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The Accessory Subunit KChIP2 Modulates the Cardiac L-type Calcium Current

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Abstract

Complex modulation of voltage-gated Ca^{2+} currents through the interplay among Ca^{2+} channels and various Ca^{2+}-binding proteins (CaBPs) is increasingly being recognized. The K^+ channel interacting protein 2 (KChIP2), originally identified as an auxiliary subunit for $K_V4.2$ and a component of the transient outward K^+ channel (I_{to}), is a CaBP whose regulatory functions do not appear restricted to $K_V4.2$. Consequently, we hypothesized that KChIP2 is a direct regulator of the cardiac L-type Ca^{2+} current ($I_{Ca,L}$). We found that $I_{Ca,L}$ density from KChIP2$^{-/-}$ myocytes is reduced by 28% compared to $I_{Ca,L}$ recorded from WT myocytes ($P<0.05$). This reduction in current density results from loss of a direct effect on the Ca^{2+} channel current, as shown in a transfected cell line devoid of confounding cardiac ion currents. $I_{Ca,L}$ regulation by KChIP2 was independent of Ca^{2+} binding to KChIP2. Biochemical analysis suggested a direct interaction between KChIP2 and the $Ca_V1.2$ α_{1C} subunit N-terminus. We found that KChIP2 binds to the N-terminal inhibitory (NTI) module of α_{1C} and augments $I_{Ca,L}$ current density without increasing $Ca_V1.2$ protein expression or trafficking to the plasma membrane. We propose a model in which KChIP2 impedes the NTI module of $Ca_V1.2$, resulting in increased $I_{Ca,L}$. In the context of recent reports that KChIP2 modulates multiple K_V and Na_V currents, these results suggest that KChIP2 is a multimodal regulator of cardiac ionic currents.

Keywords

Ion channels; mouse models; amino terminal inhibitory module; auxiliary subunit; $Ca_V1.2$

Introduction

The K_V4 subfamily of voltage-gated K^+ channels underlies the A-currents in neuronal tissue (I_A)[1] and the fast-inactivating transient-outward current in the heart ($I_{to,f}$).[2] The K^+ Channel Interacting Proteins (KChIPs) were discovered as a family of four alternatively spliced accessory subunits for the A-type K^+ currents in neuronal tissue.[3, 4] Whereas all KChIPs are expressed in brain[3, 4], only KChIP2 is found in heart, where it interacts with $K_V4.2$ and $K_V4.3$ to form the native $I_{to,f}$.[5, 6] K_V4 subunits co-localize with all KChIP subtypes when expressed in cell lines, and the addition of any KChIP increases the K_V4-current density.[7, 8]

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Further, KChIP1, KChIP2 and KChIP3 slow K_V-current inactivation and speed recovery from inactivation.[3, 5, 9] KChIP4 has been described as an inactivation suppressor of the K_V4 current, where it abolishes fast inactivation.[10]

The KChIPs are neuronal calcium-sensor (NCS) proteins[11] and contain four Ca^{2+}-binding EF-hand motifs, of which the last three bind Ca^{2+}.[3] EF-hand mutations that ablate Ca^{2+} binding disrupt K^+ channel modulation, but preserve binding affinity to K_V4.[3] NCS proteins and KChIPs share structural homology with the superfamily of Ca^{2+}-binding proteins (CaBP)[12, 13], several of which regulate $I_{Ca,L}$ in the heart. Most prominently, calmodulin (CaM)[14-17] and Ca^{2+}-binding protein 1 (CaBP1)[18] differentially govern Ca^{2+}-dependent inactivation of $I_{Ca,L}$ via interactions with the amino- (N) or carboxy- (C) termini of the pore-forming α_{1C} subunit, $Ca_V1.2$. Other members of the CaBP superfamily variously affect different L-type Ca^{2+} channels (e.g. $Ca_V1.3$)[19, 20] or non-L-type Ca^{2+} channels (e.g., $Ca_V2.1$)[21], suggesting an extensive interplay among various Ca^{2+} channels and CaBPs.

Whether KChIP2 regulates Ca^{2+} channels has not been explored, although emerging evidence implicates KChIP2 as a modulator of channels other than K_V4, particularly in the heart. For example, in cell lines KChIP2 impairs trafficking of $K_V1.5$[22], and KChIP2 silencing has been reported to eliminate the sodium current (I_{Na}) in cultured neonatal cardiomyocytes.[23] Thus, in the present study, we hypothesized that KChIP2 modulates the L-type Ca^{2+} current. Using both ventricular myocytes and a heterologous expression system, we show that KChIP2 increases $I_{Ca,L}$. Furthermore, biophysical and functional experiments show that KChIP2 binds specifically to the amino-terminal inhibitory (NTI) domain of $Ca_V1.2$ in a Ca^{2+}-independent manner. This KChIP2-NTI association causes an increase in the Ca^{2+}-current density, independent of protein synthesis and trafficking.

Materials and Methods

Electrophysiological recordings in disaggregated ventricular myocytes

We studied adult (10-12 weeks), male C57BL6 WT and re-derived KChIP2$^{-/-}$ mice.[24] All animal experiments were approved by the Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health). Genotyping was performed using DNA isolated from tail samples, followed by PCR amplification of KChIP2 and neomycin. ECGs obtained from anesthetized WT and KChIP2$^{-/-}$ mice (Online Figure I) showed ST-segment elevation specific to the KChIP2$^{-/-}$ mice, consistent with earlier reports from the KChIP2$^{-/-}$ mice originally generated in the 129SV background.[24]

Hearts were excised from anesthetized (0.1 mg/g ketamine, 0.01 mg/g xylazine, IP) mice and myocytes were disaggregated from the left ventricular free wall. Membrane recordings were made within 6 hours of myocyte isolation using standard whole-cell voltage-clamp protocols. Patch pipettes (1-2 MΩ) contained (in mM): 80 aspartic acid, 10 NaCl, 70 CsOH, 40 CsCl, 2 $MgCl_2$, 10 EGTA, 10 HEPES-Na, 2 ATP-Na_2, and 0.1 GTP-Na_2 (pH 7.2). Cells were superfused with (in mM): 135 NaCl, 10 CsCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 HEPES-Na, 10 dextrose, and 0.01 tetrodotoxin (pH 7.4; T 37°C). Current-voltage curves were generated as nifedipine-sensitive (5 μM) difference-currents using 400-ms depolarizing steps from a holding potential of −40 mV as described.[25] Currents are expressed as peak-inward density; cell capacitances were similar for WT and KChIP2$^{-/-}$ myocytes.

