

Full-length article

Co-expression of KCNE2 and KChIP2c modulates the electrophysiological properties of Kv4.2 current in COS-7 cells¹Wen-juan LIU², Hai-tang WANG², Wei-wei Chen, Jian-xin DENG, Yong JIANG, Jie LIU³*Department of Pathophysiology, Southern Medical University, Guangzhou 510515, China***Key words**transient outward current; Kv4.2; K⁺-channel interacting protein; KCNE2; electrophysiology¹ Project supported by the National Natural Science Foundation of China (No 30570418 and 30570940).² These authors contributed equally to this work.³ Correspondence to Dr Jie LIU.

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Abstract

Aim: Several β -subunits have been suggested to modulate the electrophysiological properties of the transient outward current (I_{to}) in cardiac myocytes, including the obligatory β -subunit K⁺-channel interacting protein (KChIP2) and KCNE2. However, neither KChIP2 nor KCNE2 modulation of Kv4.x (x=2 and/or 3) can fully recapitulate the electrophysiological properties of native I_{to} . The present study is to investigate how I_{to} current is modulated when both KChIP2 and KCNE2 are co-expressed. **Methods:** Kv4.2, KChIP2c, and KCNE2 cDNA were simultaneously transfected into COS-7 cells at a molar ratio of 3:1:1. Whole-cell currents were recorded by the patch-clamp method. **Results:** In comparison with the current regulated by KChIP2c alone, the co-expression of KCNE2 further slowed Kv4.2 current inactivation kinetics, but diminished KChIP2c-induced positive shift of the voltage-dependent activation of Kv4.2 current. Importantly, co-expression of KCNE2 accelerated the current recovery from inactivation, and caused an “overshoot” of peak current amplitude during Kv4.2 current recovery, a phenomenon which has been uniquely described for human I_{to} . However, co-expression of KCNE2 exerted no further effect on Kv4.2 current amplitude, the rate of Kv4.2 current activation and voltage-dependent inactivation. **Conclusion:** Co-expression of Kv4.2 with KChIP2c and KCNE2, but not with KChIP2c or KCNE2 alone, yields a current profile similar to native I_{to} . Both KChIP2c and KCNE2 simultaneously participate in recapitulation of the electrophysiological properties of I_{to} in cardiac myocytes.

Introduction

The transient outward current (I_{to}) underlies the early repolarization phase of cardiac action potential and contributes to the determination of the overall repolarization phase as well as excitation–contraction coupling via modulating Ca²⁺ and other K⁺ currents^[1,2]. It is well established that Kv4.x (x=2 and/or 3) are the major α -subunits of I_{to} channels in the heart^[1,2]. The electrophysiological properties of I_{to} are modulated by several β -subunits, among them the K⁺-channel interacting protein (KChIP2) is the most thoroughly investigated. KChIP2 can increase peak I_{to} current density by promoting trafficking of Kv4.x to the cell membrane, slow inactivation, and accelerate recovery from inactivation^[3]. Co-expression of Kv4.x and KChIP2 in mammalian cell lines yields

current with a time course of inactivation similar to native cardiac I_{to} , but markedly slows recovery from inactivation and steady-state inactivation in a more positive potential range, suggesting that KChIP2 may not be the only accessory subunit of native I_{to} in cardiomyocytes^[4].

Recently, KCNE2 protein has been found to be readily detectable in the ventricles of humans, dogs, and rats, and forms stable complex with the cardiac Kv4.2 protein^[5]. In *Xenopus* oocyte expression system, KCNE2 slowed the rates of Kv4.2 activation and inactivation and shifted the voltage dependence of channel gating in the positive direction, but did not affect the current amplitude^[6]. Our previous data also demonstrated that KCNE2 had a similar modulation effect on Kv4.3 in COS-7 expression system^[7]. This

evidence indicates that KCNE2 may also serve as a regulatory (b) subunit of I_{to} channels in the heart.

However, it remains elusive how Kv4.2 current is regulated in the context of cardiac electrophysiology in which both KChIP2 and KCNE2 are expressed. It is unknown whether KCNE2 can still exert its regulation function on Kv4.2 in the presence of KChIP2, the obligatory β -subunit of I_{to} . To address these questions, we co-expressed these 2 regulatory subunits with Kv4.2, and compared the current properties with Kv4.2+KChIP2c current. We found that co-expression of KCNE2 produced a current profile with slower inactivation and faster recovery from inactivation as compared with Kv4.2+KChIP2c current. Moreover, co-expression of KCNE2 diminished the positive shift in voltage dependence of activation, but had no effect on the positive shift in voltage dependence of inactivation induced by KChIP2c alone.

