Modulation of Kv4.3 current by accessory subunits

Isabelle Deschênes, Gordon F. Tomaselli*

Department of Medicine, Institute of Molecular Cardiobiology, Division of Cardiology, Johns Hopkins University, 720 N Rutland Avenue, Ross 844, Baltimore, MD 21205, USA

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Abstract **Kv4.3 encodes the pore-forming subunit of the cardiac transient outward potassium current (I_{to}). hKv4.3-encoded current does not fully replicate cardiac I_{to}, suggesting a functionally significant role for accessory subunits. KChIP2 associates with Kv4.3 and modifies hKv4.3-encoded currents but does not replicate native I_{to}. We examined the effect of several ancillary subunits expressed in the heart on hKv4.3-encoded currents. Remarkably, the ancillary subunits Kvβ_3, minK, MiRP-1, the Na channel β_1 and KChIP2 increased the density and modified the gating of hKv4.3 current. hKv4.3 promiscuously assembles with ancillary subunits in vitro, functionally modifying the encoded currents; however, the physiological significance is uncertain.**

Key words: Ion channel; Potassium; Accessory subunit

1. Introduction

Voltage-gated K^+ channels open in response to membrane depolarization and are crucial determinants of the cardiac action potential duration and profile. A large number of K^+ channel pore-forming (α) and accessory (β) subunits have been identified [1,2]. The pore-forming subunits are transmembrane proteins that assemble as tetramers or dimers and are sufficient to form a K^+ selective pore. At least four general classes of K channel accessory subunits have been described; the type of interaction and effects on α subunit function differ among the potassium channel accessory subunits. The Kvβ family of subunits associates with the cytoplasmic amino terminus of the Kv1 family of α subunits and carboxyl terminus of the Kv4 family, increasing current density and in the case of Kv1 altering gating [3]. The K channel interacting protein (KChAP) interacts with Kv4.3 and Kv1.3, increasing current density without altering gating [4]. KvLQT1 and human ether-a-go-go related gene (HERG) interact with minK [5,6] and minK related protein (MiRP)-1 [7], respectively; these accessory subunits span the membrane and in the case of minK, alter the permeation properties of KvLQT1 [8,9]. Kv channel interacting proteins (KChIPs) interact with the amino terminus of the Kv4 family, increasing peak current density and altering the voltage dependence and kinetics of gating [10–14].

Kv4.3 [15,16] underlies the Ca^{2+}-independent transient outward K^+ current (I_{to}) in human ventricle. When the cloned α subunit of this gene is expressed in heterologous systems, the currents are similar to, but do not replicate I_{to} with complete fidelity, suggesting that accessory subunits may play a significant role in the phenotype of the native current. KChIPs are Ca^{2+}-binding ancillary subunits that modify Kv4-encoded currents in heterologous expression systems. However, even the coexpression of KChIP2 with Kv4.3 does not reproduce native I_{to} currents with complete fidelity [10–14]. Indeed, some effects of KChIP2 serve to make the expressed current more dissimilar to native cardiac I_{to} than Kv4.3 expressed alone.

In order to understand the molecular basis of I_{to} and verify the specificity of the gating modulation of hKv4.3 by KChIP2 [14] we have coexpressed hKv4.3 with other accessory subunits (Kvβ_3, minK, MiRP-1 and the Na channel β_1 subunit) in HEK293 cells and characterized the currents. All β subunits tested increased the density of the expressed Kv4.3 currents as previously described for KChIP2 [10–14]. Remarkably, coexpression of any of the ancillary subunits with Kv4.3 altered either the kinetics or voltage dependence of one or more gating processes. Almost certainly most of these channel subunit interactions do not occur in native cells and tissues, therefore the modulation of K channel α subunits by ancillary proteins in heterologous expression systems may not be relevant to native myocyte physiology.

2. Materials and methods

2.1. Heterologous expression of hKv4.3 and the β subunits in HEK293 cells

The full-length cDNA encoding hKv4.3-L was subcloned into pREP4 (Invitrogen). Full-length cDNAs encoding the different KChIP2s, minK and Kvβ_3 were subcloned into the pIRES-GFP (Clontech, Palo Alto, CA, USA) vector for bi-cistronic expression of these subunits and green fluorescence protein. MiRP-1 was subcloned in pCINeo [7] and the Na channel β_1 subunit was subcloned in pCMV5 [17]. MiRP-1 and the Na channel β_1 subunit were co-transfected with hKv4.3-L and pDsRed (Clontech) for identification by epifluorescence. Human embryonic kidney cells (HEK293) were grown in Dulbecco's modified Eagle's medium glucose supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (10 mg/ml) (Life Technologies, Rockville, MD, USA) in a humidified 5% CO_2 atmosphere. Transient transfections were performed using lipofectamine (Life Technologies) according to the manufacturer's instructions.