Molecular biology

cDNA constructs consisted of rabbit α_{1C} ($Ca_V1.2$), and rat $Ca_V\beta_2$, $Ca_V\alpha_2\delta_2$, and GFP-KChIP2b (IRES) and GFP in pcDNA3.1. The ΔNT-$Ca_V1.2$ (amino acids 140-2171), and KChIP2-ΔEF

(Asp residues at position 1 in EF-hands 2, 3 and 4 of KChIP2b mutated to Ala) were generated by standard molecular biology techniques. The GFP-α_{1C} construct was constructed as previously described.[26]

Electrophysiological recordings in tsA201 cells

Transfection of 5 μg total DNA (35 mm culture dish) into tsA201 cells (ECACC, Wiltshire, UK) was performed using calcium-phosphate precipitation (Chemicon, Phillipsburg, NJ). The tsA201 cell line is an SV-40 transformed variant of the HEK293 cell line that produces large amounts of recombinant protein. Transfected cells were identified by green fluorescent protein. Patch-clamp experiments were conducted 48 hours after transfection. Pipettes (3-5 MΩ) contained (in mM): 135 Cs-$MeSO_3$, 5 CsCl, 5 EGTA, 1 $MgCl_2$, 10 HEPES-Na, 4 mM ATP-Na_2 (pH 7.2). External solution contained (in mM): 140 TEA-Cl, 10 HEPES-Na, 5 $CaCl_2$ (pH 7.3; T 22°C). Ba^{2+} containing superfusate was prepared by equimolar substitution of $BaCl_2$ for $CaCl_2$. Expressed currents were recorded using 400-ms depolarizing steps to −40 to +60 mV from a holding potential of −90 mV.

Protein chemistry

Immunoblotting was done using previously described methods.[27] Briefly, whole-cell protein lysates were obtained from WT and KChP2$^{-/-}$ ventricular tissue and from transfected tsA201 cells. Myocardial protein lysates were fractionated using a protocol from Ventura et al.[28] Proteins were separated on 4-20% gradient SDS-PAGE gels and transferred to PVDF membranes. Blots were probed with primary antibodies against $Ca_V1.2$ (NeuroMab, CA), GAPDH or actin (SantaCruz, CA). Fusion proteins (GST-tagged N terminus and 6xHis-tagged C terminus) were co-expressed with KChIP2 and purified as described earlier.[29]

Statistical analysis

Data are expressed as means±SEM. One-way ANOVA followed by Bonferroni's *t* test, when appropriate, was used for statistical comparison. $P<0.05$ was considered statistically significant.

Results

Mice Lacking KChIP2 Have Reduced $I_{Ca,L}$

We compared patch-clamp recordings from disaggregated left-ventricular myocytes from WT and KChIP2$^{-/-}$ mice to investigate whether KChIP2 regulates $I_{Ca,L}$. Figure 1A (left) shows representative nifedipine-sensitive current traces. The current-voltage relationship demonstrates reduced $I_{Ca,L}$ in KChIP2$^{-/-}$ myocytes compared to WT myocytes, independent of test voltage (Figure 1A, right). For example, at a test voltage (V_{test}) of +10 mV, $I_{Ca,L}$ density was −16.6±1.0 pA/pF in WT myocytes (n=15) versus −12.0±0.7 pA/pF (n=18; $P<0.05$) in KChIP2$^{-/-}$, a 28% reduction. There were no changes in $I_{Ca,L}$ activation or steady-state inactivation (Figure 1B). The decreased current amplitude in KChIP2$^{-/-}$ myocytes was associated with slower Ca^{2+}-dependent inactivation. The decay phase of the current trace was best fit with 2 exponentials. At voltage steps to +10 mV, τ_{fast} and τ_{slow} for WT myocytes were 10±1 and 61±10 ms, respectively, compared with 13±1 ($P<0.05$) and 81±5 ms ($P=0.07$), respectively, for KChIP2$^{-/-}$ myocytes.

The reduced $I_{Ca,L}$ could not be explained by less $Ca_V1.2$ protein: rather, we found a 64% increase in $Ca_V1.2$ protein in whole-cell lysates from KChIP2$^{-/-}$ ventricular tissue, compared to WT ($P<0.05$; Figure 1C). When we partitioned protein lysates from WT and KChIP2$^{-/-}$ ventricles into cytosolic, light membrane (including the plasma membrane), heavy membrane (including the sarcoplasmic reticulum) and debris (predominantly nuclei) fractions, we

observed significantly more $Ca_V1.2$-immunoreactive protein in all KChIP2$^{-/-}$ fractions (Figure 1D), suggesting that the decreased $I_{Ca,L}$ in KChIP2$^{-/-}$ mice does not result from deficient protein trafficking. Thus, despite an increase in $Ca_V1.2$ protein, the absence of KChIP2 attenuates $I_{Ca,L}$ in myocytes.

KChIP2 Augments Ca^{2+} Currents in a Heterologous Expression System

The reduced $I_{Ca,L}$ in KChIP2$^{-/-}$ myocytes could be due to either loss of a direct modulatory effect upon $Ca_V1.2$ or secondary to unidentified electrical remodeling processes that diminish $I_{Ca,L}$. To distinguish between these possibilities, we tested whether KChIP2 directly modulates $I_{Ca,L}$ in a heterologous expression system. The $Ca_V1.2$ α_{1C} subunit and the Ca^{2+} channel accessory subunits β_2 and $\alpha_2\delta$ were expressed with or without KChIP2 in tsA201 cells and whole-cell patch-clamp recordings were compared. Currents from cells expressing KChIP2 showed a ~2-fold increase in peak density (Figure 2A) without any change in the activation or steady-state inactivation kinetics (Figure 2B). Scaling peak current to unity revealed no effect of KChIP2 co-expression on current decay (Figure 2C, top), and pedestal currents measured at 300 ms were comparable between KChIP2-positive and -negative cells (Figure 2C, bottom).