Materials and methods

Cell culture and transfection Kv4.2 gene was subcloned into pcDNA3.1 vector, and KChIP2c- and c-myc-tagged KCNE2 genes were subcloned into pALTER-MAX vector. All the plasmid cDNA were kindly supplied by Dr Gea-Ny TSENG (Virginia Commonwealth University, Richmond, VA, USA). COS-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum in a moist 5% CO₂ chamber at 37 °C. Cells were plated at a subconfluence level the day before transfection and were divided into Kv4.2, Kv4.2+KChIP2c, and Kv4.2+KChIP2c+KCNE2 groups. Each 35-mm dish of cells in Kv4.2+KChIP2c+KCNE2 group was transfected with the following amounts of plasmid cDNA: Kv4.2, 2 μ g; KChIP2c- and c-myc-tagged KCNE2, 0.58 μ g each; and CD8, 0.2 μ g, to reach a cDNA molar ratio of 3:1:1:0.5 using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. In the other 2 groups, the amount of cDNA for the transfection was the same as that in Kv4.2+KChIP2c+KCNE2 group, except neither KCNE2 nor KChIP2c was added in Kv4.2 group, and no KCNE2 was added in Kv4.2+KChIP2c group. Before current recording, the cells were incubated with CD8 monoclonal antibody (mAb)-coated beads (Dynal Biotech, Norway) at room temperature for 10 min, and the cells decorated with CD8 beads were used for patch clamping.

Whole-cell current recording Twenty-four to forty-eight hours after transfection, whole-cell currents were recorded by the patch-clamp method using an Axopatch 200B amplifier (Axon Instruments, USA) at room temperature. The pipette solution contained the following (in mmol/L): KOH 120, aspartic acid 120, KCl 20, ATP(K) 10, EGTA 10, HEPES 10,

and MgCl₂ 1, with pH adjusted to 7.3 with KOH. The pipette tip resistance was 2–5 M Ω when filled with pipette solution. Myocytes were superfused with Tyrode's solution containing the following (in mmol/L): NaCl 126, MgCl₂ 0.5, KCl 4, CaCl₂ 2, and HEPES 5, and dextrose 1 mg/mL, with pH adjusted to 7.3 with NaOH. Currents were low-pass filtered at 1 kHz, stored on a computer, and analyzed offline. Clampfit (Axon, USA), EXCEL (Microsoft), and PeakFit were used for the data analysis.

Immunocytochemistry and confocal experiments COS-7 cells cotransfected with Kv4.2, KChIP2c and KCNE2 plasmids were fixed on coverslips with 4% paraformaldehyde and permeabilized by 0.5% Triton X-100. The cells were incubated with anti-c-myc-tagged mAb (Cell Signaling, Danvers, MA, USA) at a 1:2000 dilution and an anti-KChIP2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a 1:50 dilution overnight at room temperature. The slides were then washed 3 times with phosphate-buffered saline and incubated with secondary antibodies (goat antimouse immunoglobulin G [IgG] labeled with fluorescent Alexa Flour-488 for c-myc KCNE2 and donkey antigoat IgG with Alexa Flour-594 for KChIP2c). Immunofluorescence was imaged with a fluorescent microscope. The excitation wave length for Alexa Flour-488 and Alexa Flour-594 was 488 nm and 543 nm, emitting fluorescence at >505 nm and >605 nm, respectively.

Statistics Data were expressed as mean \pm SEM. Two-tailed unpaired Student's *t*-test and one-way ANOVA were applied when appropriate. *P*<0.05 was considered statistically significant.

Results

Effects of co-expression of KCNE2 and KChIP2c on Kv4.2 current density and gating kinetics Before patch-clamp experiment, we needed to address whether the cells studied by patch-clamp expressed all the proteins encoded by the cDNAs. Given that cells not expressing Kv4.2 yield no current, we needed to determine whether COS-7 cells transfected with Kv4.2, KChIP2c, and KCNE2 cDNA co-expressed KChIP2c and KCNE2 proteins. These 2 proteins were simultaneously probed with specific antibodies using immunocytochemistry. KChIP2c and KCNE2 were co-expressed in the same cells, as shown in Figure 1. The statistical analysis revealed 96% cells (*n*=200) with fluorescence were positive for both KChIP2c and KCNE2, whereas only 1% and 3% of cells were positive for KChIP2c and KCNE2 alone, respectively. It implicates that the currents we recorded in Kv4.2+KChIP2c+KCNE2 expressing cells were mainly Kv4.2+KChIP2c+KCNE2 currents, and the interference of Kv4.2+KChIP2c or Kv4.2+KCNE2