2.2. Electrophysiology

Cells were used for patch-clamp experiments 24–72 h after trans-

*Corresponding author. Fax: (1)-410-955 7953.
E-mail address: gtomasel@jhmi.edu (G.F. Tomaselli).

Abbreviations: I_{to}, transient outward potassium current; KChAP, K channel interacting protein; KChIP, Kv channel interacting protein; MiRP, minK related protein; HERG, human ether-a-go-go related gene

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fection. Macroscopic currents from transfected cells were recorded using the whole-cell configuration of the patch-clamp technique [18]. Cells that emitted green or red fluorescence and that expressed I_{to}-like current were considered to express both hKv4.3 and the respective β subunit. Currents were clamped using an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA, USA) interfaced to a personal computer. Voltage commands were issued and data collected with custom-written software. Patch electrodes were pulled from borosilicate glass and had 2–4 MΩ tip resistance when filled with an internal solution containing (in mM): 110 KCl, 1 $MgCl_2$, 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 1 ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 2 Mg-ATP with pH adjusted to 7.2 with KOH. The bath solution contained (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, and 10 glucose adjusted to pH 7.4. Membrane currents were filtered at 5 kHz and digitized with 12-bit resolution. Experiments were performed at room temperature (22–23°C).

Cell capacitance was calculated by integrating the area under an uncompensated capacity transient elicited by a 10 mV hyperpolarizing test pulse from a holding potential of −80 mV. Whole-cell currents were elicited by a family of depolarizing voltage steps from a holding potential of −80 mV. The decay rates were determined by a single exponential fit to the failing phase of the current over a range of voltages from 0 to +60 mV. Steady-state inactivation curves were generated by a standard two-pulse protocol with a conditioning pulse of 500 ms. The steady-state inactivation curves were fit with a Boltzmann function. Recovery from inactivation was assessed by a standard paired pulse protocol: a 500 ms test pulse to +50 mV (P1) was followed by a variable recovery interval at −80 mV, then by a second test pulse to +50 mV (P2). The plot of P2/P1 was fit by a single exponential function to determine the rates of recovery from inactivation.

2.3. Statistical analysis

Comparisons of the biophysical properties of expressed hKv4.3 current in the presence and absence of the different accessory subunits were performed using an unpaired *t*-test. Data were considered significantly different at $P < 0.05$.

3. Results

3.1. Coexpression of hKv4.3 with minK

The coassembly of KvLQT1 and minK (*KCNE1*) proteins forms the slow component of the delayed rectifier current, I_{Ks} [5,19]. When minK is coexpressed with hKv4.3 the current density increases about five-fold compared to hKv4.3 expressed alone (Fig. 1B,G) without a significant change in the current–voltage (*I–V*) relationship (Fig. 2A). MinK significantly slows the whole-cell gating kinetics of hKv4.3. The activation time constant, and therefore time to peak current, were prolonged. The current decay was also significantly slowed in presence of minK, with a time constant at +60 mV of 95 ± 12 ms compared to 70 ± 12 ms for hKv4.3 expressed alone (Figs. 1 and 2 and Table 1). Recovery from inactivation of Kv4.3 currents was significantly slowed in the presence of minK; τ_{rec} was increased more than two-fold (hKv4.3: τ_{rec} = 75 ± 2 ms; hKv4.3+minK: τ_{rec} = 168 ± 11 ms) (Fig. 2 and Table 1). The steady-state inactivation curve, however, was not significantly modified (Fig. 2 and Table 1).

3.2. Coexpression of hKv4.3 with MiRP-1

MiRP-1 is known to interact with HERG and is believed to be a component of the rapid component of the delayed rectifier current, I_{Kr} [7]. MiRP-1 increased the density of expressed hKv4.3 current about three-fold (Fig. 1C,G) without a significant change in the *I–V* relationship. A modest but significant slowing of the current decay was present over the entire voltage range tested (Fig. 2B and Table 1). Activation kinetics, recovery from inactivation and steady-state availability were unchanged compared to hKv4.3 expressed alone (Figs. 1 and 2, and Table 1).