As in myocytes, the reduced current density in the absence of KChIP2 could not be attributed to reduced $Ca_V1.2$ protein expression or decreased trafficking to the plasma membrane: we observed no differences in protein levels with or without KChIP2 co-expression (Figure 2D). Further, analysis of GFP-labeled $Ca_V1.2$ distribution in tsA201 cells showed that membrane targeting of $Ca_V1.2$ was unaffected by KChIP2 (Figure 2E). The membrane-to-whole-cell GFP fluorescence ratio normalized to cell size was comparable in KChIP2-negative (5.9±1.6 arbitrary units; n=18) and KChIP2-positive cells (5.6±0.9 arbitrary units, $P>0.05$; n=19).

To test specifically for a role for KChIP2 in trafficking $Ca_V1.2$ to the plasma membrane, we compared currents from cells transfected with $Ca_V1.2$ (without β_2) to cells transfected with $Ca_V1.2$ and KChIP2. As expected, in the absence of a β_2 subunit, currents were barely detectable (Online Figure II). The addition of KChIP2 did not generate larger currents, suggesting that KChIP2 cannot replace the role of β subunits in trafficking the pore-forming β subunits to the plasma membrane. Since KChIP2 modulated $I_{Ca,L}$ in a heterologous expression system essentially stripped of all other ionic currents, we conclude that KChIP2 acts directly upon the $Ca_V1.2$ channel.

KChIP2 affects $Ca_V1.2$ currents in a Ca^{2+}-independent manner

Next, we determined whether the modulatory effects of KChIP2 are dependent upon Ca^{2+}. When we substituted Ba^{2+} for Ca^{2+}as charge carrier, the addition of KChIP2 still increased current density of $Ca_V1.2$ channels expressed in tsA201 cells (Figure 3A). This suggests that Ca^{2+} permeation through the channel does not alter KChIP2-mediated effects. To test for a contribution of Ca^{2+} binding to the KChIP2 EF hands, we constructed a Ca^{2+}-insensitive KChIP2 with Asp -to-Ala mutations in EF hands 2-4 KChIP2-ΔEF.[3] Compared to WT-KChIP2, this triple EF-hand mutant produced a similar increase in the peak-current density when expressed in tsA201 cells in the presence of $Ca_V1.2$, β_2 and $\alpha_2\delta$ (Figure 3B). Thus, Ca^{2+} binding to KChIP2 does not influence the KChIP2-mediated increase in $Ca_V1.2$ current density. Interestingly, we observed a 7±2 mV depolarizing shift in the activation curve of the Ca^{2+} current with this mutant KChIP2 ($P<0.05$ versus WT-KChIP2). This shift persisted when Ba^{2+} replaced Ca^{2+} as the charge carrier (data not shown).

KChIP2 modulates $I_{Ca,L}$ through binding to the N-terminal domain of $Ca_V1.2$

Other Ca^{2+}-binding proteins affecting $Ca_V1.2$ function, like CaM and CaBP1, associate with $Ca_V1.2$ at the C- and/or the N-termini[14, 15, 17, 30, 31], suggesting likely targets for KChIP2

interaction. Hence, we expressed the C- and N-termini of $Ca_V1.2$ as fusion proteins tagged with 6xHis and GST, respectively, and tested whether they were able to bind KChIP2 in a recombinant bacterial co-expression system. Figure 4A shows a representative Coomassie-stained SDS-PAGE of the proteins purified after affinity chromatography: KChIP2 co-purifies with the region of the N terminus previously shown to support CaM binding (amino acids 60-120; [17]), but not with GST alone. A construct with amino acids 1-70 of $Ca_V1.2$ showed no KChIP2 co-purification (data not shown). In contrast, KChIP2 did not interact with the $Ca_V1.2$ C terminus. The C-terminal domain of $Ca_V1.2$ cannot be purified as a soluble protein in the absence of a true binding partner[32] and we did not recover any soluble $Ca_V1.2$ C terminus when KChIP2 was co-expressed (Figure 4A). In contrast, the $Ca_V1.2$ C-terminal domain was purified when CaM was co-expressed as a binding partner. Thus, KChIP2 likely mediates its effect on $I_{Ca,L}$ through an interaction with the $Ca_V1.2$ N terminus. To address the importance of the calcium-binding properties of KChIP2, we repeated the co-purification experiments when Ca^{2+} was chelated by EGTA. Under this condition, KChIP2 still efficiently associated with the N-terminal domain of $Ca_V1.2$ (data not shown), consistent with the absence of a Ca^{2+}-dependent effect upon $I_{Ca,L}$ amplitude (Figure 3).

To confirm functionally that KChIP2 modulates $Ca_V1.2$ through its interaction with the α_{1C} N-terminus, we deleted the first 139 amino acids of α_{1C} (ΔNT-$Ca_V1.2$) and tested this truncated channel in patch-clamp experiments. The current recorded from the ΔNT-$Ca_V1.2$ construct in the absence of KChIP2 was increased about 3 fold compared to WT-$Ca_V1.2$ (Figure 4B), as previously reported.[33] The increased current density seen with the ΔNT-$Ca_V1.2$ construct relative to WT-$Ca_V1.2$ was not secondary to changes in protein levels of $Ca_V1.2$ (Figure 4C), consistent with pervious results.[33, 34] With its binding site in the $Ca_V1.2$ N-terminus now absent, KChIP2 no longer produced an increase in $I_{Ca,L}$ current density (Figure 4B). These data suggest that KChIP2 affects $I_{Ca,L}$ through a direct association with the N-terminal domain of $Ca_V1.2$. Since these heterologous expression systems contain β and $\alpha_2\delta$ subunits, we cannot formally exclude the possibility that KChIP2 interactions with these auxiliary subunits contribute to the observed modulation.