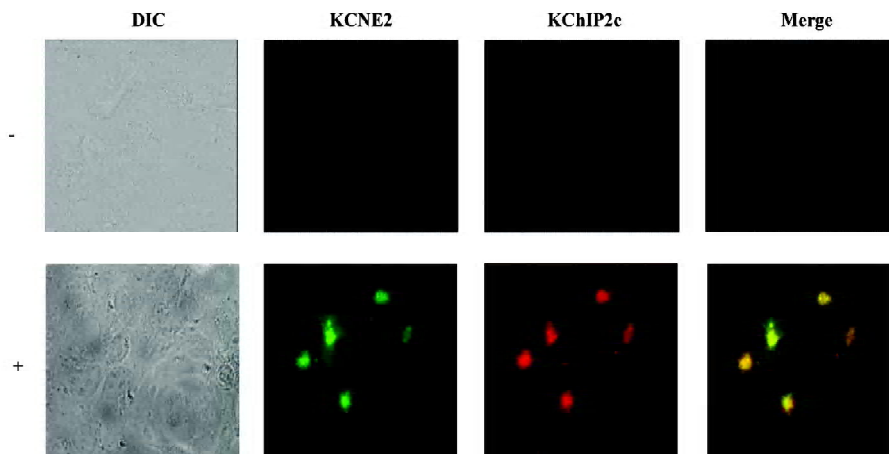


Figure 1. KCNE2 and KChIP2c were co-expressed in COS-7 cells co-transfected with Kv4.2, KCNE2, and KChIP2c. DIC, image of differential interference contrast; KCNE2, fluorescent image of Alexa Flour-488 (detecting KCNE2, green); KChIP2c, fluorescent image of Alexa Flour-594 (detecting KChIP2c, red); Merge, merge of fluorescent images from KCNE2 and KChIP2c; (-), negative control. COS-7 cells transfected with no DNA. (+), COS-7 cells co-transfected with Kv4.2, KCNE2, and KChIP2c genes.

current can be ignored.

It is known that KChIP dramatically increases Kv4.2 current density, whereas KCNE2 has no significant effect on it. We first examined how the current density was regulated when both KChIP2c and KCNE2 were expressed with Kv4.2. Consistent with previous studies, KChIP2c alone significantly increased Kv4.2 current density (Figure 2A and 2B). The current density was increased by 1.3-fold, from 194.7 ± 30.5 pA/pF ($n=20$) in Kv4.2 group to 449.0 ± 40.5 pA/pF in Kv4.2+KChIP2c group ($n=37$, $P<0.01$). In comparison with the effect of KChIP2c alone, co-expression of KCNE2 did not change the current density. In Kv4.2+KChIP2c+KCNE2 group, the current density was 409.2 ± 90.5 pA/pF ($n=15$), not significantly different from that in Kv4.2+KChIP2c group ($P>0.05$). These data suggest that KCNE2 might not interfere with KChIP2c regulation of Kv4.2 current density. In other words, KChIP2c dominantly regulates Kv4.2 current density when it is co-expressed with KCNE2.

KCNE2 and KChIP have both been reported to slow the rates of Kv4.2 activation and inactivation. We next explored whether KCNE2 would further slow Kv4.2 activation and inactivation kinetics in the presence of KChIP2c. The activation and inactivation rates of Kv4.2, as indexed by the time to peak (TTP) and half-time ($t_{0.5}$) of decay recorded at +60 mV potential were slowed down by the expression of KChIP2c alone (Figure 2C,2D). The TTP and $t_{0.5}$ of decay in Kv4.2+KChIP2c group were 6.7 ± 0.2 ms and 40.9 ± 2.7 ms ($n=37$), much longer than 4.5 ± 0.2 ms and 17.0 ± 1.2 ms in Kv4.2 group ($n=20$, $P<0.01$). When co-expressed with KChIP2c, KCNE2 did not further slow the Kv4.2 activation rate. The TTP was 6.8 ± 0.9 ms in Kv4.2+KChIP2c+KCNE2 group, not

significantly different from that in Kv4.2+KChIP2c group ($P>0.05$). In contrast, co-expression of KCNE2 further slowed Kv4.2 inactivation kinetics. The $t_{0.5}$ of the current decay was 56.0 ± 0.7 ms in Kv4.2+KChIP2c+KCNE2 group, significantly longer than that in Kv4.2+KChIP2c group ($P<0.01$). These data indicate that KCNE2 does not homogeneously regulate Kv4.2 current activation and inactivation kinetics in the presence of KChIP2c.