3.3. Coexpression of hKv4.3 with the Na channel β_1 subunit

Although the distinct profile of gating changes produced by minK and MiRP-1 suggested that the effects of these subunits on hKv4.3 current were specific we wanted to examine the effects of an ancillary subunit with a similar transmembrane topology from an unrelated channel family. The NaCh β_1 subunit is a single membrane spanning repeat subunit that coassembles with a number of the voltage dependent Na channel isoforms increasing current density and altering channel gating. Coexpression of NaCh β_1 with hKv4.3 increased current density about four-fold (Fig. 1D,G), as much as any of the K channel-specific ancillary subunits. NaCh β_1 did not alter the *I–V* relationship but significantly affected the voltage dependence and kinetics of channel gating compared to hKv4.3 expressed alone. Activation of the current was hastened through all the voltages (time to peak = 10.5 ± 0.7 ms for Kv4.3+NaCh β_1 compared to 14.2 ± 1.1 ms for Kv4.3 alone at +40 mV). The current decay was sped two-fold (τ_h = 34 ± 4 ms) (Figs. 1 and 2, and Table 1), recovery from inactivation was two-fold slower (τ_{rec} = 135 ± 14 ms) (Fig. 2 and Table 1). The NaCh β_1 subunit shifted the steady-state inactivation relationship 10 mV in the hyperpolarized direction ($V_{1/2}$ = −56 ± 4 mV) (Fig. 2 and Table 1).

3.4. Coexpression of hKv4.3 with $Kv\beta_3$

Auxiliary Kvβ subunits form complexes with Kv1 family voltage-gated K channels by binding to the N-terminus of the channel protein. This association influences expression and gating of these channels. The Kvβ family also associates with the C-terminus of Kv4.3, increasing current density [3] without affecting gating. As with the other accessory subunits, $Kv\beta_3$ increased the hKv4.3 current density (Fig. 1E,G) without changing the *I–V* relationship (Fig. 3A). The rate of decay of the currents (Fig. 3B) was unaffected but the recovery from

Table 1
Functional effects of different accessory subunits on hKv4.3 currents expressed in HEK293 cells

Biophysical parameter	hKv4.3	hKv4.3+ minK	hKv4.3+ MiRP-1	hKv4.3+ NaCh β_1	hKv4.3+ $Kv\beta_3$	HKv4.3+ KChIP2	hKv4.3+ KChIP2+$Kv\beta_3$
Inactivation time constant (τ_h in ms at +60 mV)	70 ± 11	95 ± 12*	82 ± 3*	34 ± 4*	66 ± 4	148 ± 17*	104 ± 8*
Recovery from inactivation (τ_{rec} in ms)	75 ± 2	168 ± 11*	78 ± 3	135 ± 14*	175 ± 14*	46 ± 3*	44 ± 4*
Steady-state inactivation ($V_{1/2}$ in mV)	−46 ± 3	−52 ± 3	−49 ± 3	−56 ± 4*	−55 ± 3*	−50 ± 3	−52 ± 3
Number of cells (*n*)	6	5	5	5	5	5	5

*Significantly different from hKv4.3 ($P < 0.05$).