Discussion

The novel findings in this study are that KChIP2 modulates $I_{Ca,L}$ by interacting with the N-terminal domain of $Ca_V1.2$ and does so in a Ca^{2+}-independent manner. Moreover, this modulation increases $I_{Ca,L}$ amplitude without requiring additional $Ca_V1.2$ channel protein.

Generally, amplitudes of ionic currents can be augmented by adding functional ion channels to the sarcolemma (*N*), by increasing the likelihood of the channel to be in the open state (P_O), or by improving conductance of the individual channel (*i*). Although the present study did not determine which (combination) of these 3 mechanisms was underlying the KChIP2-mediated increase in whole-cell Ca^{2+}-current amplitude, the unaltered protein expression (Figure 2D) and localization (Figure 2E) make it unlikely that the KChIP2-mediated increase in amplitude derives from improved trafficking of functional channels to the membrane. Consistent with this logic, sub-cellular fractions from WT myocytes actually had lower levels of $Ca_V1.2$ protein than KChIP2$^{-/-}$ myocytes (Figure 1D) even though current amplitude was higher in the WT myocytes.

Instead of affecting the parameter *N*, we propose a model in which KChIP2 association with $Ca_V1.2$ increases P_O by relieving the inhibitory effects of the α_{1C} N terminus that have previously been characterized by Dascal and colleagues.[30, 34] As described, α_{1C} contains a N-terminal inhibitory (NTI) module[35] that decreases the channel's maximal open probability ($P_{O,max}$), in a manner that is partially relieved by the β subunit.[34] We propose that KChIP2 interacts with the α_{1C} N terminus, reducing the NTI module's effect on channel gating beyond

that provided by the β subunit (Figure 5). Consistent with this hypothesis, KChIP2 binding to the N-terminal domain was established both biochemically (Figure 4A) and functionally (Figure 4B). Further, KChIP2 caused an increased current density in the absence of a shift in the activation curve (Figure 2B), consistent with modulation of the NTI module.[34] Finally, KChIP2 did not increase $Ca_V1.2$ current density in the absence of the β subunit (Online Figure II), also consistent with an action on $P_{O,max}$ rather than an effect upon channel trafficking; however, this hypothesis has to be verified by single-channel recordings.

As a member of the CaBP family, KChIP2 is the newest example of a Ca^{2+} binding protein that regulates voltage-gated Ca^{2+} channels in general and $Ca_V1.2$ channels in particular. KChIP2 is unique among these CaBPs, however, in that Ca^{2+} binding does not appear to be required for dynamic regulation of Ca^{2+} channel function: we observed comparable KChIP2 influence of I_{Ba} (Figure 3A) and no change in I_{Ca} amplitude when the Ca^{2+}-binding EF hands of KChIP2 were mutated (Figure 3B). Furthermore, KChIP2 co-purified with the N terminus both in the presence and in the absence of free Ca^{2+}. The Ca^{2+}-insensitive KChIP2-ΔEF mutant also binds to Kv4.2, but does not modulate the K^+ current[3], suggesting distinct Ca^{2+}-dependent and –independent regulatory functions of KChIP2. Nonetheless, the depolarizing shift in the activation curve induced by the ΔEF mutant hints at an even more complex regulation of $I_{Ca,L}$ by Ca^{2+} and KChIP2.

The increased $Ca_V1.2$ protein level in KChIP2$^{-/-}$ mice is intriguing in that KChIP family members, including KChIP2, can also act as Ca^{2+}-dependent transcriptional repressors.[36] Whether loss of KChIP2-mediated repression of $Ca_V1.2$ transcription explains the observed increase in $Ca_V1.2$ protein in the KChIP2$^{-/-}$ myocytes is not known but, if correct, would indicate that KChIP2 modulates $Ca_V1.2$ currents via multiple and independent mechanisms and would place more emphasis on the additional roles attributed to KChIP2, such as the modulation and regulation of cardiac Na_V and K_V currents.[3, 6, 23] Thus, KChIP2 appears to be a multimodal regulator of several cardiac ionic currents rather than a contributor solely to I_{to}.

The $Ca_V1.2$ gene gives rise to multiple, tissue-specific isoforms as a consequence of alternative splicing. The NTI module is absent in short N-terminus isoforms, in which the initial 46 amino acids encoded by exon 1a are replaced by a partially homologous sequence of 16 amino acids encoded by exon 1b.[34] Whereas the long isoforms containing the NTI module are predominant in the heart, vascular smooth muscle cells express only the short isoforms lacking the NTI module.[37] Both short and long N-terminal isoforms are found in the brain. At present it is unknown whether KChIP2 interacts with $Ca_V1.2$ isoforms lacking the NTI module, however such association would have implications for Ca^{2+} entry in vascular tissue and in neuronal tissue expressing KChIP2.

Our findings may have even more relevance to arrhythmogenesis. Initially described as an auxiliary subunit for the neuronal A-type current[3], KChIP2 was later shown to be important for the expression of I_{to} in the human heart.[5] Recently, it was reported that post-transcriptional silencing of KChIP2 decreases I_{to} and I_{Na}, suggesting that the two channels form a structural and functional channel complex.[23] The present data show that KChIP2, in addition to its other regulatory functions, modulates the cardiac Ca^{2+} current, implicating a broad physiological role of KChIP2 in cardiac electrophysiology. Although the arrhythmia susceptibility of KChIP2$^{-/-}$ knockout mice[23] already pointed to a key physiological role for KChIP2, the data reported herein offer new hypotheses about the observed arrhythmogenesis. This concept is particularly relevant since mice lacking the subunit for the I_{to} current ($K_V4.2$) showed no arrhythmia susceptibility[38]; likely one of the other modulatory roles played by KChIP2 is paramount.