Effects of co-expression of KCNE2 and KChIP2c on voltage dependence of Kv4.2 current activation Previous studies have demonstrated that both KCNE2 and KChIP shift the voltage dependence of Kv4.2 current activation in a positive direction^[4,5]. We examined how the voltage dependence of Kv4.2 current activation was regulated when both KCNE2 and KChIP2c were co-expressed. Voltage dependence of activation was obtained by normalizing steady-state activation (g_m) curves calculated from current-voltage ($I-V$) curves elicited by test steps (200 ms) from -50 to 60 mV from a holding potential (V_h) of -80 mV, assuming an reversal potential (E_{rev}) of -60 mV (Figure 3A,3B). The voltage of half activation ($V_{0.5,act}$) was estimated by fitting the activation curves with Boltzman function. As shown in Figure 3C, the expression of KChIP2c caused an 8.5 mV positive shift of Kv4.2 current activation ($V_{0.5,act}$ was -12.7 ± 1.8 mV, $n=5$ in Kv4.2 group, and -4.2 ± 1.6 mV, $n=10$ in Kv4.2+KChIP2c group), which was consistent with a previous report^[4]. When KCNE2 was co-expressed, the effect of KChIP2c on the voltage dependence of activation, the effect of KChIP2c on the voltage dependence of activation was weakened. The $V_{0.5,act}$ in Kv4.2+KChIP2c+KCNE2 group was -10.0 ± 3.8 mV ($n=5$), which was only a 2.7 mV positive shift versus the value in Kv4.2 group ($P>0.05$).

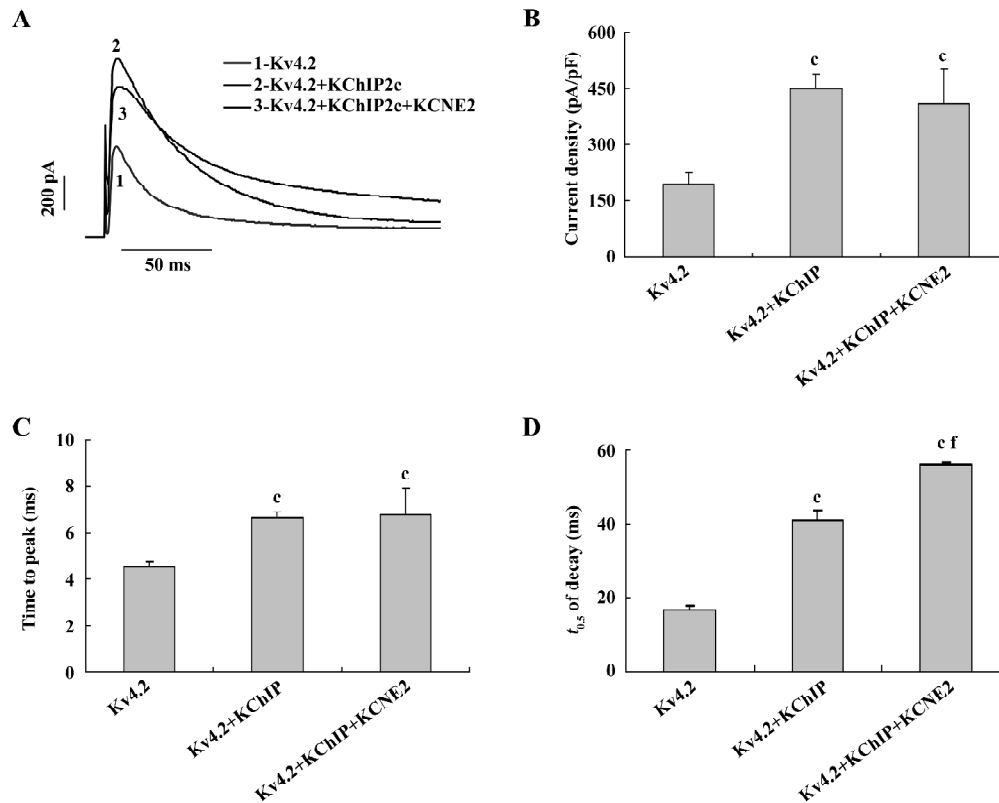


Figure 2. Effects of co-expression of KCNE2 and KChIP2c on Kv4.2 current amplitude and kinetics of activation and inactivation. (A) representative current traces were recorded at +60 mV in COS-7 cells expressing Kv4.2, or Kv4.2+KChIP, or Kv4.2+KChIP+KCNE2. (B–D) statistics of peak current density (B), TTP of Kv4.2 activation (C), and $t_{0.5}$ of decay of Kv4.2 inactivation (D). Co-expression of KCNE2 had no further effect on the current density and TTP, but significantly prolonged the $t_{0.5}$ of decay of Kv4.2 current in comparison with Kv4.2 current regulated by KChIP2c alone. ^c $P < 0.01$ vs Kv4.2. ^f $P < 0.01$ vs Kv4.2+KChIP.

