it does not appear that HEK293 cells express a significant amount of KChIP2 protein, because no specific bands were identified on Western blots of naive cells (Figure 4E). The increase in Kv4 current density may be related to several factors. First, there may be increased protein expression of Kv4.3 attributable to increased transcription or translation of Kv4.3 mRNA. KChIP3 has been noted to be 99% similar in sequence to the calcium-regulated repressor of transcription DREAM.\(^{16,17}\) It is possible that KChIP2 likewise functions at a transcriptional level, increasing Kv4.3 mRNA production. KChIP2 may also act as a chaperone similar to that of KChAP,\(^{18}\) where the protein is protected from degradation during assembly. Ancillary subunits have also been shown to enhance trafficking of channels to cell surface.\(^{19–22}\) It is possible that KChIP2 has a similar effect on Kv4.3 channels.

Independent of the effect of KChIP2 on Kv4.3 mRNA or immunoreactive protein, this subunit modulates channel gating in heterologous expression systems. Each KChIP2 splice variant has a unique constellation of effects on hKv4.3 currents. The most prominent gating effect of all of the variants is slowing the decay of the current (Figure 2B), similar to previous reports.\(^4–6\) As previously described,\(^4–6\) KChIP2 and 2S hasten recovery from inactivation of Kv4.3-encoded currents; however, KChIP2T did not have an effect on recovery from inactivation (Figure 2C). In contrast to other studies,\(^5,6\) we observed no significant effect of any of the splice variants on the steady-state availability (Figure 2E). The differences may be related to the varied expression systems and experimental conditions used in each of the studies. Several lines of evidence argue against the absence of an effect on the availability curve as the result of poor expression of KChIP2. First, we identified cells transfected with KChIP2 variants by epifluorescence and parallel Western blots. More importantly, the absence of an effect on the voltage dependence of inactivation was observed in the presence of a clear-cut slowing of current decay.

The presence of KChIP2 provides a mechanism for Ca\(^{2+}\)-mediated regulation of Kv4.3 current. The 3 KChIP2 splice variants differ in their amino termini in the region preceding the first of 4 EF hands\(^6\) and do exhibit distinct effects on Kv4.3 current in both the presence and absence of Ca\(^{2+}\). The enhancement of current density and hardening of recovery from inactivation (KChIP2, 2S) do not require Ca\(^{2+}\), but buffering of Ca\(^{2+}\) drastically reduces KChIP2-induced slowing of current decay by all KChIP2 variants (Figures 2B and 2D, Table 1). Interestingly, without Ca\(^{2+}\) buffering, currents comprised of KChIP2+Kv4.3 do not exhibit voltage dependence of the current decay, and buffering Ca\(^{2+}\) enhances the voltage dependence of decay. Buffering Ca\(^{2+}\) also imparts voltage dependence of decay to the currents produced by coexpression of Kv4.3+KChIP2S. These data highlight the importance of the N-terminus in functional modulation of Kv4 current. Additional experiments will be required to confirm these hypotheses.
Deschênes et al. Regulation of Kv4.3 Current by KChIP2

The net effect of slowing the current decay (Figures 1 and 2B) and hastening recovery from inactivation (Figure 2C) is a destabilization of inactivation. Such a destabilization would tend to make more I\textsubscript{K} available and is qualitatively consistent with the transmural gradient of KChIP2 mRNA expression\textsuperscript{2} and the well-described increase in I\textsubscript{K} density in the midmyocardium and epicardium compared with the endocardium.\textsuperscript{23} However, the magnitude of the mRNA gradient significantly exceeds that of I\textsubscript{K} density; furthermore, there is no significant gradient of protein expression of any of the KChIP2 splice variants across the layers of the left ventricular in either humans or dogs (Figure 4), although recent work by Patel et al.\textsuperscript{24} has demonstrated a gradient of protein expression of novel KChIP2 splice variant in the ferret heart. Thus, the gradient in I\textsubscript{K} density (Figures 5A through 5C)\textsuperscript{23} in the midmyocardium and epicardium is not explained by a gradient of expression of KChIP2 proteins. It is unclear how the gradient of KChIP mRNA previously described\textsuperscript{2} and confirmed in our work using real-time PCR (Figure 4A) relates to I\textsubscript{K} modulation. Also, the slowing of current decay produced by coexpression of KChIP2 with hKv4.3 compared with hKv4.3 alone is inconsistent with data from native cardiac myocytes, where the time constants of macroscopic current decay under similar recording conditions are \(<50\) ms\textsuperscript{1,25} (Figure 5). Other constituents of the myocardial cell environment, such as additional ancillary subunits or arachidonic acid,\textsuperscript{26} may also contribute to the function of the native I\textsubscript{K} and its modulation by KChIP2.

If functional KChIP2 protein were differentially expressed across the ventricular wall, one would anticipate a difference in the biophysical signature of I\textsubscript{K} currents from different layers of the left ventricle. In cells isolated from the canine ventricle, we do not observe changes in the kinetics of I\textsubscript{K} that suggest differential functional expression of KChIP2 splice variants.

In conclusion, KChIP2 splice variants modulate hKv4.3 function in an isoform-specific manner and require Ca\textsuperscript{2+} for some, but not all, of their functional effects. The calcium dependence provides yet another link between Ca\textsuperscript{2+} signaling and cell electrophysiology. The homology to transcriptionally active proteins, subcellular localization, and absence of density in the midmyocardium and epicardium is not explained by a gradient of expression of KChIP2 protein and cell electrophysiology. The homology to transcriptionally active proteins, subcellular localization, and absence of density in the midmyocardium and epicardium is not explained by a gradient of expression of KChIP2 protein and cell electrophysiology. The homology to transcriptionally active proteins, subcellular localization, and absence of density in the midmyocardium and epicardium is not explained by a gradient of expression of KChIP2 protein and cell electrophysiology.

Acknowledgments

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References

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