Our findings also suggest a role for KChIP2 in arrhythmogenesis associated with heart failure. Since KChIP2 is downregulated in patients with heart failure[39], the present findings in combination with the previous reports of KChIP2-mediated actions of K_V and Na_V currents[23, 40], suggest that KChIP2-downregulation will have widespread effects on electrophysiology and contractile performance. Our results specifically predict that KChIP2 downregulation would decrease $I_{Ca,L}$, thereby highlighting a recent report in which loss-of-function mutations in the $Ca_V1.2$ α_{1C} or the β_2 subunits are associated with sudden cardiac death.[41] Of particular interest, one of the mutations reported in that study, A39V in α_{1C}, resides in the NT of α_{1C}. It is reasonable to speculate that the reduced current observed in cells expressing the A39V mutant results from an effect upon the α_{1C} NTI module. It is also intriguing that a common ECG phenotype reported for these patients with $I_{Ca,L}$ loss of function was J-point elevation: as previously observed[24] and as confirmed by us (Online Figure I and Online Table I), ECGs from KChIP2$^{-/-}$ mice also display J-point elevation. That this may be secondary to effects upon $I_{Ca,L}$ rather than upon I_{to} as suggested by a report in which ECGs from Kv4.2$^{-/-}$ mice are indistinguishable from WT.[38]

Conclusion

These data provide new insights into the function of the cardiac $Ca_V1.2$ calcium channel. The effects of KChIP2 on $I_{Ca,L}$ in both native myocytes and in heterologous expression systems strongly suggest that this protein directly augments current amplitude in the absence of altered channel density, activation or inactivation kinetics. Our data, taken together with previous studies, suggest that KChIP2 is a multimodal regulator of a range of voltage-gated cardiac K^+, Na^+ and Ca^{2+} channels.

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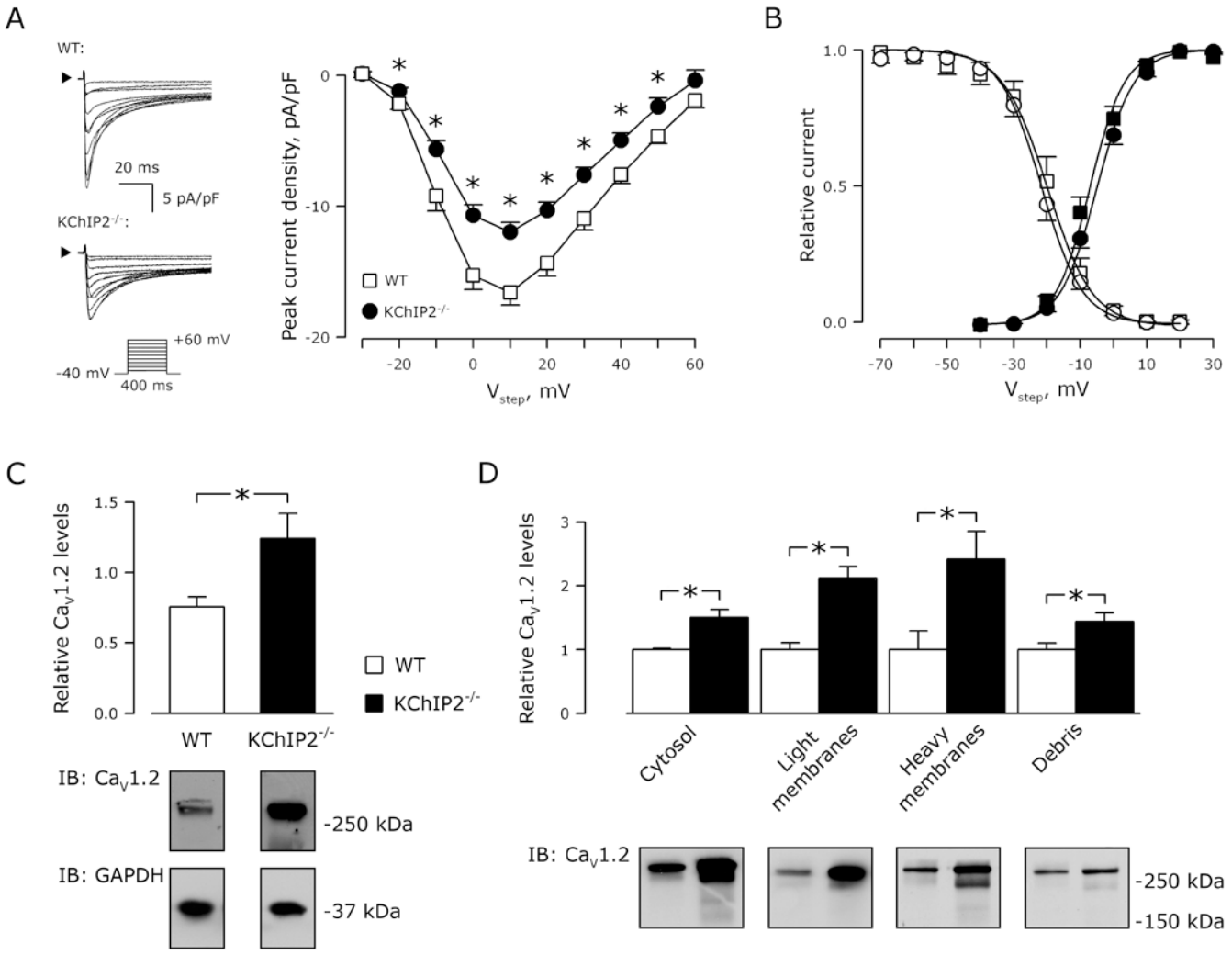

Figure 1.