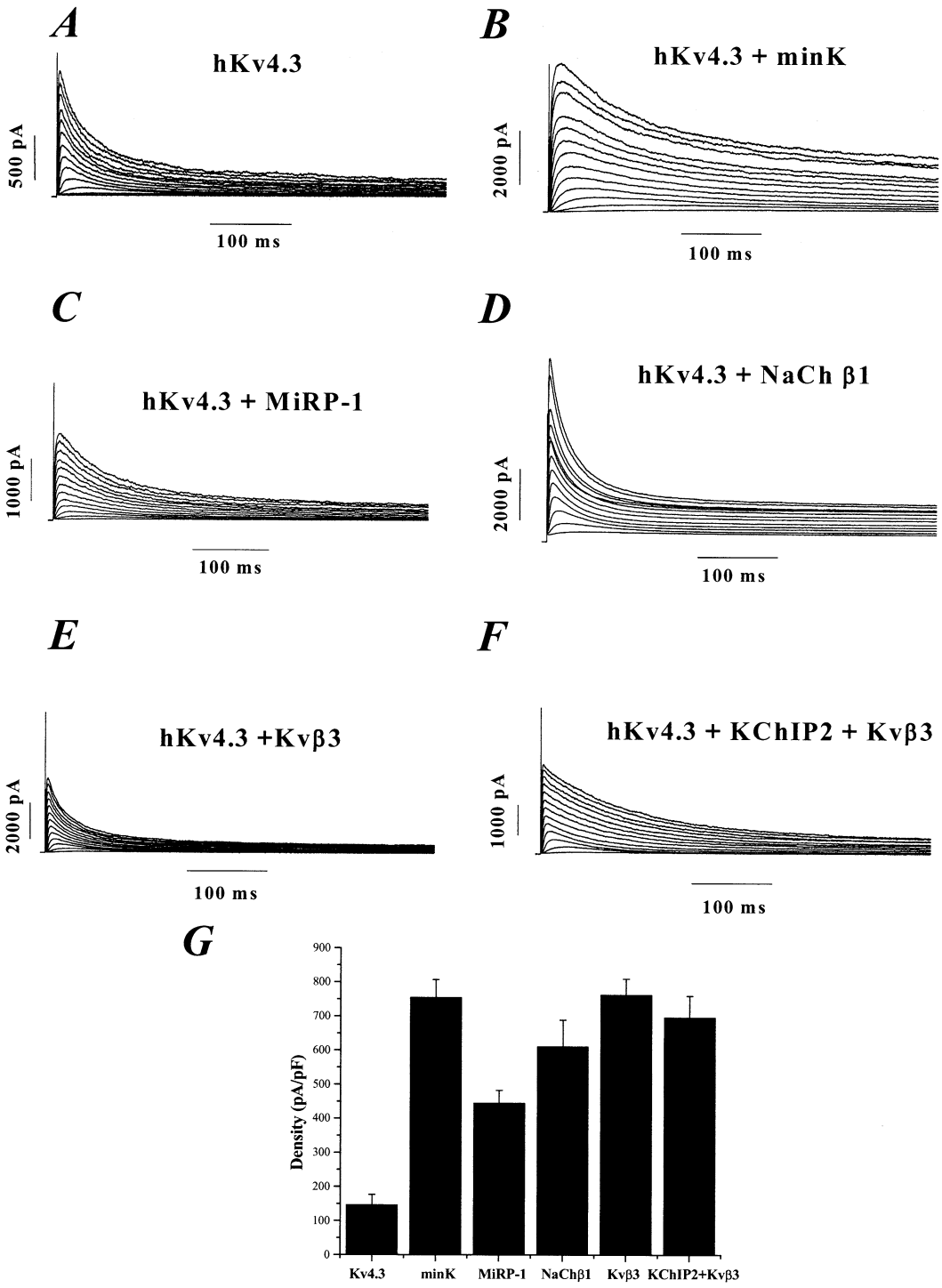

Fig. 1. Representative families of currents encoded by either Kv4.3 alone (A) or coexpressed with minK (B), MiRP-1 (C), NaCh β_1 (D), Kvβ3 (E), or KChIP2 plus Kvβ3 (F). The current records were elicited by 500 ms voltage steps from −40 to +80 mV in increments of 10 mV from a holding potential of −80 mV. (G) Current density of Kv4.3 expressed alone and with the other ancillary subunits. There is at least a three-fold increase in density with all subunits that were tested ($N \geq 5$ cells for each determination).

inactivation was significantly slowed ($\tau_{rec} = 175 \pm 14$ ms)(Fig. 3C). The voltage dependence of the steady-state inactivation relationship was significantly shifted in the hyperpolarizing direction ($V_{1/2} = -55 \pm 3$ mV) (Fig. 3D and Table 1).