Effects of co-expression of KCNE2 and KChIP2c on voltage dependence of Kv4.2 current inactivation KChIP2c and KCNE2 have also been reported to cause a significant positive shift in the voltage dependence of Kv4.2 current inactivation. We next investigated whether KCNE2 influenced KChIP2c regulation of voltage-dependent Kv4.2 inactivation in a similar way as it did on voltage-dependent Kv4.2 activation. The voltage-dependent inactivation was estimated by $V_{0.5, \text{inact}}$ calculated by fitting the inactivation curve with Boltzman function (Figure 4A). In comparison with the effect of KChIP2c, co-expression of KCNE2 caused no further alteration in the voltage dependence of inactivation. $V_{0.5, \text{inact}}$ was -42.8 ± 1.6 mV in Kv4.2+KChIP2c+KCNE2 group ($n=10$), not significantly different from -39.6 ± 1.8 mV in Kv4.2+KChIP2c group ($n=15$, $P > 0.05$; Figure 4B).

Effects of co-expression of KCNE2 and KChIP2c on voltage dependence of Kv4.2 current recovery from inactivation Double pulses of depolarization stimulation (V_1 and V_2 , +60 mV) with different time intervals ranging from 12 ms to

2 s were applied (Figure 5) for studying the time course of Kv4.2 recovery from inactivation. The peak Kv4.2 current amplitude recovered from inactivation gradually with the prolongation of the V_1 - V_2 interval, in a monoexponential function (Figure 5A and 5C). Consistent with previous reports, KChIP2c accelerated Kv4.2 recovery from inactivation. The time constant (τ) of recovery was shortened from 344.9 ± 36.8 ms ($n=9$) in Kv4.2 group to 66.7 ± 8.9 ms ($n=9$) in Kv4.2+KChIP2c group. When KCNE2 was co-expressed, the recovery was further accelerated. The time constant of recovery was reduced to 34.1 ± 5.5 ms in Kv4.2+KChIP2c+KCNE2 group ($n=6$), significantly shorter than that in Kv4.2+KChIP2c group ($P < 0.05$; Figure 5D).

It is noteworthy that the co-expression of KCNE2 induced an overshoot of Kv4.2 peak current amplitude during recovery from inactivation, that is, the peak V_2 current amplitude (P_2) was approximately 4% larger than the V_1 current amplitude (P_1 ; Figure 5B). This phenomenon was not found in Kv4.2 and Kv4.2+KChIP2c-expressing cells.