KChIP2$^{-/-}$ myocytes have decreased $I_{Ca,L}$. A, Representative nifedipine-sensitive current traces normalized to cell capacitance (left panels; arrowheads indicate 0 pA), and current-voltage relationship for whole-cell Ca^{2+} currents (right panel) from cardiomyocytes isolated from WT (squares, n=15) and KChIP2$^{-/-}$ myocytes (circles, n=18). *, $P<0.05$ versus WT. Inset, voltage protocol. B, Current activation (filled symbols) and steady-state inactivation (non-filled symbols) recorded from WT (squares) and KChIP2$^{-/-}$ (circles) myocytes. Solid lines represent sigmoidal fits to the data, where comparable $V_{0.5}$-values (activation, WT: −7 ±1 versus KChIP2$^{-/-}$: −5±1 mV; inactivation, WT: −20±2 versus KChIP2$^{-/-}$: −22±1 mV; $P>0.05$ for both) and k-values (activation, WT: 5±1 versus KChIP2$^{-/-}$: 6±2; inactivation, WT: −7±1 versus KChIP2$^{-/-}$: −6±1; $P>0.05$ for both) were obtained. C, Representative immunoblot for $Ca_V1.2$ (below) and quantification (above) of whole-cell lysates from WT (n=4) and KChIP2$^{-/-}$ (n=4) ventricles. GAPDH was used as control for equal loading, and was used for normalizing $Ca_V1.2$ levels. *, $P<0.05$. D, Protein fractionation and immunostaining for $Ca_V1.2$. Quantification of immunoblots of $Ca_V1.2$ (above) from fractions predominantly containing cytosol, light membranes, heavy membranes, and debris, respectively. *, $P<0.05$. Below: Representative immunoblots.

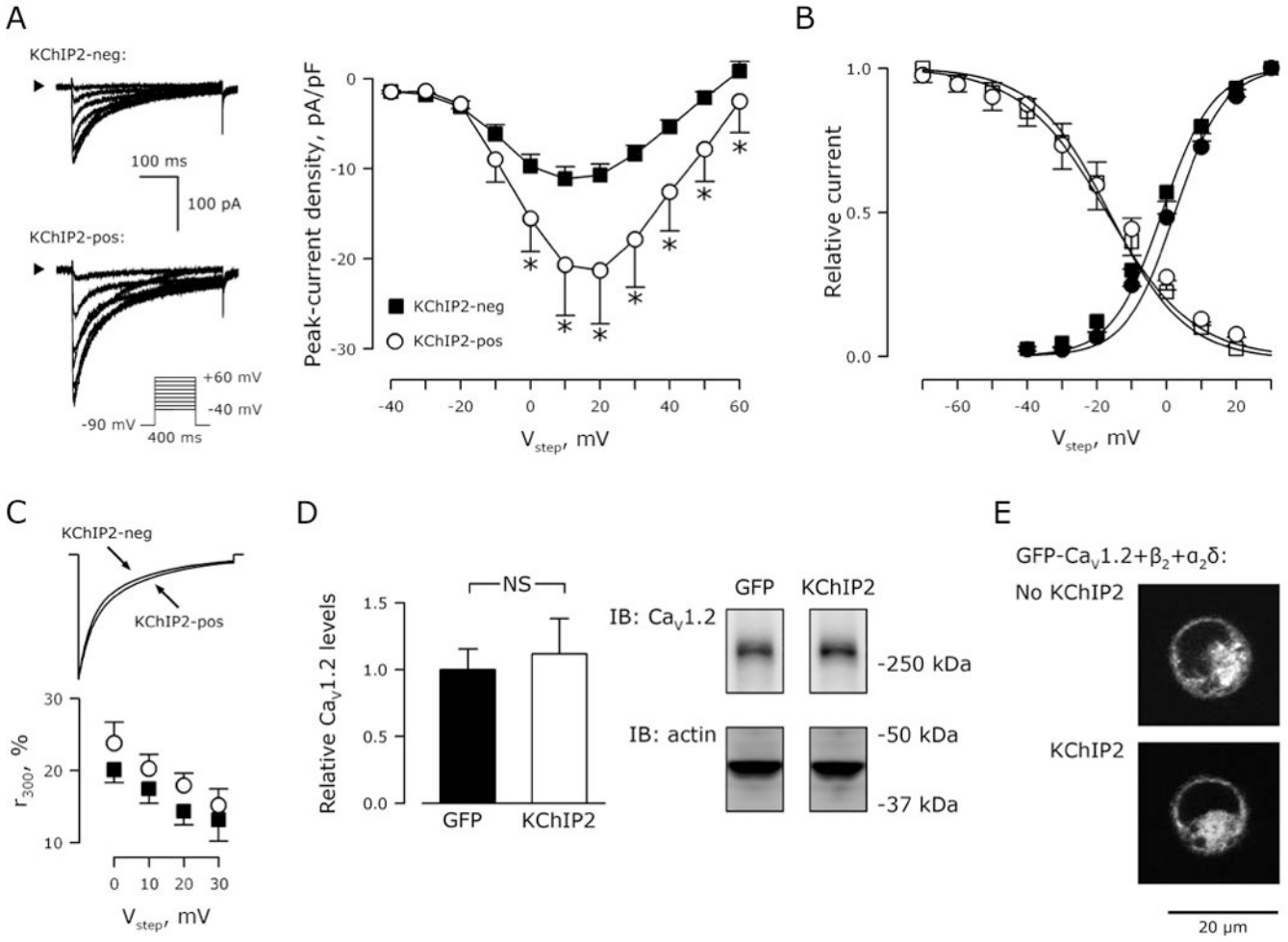

Figure 2.

KChIP2 augments peak $Ca_V1.2$ current in transfected tsA201 cells. A, Representative current traces from tsA201 cells transfected with the $Ca_V1.2$ α_{1C}, β_2, and $\alpha_2\delta$ subunits with and without KChIP2. Arrowheads indicate 0 pA. Inset, voltage protocol. Right, Ca^{2+} current-voltage relationship from KChIP2-transfected cells (circles, n=14) and GFP-transfected cells (KChIP2-neg; squares, n=18). Data presented as mean±SEM; *, $P<0.05$ versus KChIP2-neg). B, Current activation (filled symbols) and steady-state inactivation (non-filled symbols) recorded in KChIP2-negative (squares) and -positive (circles) cells. Solid lines represent sigmoidal fits to the data, where comparable $V_{0.5}$- and *k*-values were obtained (not shown). C, Comparable current-decay time course exemplified with scaled amplitudes. Below, residual current at 300 ms relative to peak current amplitude in KChIP2-negative (squares) and -positive (circles) cells. D, Representative immunoblot for $Ca_V1.2$ (right) and quantification (left) of whole-cell lysates from tsA201 cells transfected with $Ca_V1.2$, β_2, and $\alpha_2\delta$ with GFP or KChIP2. Transfections were done in triplicates. Actin was used as control for equal loading, and was used for normalizing $Ca_V1.2$ levels. NS, $P>0.05$. E, Representative fluorescent confocal images of tsA201 cells transfected with GFP-labeled $Ca_V1.2$, β_2, and $\alpha_2\delta$ with and without KChIP2.