3.5. Coexpression of hKv4.3 with both $Kv\beta_3$ and KChIP2 subunits

Previous studies have demonstrated that KChIP2 coexpression modulates expressed Kv4 family currents; however, even this combination of channel subunits is not sufficient to recapitulate native cardiac I_{to} [10–14]. Kvβ subunits are known to associate with the C-terminus of Kv4 subunits, increasing the density of the expressed current. We tested whether the coexpression of the combination of hKv4.3, $Kv\beta_3$ and KChIP2 recapitulates native cardiac I_{to} with high fidelity. Coexpression of KChIP2 and $Kv\beta_3$ with hKv4.3 produced a five-fold in-

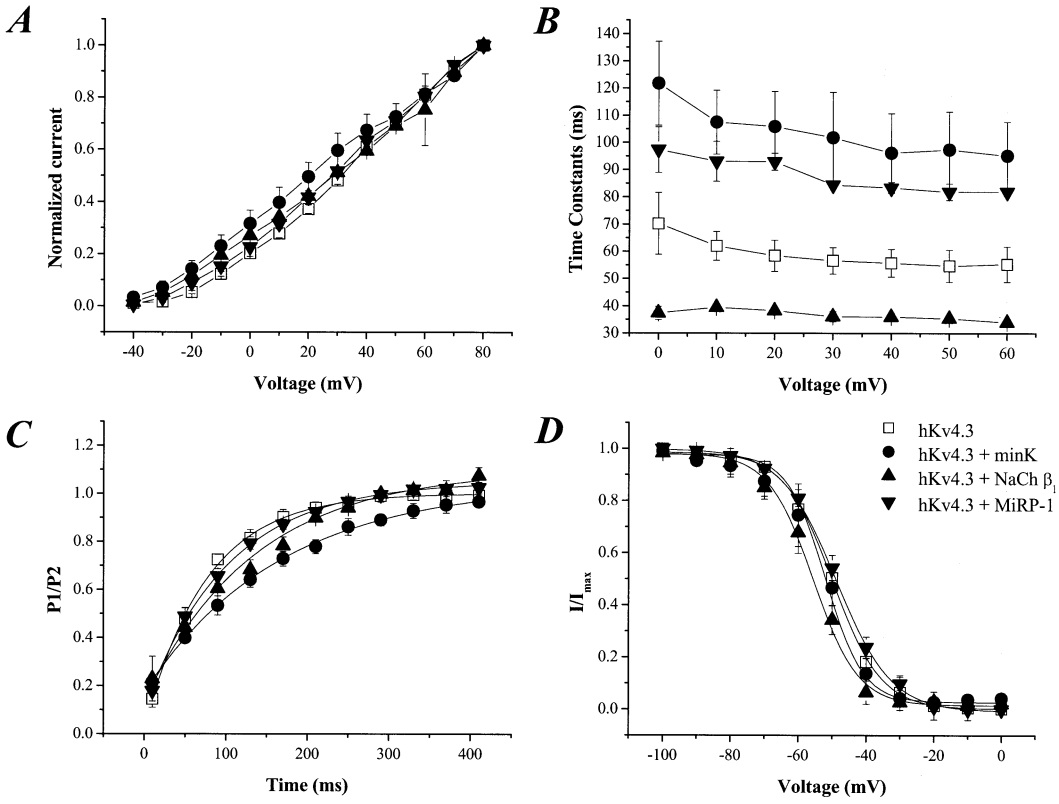

Fig. 2. Biophysical properties of hKv4.3 currents in the presence of ancillary subunits. A: There is no significant change in the current–voltage relationship in the presence of minK, NaCh β_1 and MiRP-1 compared with hKv4.3 alone. B: The time constant of decay is significantly increased for each of these ancillary subunits compared to hKv4.3 alone. C: Both minK and NaCh β_1 significantly slow the recovery from inactivation compared to hKv4.3 alone (Table 1). D: NaCh β_1 significantly shifts the steady-state inactivation curve in the hyperpolarizing direction.

crease in current density (Fig. 1F,G) without altering the *I–V* relationship compared with hKv4.3 expressed alone (Fig. 3A). The current decay was significantly slowed ($\tau_h = 104 \pm 8$ ms) similar to that of KChIP2 alone expressed with hKv4.3 (Fig. 3B). The combination of KChIP2 and Kvβ_3 hastened recovery from inactivation of hKv4.3 ($\tau_{rec} = 44 \pm 4$ ms), an effect that was similar to coexpression of KChIP2 with hKv4.3 (Fig. 3C and Table 1). KChIP2 and Kvβ_3 produce less of a hyperpolarizing shift in the steady-state inactivation curve than hKv4.3 with Kvβ_3 alone (Fig. 3D and Table 1).