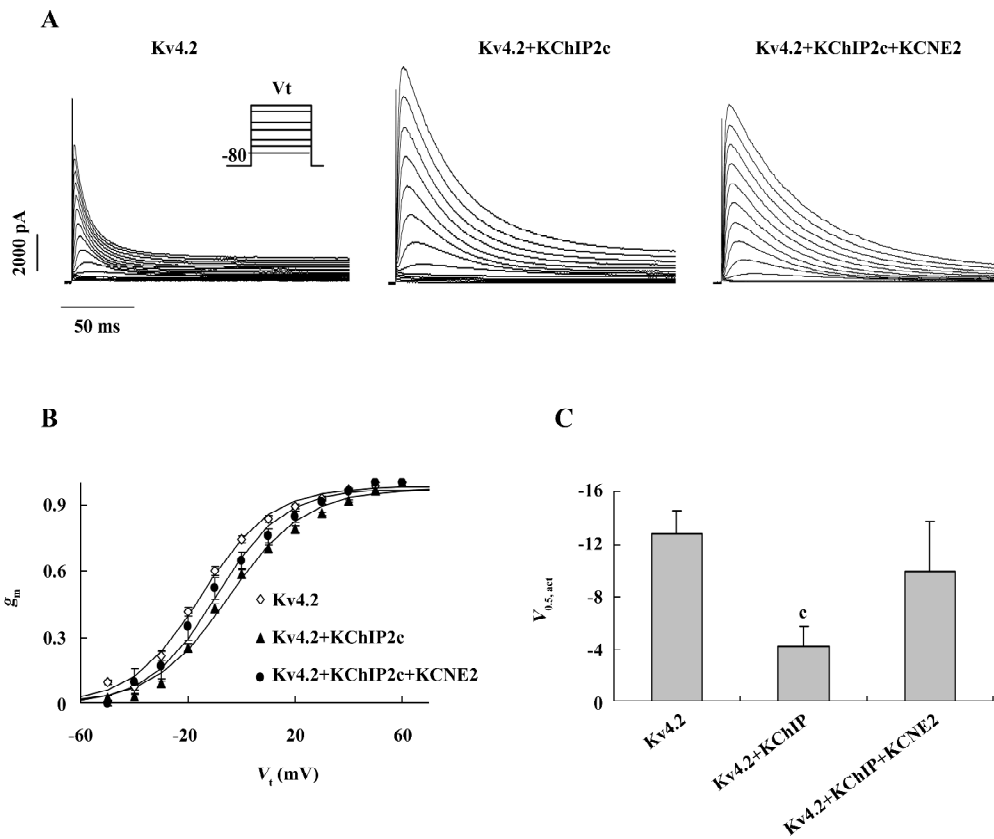


Figure 3. Effects of co-expression of KCNE2 and KChIP2c on the voltage dependence of Kv4.2 current activation. (A) typical current traces elicited by test steps (200 ms) from -50 to 60 mV at V_h of -80 mV (inset) in Kv4.2, Kv4.2+KChIP2c and Kv4.2+KChIP2c+KCNE2 groups. (B) normalized steady-state activation (g_m) curves calculated from I/V curves assuming an E_{rev} of -60 mV. (C) statistics of $V_{0.5,act}$ estimated by fitting the activation curves in B with Boltzman function. ^c $P < 0.01$ vs Kv4.2.

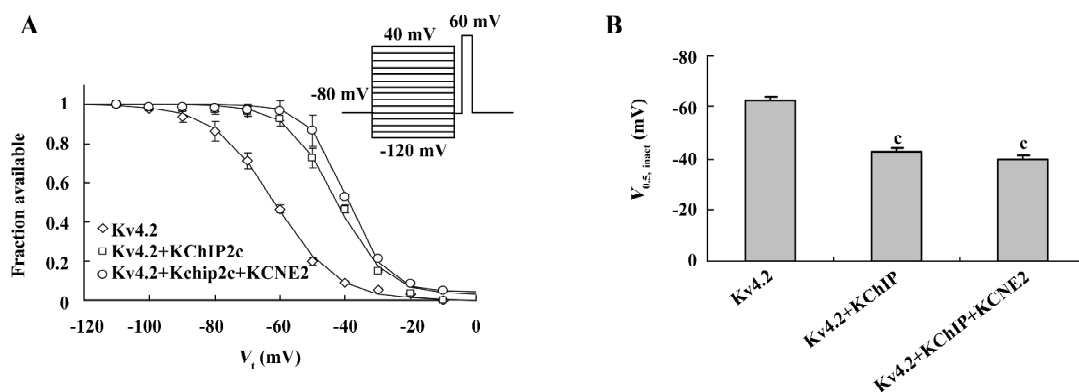


Figure 4. Effects of co-expression of KCNE2 and KChIP2c on the voltage dependence of Kv4.2 current inactivation. (A) normalized voltage dependence of inactivation curves elicited by voltage-clamp protocol (inset) as follows: from V_h of -80 mV, conditioning pulses (V_c) were applied for 2 s from -110 to -10 mV in 10 mV increments. Each V_c was followed by a test pulse (V_i) to 60 mV. Peak current amplitudes during V_i pulses at various V_c levels were normalized to that after V_c to -110 mV. This gave an estimate of fractions of channels available for activation at various V_c levels. Relationship between fraction available and V_c was fitted with Boltzman function: $1/(1+\exp\{(V_c-V_{0.5})/k\})$. (B) statistics of $V_{0.5}$ of $V_{0.5,inact}$ in the Kv4.2, Kv4.2+KChIP2c, and Kv4.2+KChIP2c+KCNE2 groups. ^c $P < 0.01$ vs Kv4.2.