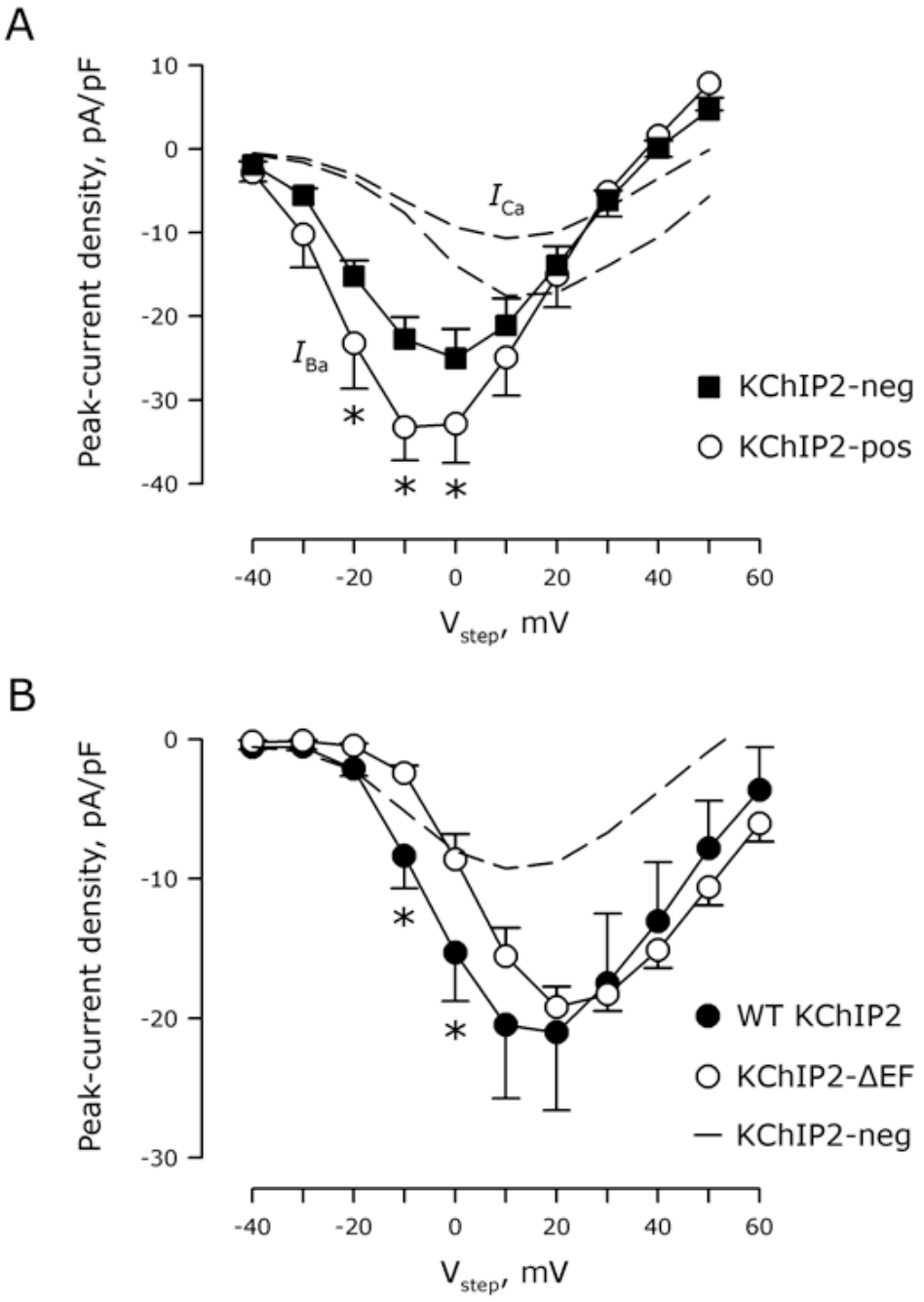

Figure 3.

KChIP2 affects $I_{Ca,L}$ in a Ca^{2+}-independent manner. A, Ba^{2+} currents obtained in KChIP2-negative (squares) and -positive (circles) cells as a function of test voltage (V_{step}). *, $P<0.05$ versus KChIP2-negative cells. For comparison, current-voltage relationships attained with Ca^{2+} as charge carrier (Figure 2A) are reproduced with dotted lines. B, Peak-current density as a function of test voltage (V_{step}) in cells transfected with $Ca_V1.2$, β_2, and $\alpha_2\delta$ subunits with either WT-KChIP2 (filled circles) or the calcium-insensitive KChIP2-ΔEF (non-filled circles). *, $P<0.05$ versus KChIP2-ΔEF ($n_{WT\text{-}KChIP2}=14$; $n_{KChIP2\text{-}\Delta EF}=10$). Dotted line illustrates the current-voltage relations in the absence of KChIP2 (Figure 2A).