4. Discussion

In humans, I_{to} is encoded by Kv4.3 [15] and possibly the Ca^{2+}-binding ancillary subunit KChIP2 [10]. Indeed, coexpression of Kv4.x and KChIP2 increases peak current density and alters the voltage dependence and kinetics of gating compared with current encoded by Kv4.x alone [10–14]. However, the slowing of current decay produced by coexpression of KChIP2 with hKv4.3 is incongruous with native cardiac I_{to} where the time constants of macroscopic current decay under similar recording conditions are significantly faster than the expressed currents [14,16,20]. The discrepancy between native and expressed currents suggests the possibility of the involvement of additional ancillary subunits in the formation of cardiac I_{to}.

We transfected multiple ancillary subunits, known to be expressed in the heart, with Kv4.3. None of the subunits dramatically improved upon the recapitulation of native I_{to} by expressed hKv4.3. Remarkably, all coexpressed ancillary subunits significantly and specifically altered expressed hKv4.3 currents. All accessory subunits tested markedly increased current density to roughly the same extent as the putative physiological partner of hKv4.3, KChIP2. Thus modulation of expression of K currents in heterologous systems is promiscuous; ancillary subunits of different K channel families and even different ion channel subfamilies (NaCh β_1) augment the expression of hKv4.3. Although the mechanism of the increase in expression of hKv4.3 current is not known, all of these ancillary subunits (Kvβ_3, minK, MiRP-1 and the Na channel β_1 subunit) have been shown to enhance trafficking of channel subunits to cell surface [3,5,7,19]. Our results suggest that the facilitation of trafficking of α subunits to the cell membrane may be a more fundamental property of ancillary channel subunits.

KChIP2 modifies the gating of Kv4.x-encoded currents. This is not a property unique to this ancillary subunit. All of the ancillary subunits studied modified the biophysical properties of hKv4.3 in distinct ways (Table 1). It appears that the effect of over-expression of these ancillary subunits on hKv4.3 is specific in that unique effects on the current are produced by each of the smaller subunits.

The question of the subunit composition of native cardiac I_{to} remains open. Coexpression of MiRP-1 with Kv4.2 and Kv4.3 in *Xenopus* oocytes slowed gating kinetics without changing current density and induced an overshoot in the recovery similar to that observed in I_{to} recorded in myocytes isolated from the epicardium of the ventricle [21]. We observe