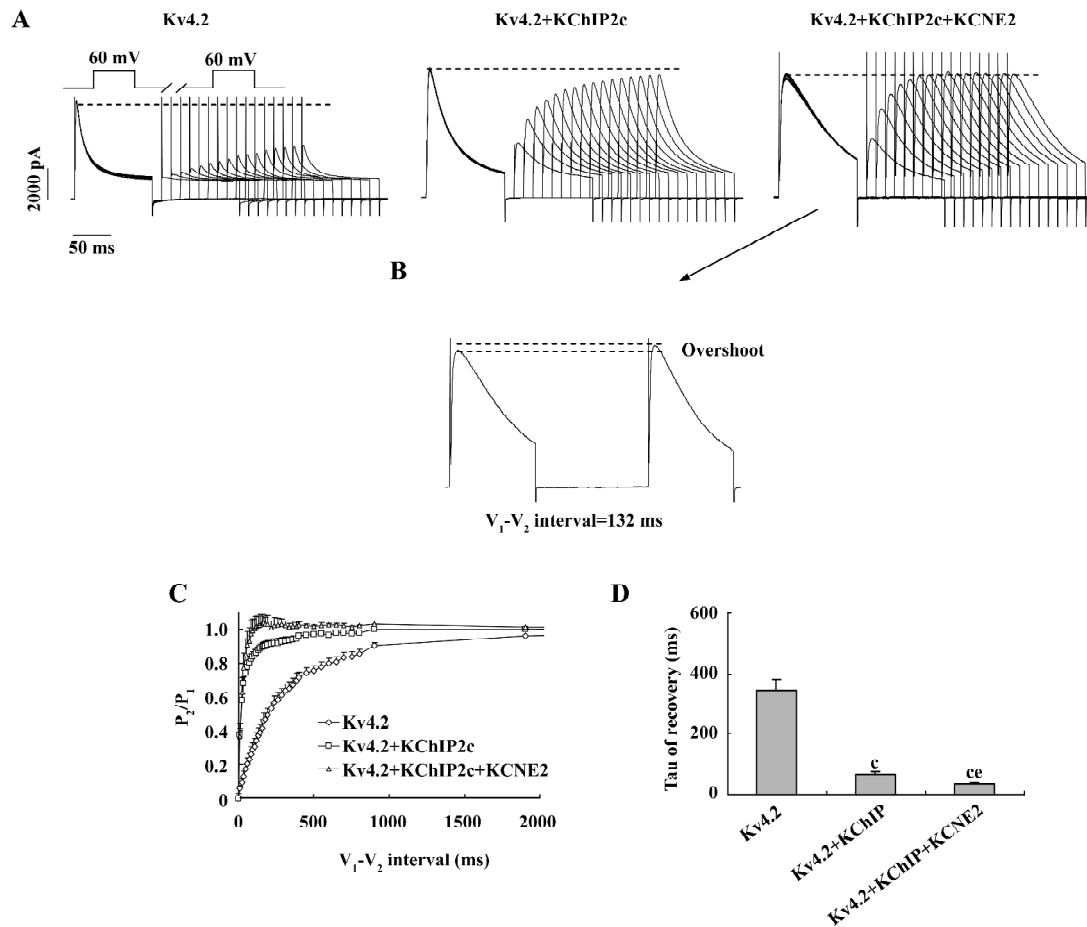


Figure 5. Effects of co-expression of KCNE2 and KChIP2c on the time course of Kv4.2 current recovery from inactivation. (A) typical current traces of Kv4.2 recovery from inactivation during V_1 - V_2 (recovery) interval ranging from 12 to 192 ms in COS-7 cells expressing Kv4.2 (left), Kv4.2+KChIP2c (middle), and Kv4.2+KChIP2c+KCNE2 (right). Upper panel demonstrates the voltage-clamp protocol: double pulse (V_1 and V_2) each to +60 mV for 500 ms applied once every 10 s. (B) current trace 11 (V_1 - V_2 interval = 132 ms) from the Kv4.2+KChIP2c+KCNE2 current shown in (A) in which the peak current amplitude during V_2 (P_2) is greater than that during V_1 (P_1). (C) time course of change in P_2/P_1 against V_1 - V_2 interval. V_1 - V_2 interval ranged from 12 to 2000 ms. (D) average of Tau of recovery from inactivation. Tau value was estimated by 1 exponential fit. * $P < 0.01$ vs Kv4.2 alone. ** $P < 0.05$ vs Kv4.2+KChIP2c.

Discussion

KChIP2 is abundantly expressed in the heart and has a prominent effect on increasing peak I_{to} current density. It is reported that KChIP2 expression in the ventricular wall of the heart parallels the gradient in transient outward current I_{to} ^[8-10]. Thus KChIP2 is well accepted as the obligatory β -subunit of I_{to} . Although KChIP2 can associate with Kv4.x and modulates the current gating, endowing Kv4.x electrical properties similar to I_{to} , co-expression of Kv4.x with KChIP2 cannot fully recapitulate the electrophysiological properties of native I_{to} in cardiac myocytes, suggesting that I_{to} is complicatedly regulated by multiple factors, rather than a single factor in the context of cardiac myocytes. KCNE2 is also expressed in ventricles and has been suggested to be a