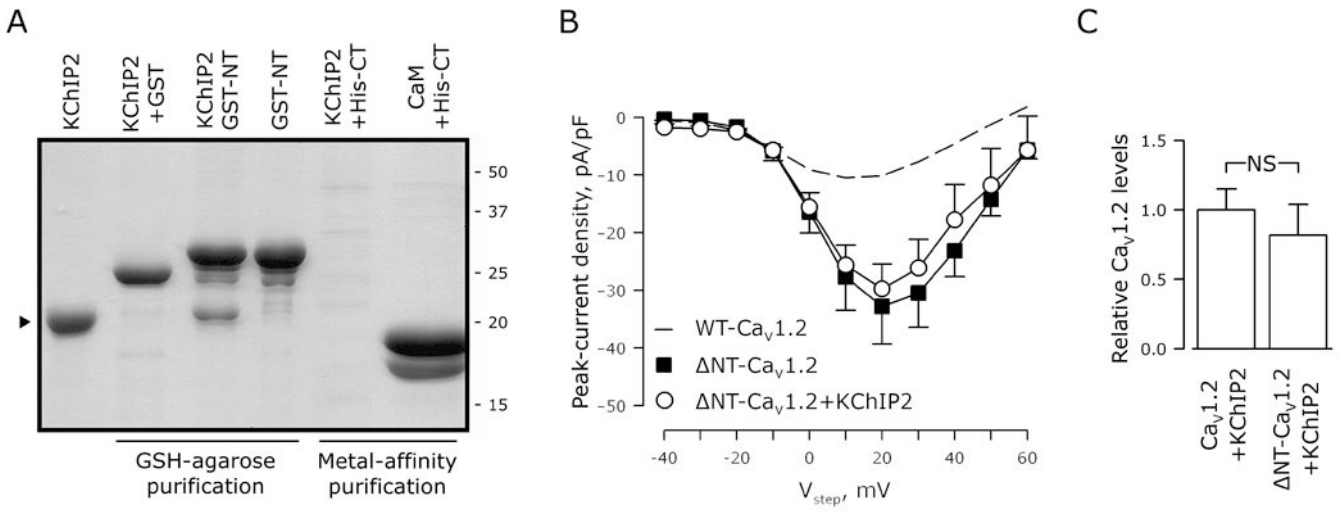

Figure 4.

The inhibiting amino terminus of $Ca_V1.2$ interacts with KChIP2. A, Coomassie-stained SDS-PAGE of GSH-agarose or metal-affinity purified protein samples. The carboxy- and amino-termini of $Ca_V1.2$ tagged with 6xHis or GST, respectively, were expressed in a recombinant bacterial system. KChIP2 or calmodulin (CaM) was co-expressed as indicated above each lane. KChIP2 co-purifies with GST-tagged $Ca_V1.2$ amino-terminus (GST-NT), but not with GST alone or with the His-tagged carboxy-terminus (His-CT). B, Peak Ca^{2+}-current density as a function of test voltage (V_{step}) in tsA201 cells transfected with ΔNT-$Ca_V1.2$, β_2, and $\alpha_2\delta$ with KChIP2 (circles) or without KChIP2 (squares). No significant differences were found ($n_{KChIP2\text{-}pos}$=8; $n_{KChIP2\text{-}neg}$=9; mean±SEM). Dotted line indicates WT-$Ca_V1.2$ in the absence of KChIP2 (from Figure 2A), to illustrate the augmented current density secondary to deletion of the N-terminal domain. C, Ablation of the amino terminus does not alter protein expression of $Ca_V1.2$ in transfected tsA201 cells. NS, P>0.05; n=3 transfections per group.

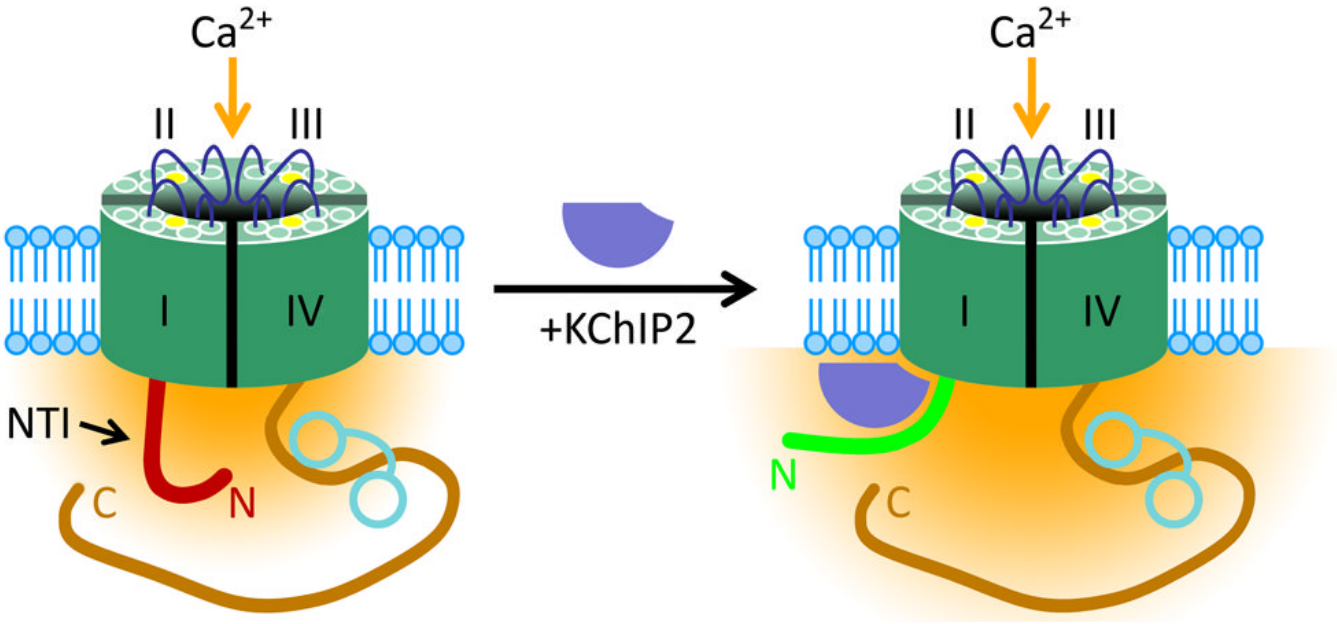

Figure 5.

Proposed model for KChIP2 modulation of $I_{Ca,L}$. The N-terminal inhibitory (NTI) module impairs the Ca^{2+}-current density (yellow) in the absence of KChIP2 (left). The N terminus translocates upon binding of KChIP2, thereby reducing the inhibitory effects on $I_{Ca,L}$ (right). Calmodulin is illustrated as a dumbbell shaped structure on the α_{1C} CT.