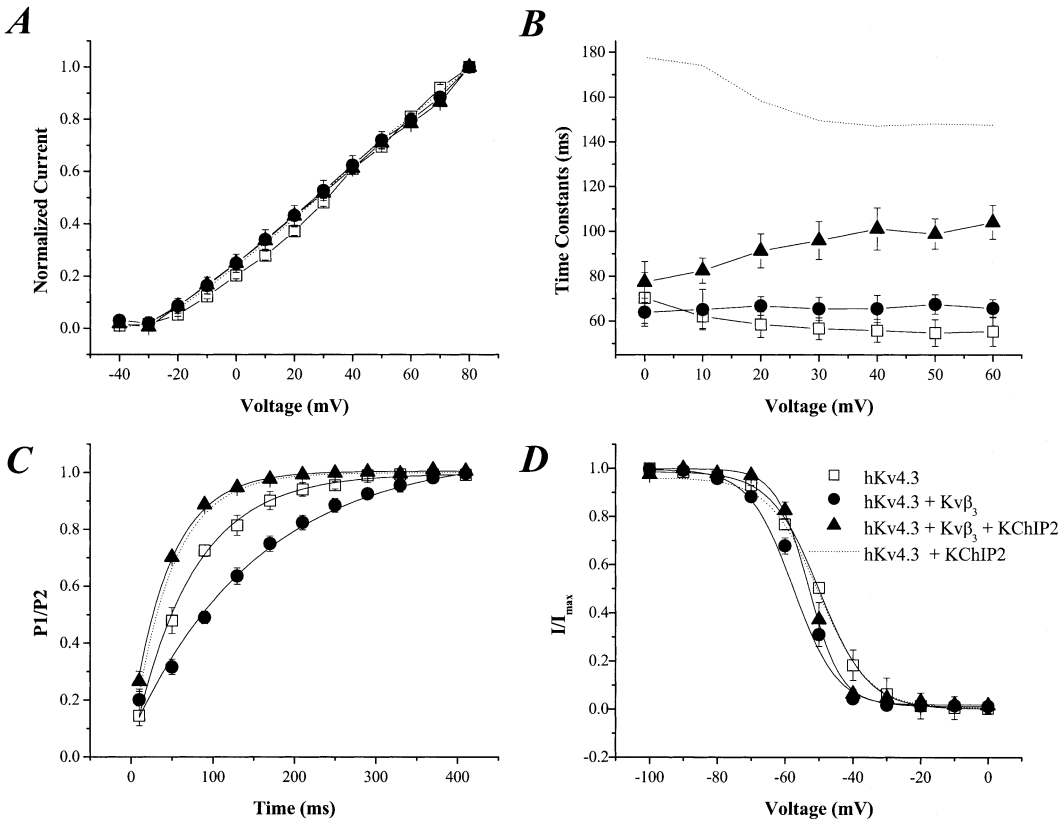

Fig. 3. The effect of coexpression of KChIP2 and $Kv\beta_3$ on hKv4.3 currents. A: There is no effect on the current–voltage relationship of either $Kv\beta_3$ or the combination of $Kv\beta_3$ and KChIP2 on hKv4.3 currents. B: $Kv\beta_3$ does not significantly alter the current decay of expressed hKv4.3 and does not antagonize the KChIP2-induced slowing of hKv4.3 current. C: $Kv\beta_3$ significantly slows recovery from inactivation and the combination of $Kv\beta_3$+KChIP2 hastens recovery. D: $Kv\beta_3$ produces a significant hyperpolarizing shift in the steady-state inactivation curve that is not observed with $Kv\beta_3$+KChIP2. The dotted lines represent hKv4.3+KChIP2 from Deschênes et al. [14].

a similar slowing of gating kinetics when MiRP-1 is expressed with hKv4.3 in mammalian cells without an overshoot during recovery from inactivation. The effect of MiRP-1 on Kv4.3 current kinetics does not improve the recapitulation of native cardiac I_{to}. Coexpression of two other β subunits known to interact with Kv4 channels modifies the current in a manner distinct from either KChIP2 or $Kv\beta_3$ alone with hKv4.3. The major gating effects of KChIP2 are to slow the rate of current decay and increase the rate of recovery from inactivation [10–14], effects that are retained in the presence of $Kv\beta_3$. In comparison, $Kv\beta_3$ slows recovery and shifts the steady-state inactivation curve in the hyperpolarizing direction. The presence of KChIP2 neutralizes the effect of $Kv\beta_3$ on recovery but does not significantly alter the shift in the voltage dependence of steady-state inactivation (Fig. 3 and Table 1). Therefore the combination of the two accessory subunits produces an expressed hKv4.3 current with a unique phenotype that is distinct from cardiac I_{to}. It is likely that other, as yet undefined subunits or factors in addition to KChIP2 and possibly Kvβ are a part of the native cardiac I_{to} channel complex.

The existence of a large number of K channel α and β subunits (and splice variants) creates a nearly limitless set of subunit combinations that contribute to the great diversity in K channels. In order to probe the relationships between K channel subunits and functional K currents expressed in native cells and tissues, in vitro and in vivo experimental approaches have been used. In most cases, expression of the pore-forming subunits alone in heterologous expression systems is sufficient to produce a reasonable facsimile of a native current. Often coexpression of an ancillary subunit will further enhance the mimicry of the native current by the expressed current. In virtually all cases, however, identical reproduction of native current by expressed channel subunits is not achieved. Differences in native and expressed currents are often ascribed to other channel-associated proteins or factors present in the native cell but not the expression system. In addition, differences in membrane composition may also contribute to the divergent biophysics and pharmacology of expressed and native currents [22].

Our results demonstrate specific but non-physiological effects of a variety of ancillary subunits on the expressed hKv4.3 current. Indeed, for most of these ancillary subunits there is no independent evidence for an association with Kv4.x channels in native cells and tissues. The diversity of K channel phenotypes in expression systems appears to exceed that observed in nature due to the ability of subunits to associate and modulate function in ways that do not appear to occur in native cells. It is therefore essential that other means of defining associations (immunoprecipitation, immunolabeling and electron microscopy or vital methods such as fluorescence resonance energy transfer between tagged subunits) among ion channel subunits be used when trying to assign a role for a channel subunit to a native current.

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