potential regulatory subunit of I_{to} in cardiac myocytes^[5-7]. In the present study, we found that co-expression of KCNE2 with KChIP2c further slowed Kv4.2 current inactivation kinetics, but diminished KChIP2c-induced positive shift of voltage-dependent activation of Kv4.2 current. Importantly, co-expression of KCNE2 accelerated the current recovery from inactivation and caused an "overshoot" of peak current amplitude during Kv4.2 current recovery, a phenomenon which has been uniquely described for human I_{to} ^[11]. This evidence collectively suggests that KChIP2c and KCNE2 are simultaneously involved in the recapitulation of the electrophysiological properties of native I_{to} in the context of cardiac myocytes.

It was noteworthy that co-expression of KCNE2 failed to

change some of the current properties in comparison with the current modulated by KChIP2c alone. There were no significant differences in the Kv4.2 current activation rate and voltage dependence of inactivation between Kv4.2+KChIP2c and Kv4.2+KChIP2c+KCNE2 groups, even though KCNE2 alone can slow the rate of Kv4.2 activation and induce a positive shift of voltage-dependent inactivation. Moreover, co-expression of KCNE2 did not change Kv4.2+KChIP2c current density in our expression system. A possible explanation for these results might be that KCNE2 does not homogeneously regulate Kv4.2 gating properties when transfected at a lower concentration with KChIP2c (molar ratio for Kv4.2: KCNE2 was 3:1), that is, it exerts a regulatory effect on the inactivation rate and recovery from inactivation, but not on the activation rate and voltage-dependent inactivation of Kv4.2 current. Furthermore, the finding that co-expression of KCNE2 diminished, rather than enhanced the KChIP2c-induced positive shift of voltage-dependent activation suggests that co-expression of KChIP2c and KCNE2 might change the pattern of KCNE2 and/or KChIP2c modulation of the Kv4.2 current.

A recent study has reported the KCNE2 modulation of I_{to} current properties in CHO cells constantly expressing both Kv4.3 and KChIP2^[12]. In this cell model, Radicke *et al* revealed that KCNE2 had no effect on current density, similar to our finding. However, in contrast to our study, these authors reported that KCNE2 had no effect on inactivation kinetics, but caused a negative shift in the voltage-dependent inactivation of Kv4.3+KChIP2c current. Although KCNE2 induced a unique “overshoot” of the peak current during recovery from inactivation, it did not increase the recovery from inactivation, which is much slower than native I_{to} . The different findings between the study from Radicke *et al* and the present one were likely to be related to the following reasons. First, Kv4.3 was used in the study of CHO cell expression, whereas Kv4.2 was used in the present study. Although Kv4.2 and Kv4.3 share many similarities in gating properties, the activation and inactivation kinetics, and the recovery of Kv4.3 channel current from inactivation are slower than those of Kv4.2^[13]. Second, in our study, KChIP2c and KCNE2 were cotransfected simultaneously, whereas KCNE2 was transiently transfected into CHO cells stably expressing KChIP2 in the study of Radicke *et al*^[12]. Third, stoichiometric transfection with Kv4.2, KChIP2 and KCNE2 was employed in the present study, but not in the study from Radicke *et al*^[12]. In the present study, KChIP2c and KCNE2 were transfected at the same molar ratio and at a lower concentration. There is no report on the stoichiometry of Kv4.x, KChIP2, and KCNE2 in native I_{to} in a normal

heart. In diseased and aging hearts, KChIP2 and/or KCNE2 expression levels have been reported to be downregulated or upregulated^[5,13,14]. Therefore, the different results from the previous study and the present one suggest that the electrophysiological properties of I_{to} might be differentially regulated under physiological and pathological conditions.

In summary, we demonstrated the regulation pattern of co-expression of KCNE2 and KChIP2c on Kv4.2 current, which produced a current similar to native I_{to} . Future experiments need to be conducted to address how I_{to} is regulated under diseased conditions by changing stoichiometry of α - and β -subunits of I_{to} to simulate diseased conditions. It is also of interest to investigate how long QT syndrome-related KCNE2 mutations, which predispose the heart to arrhythmia^[15], regulate native I_{to} (with the presence of KChIP2) properties.

Author contributions

Wen-juan LIU and Hai-tang WANG contributed equally to this work; Jie LIU designed research; Wen-juan LIU, Hai-tang WANG, Wei-wei CHEN, Jian-xin DENG, and Yong JIANG performed research; Wen-juan LIU and Jie LIU analyzed data; and Jie LIU wrote the paper.

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